ISSN 1523-5475

Journal of Agricultural and Urban Entomology

Contents

Volume 24, No. 1 October 2007

Responses of Oriental Fruit Fly (Diptera: Tephritidae) Third Instars to Desiccation and Immersion[1]

Qi Xie[2] and Runjie Zhang[3]

J. Agric. Urban Entomol. 24(1): 1–11 (January 2007)

ABSTRACT Responses of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), third instar larvae to desiccation and immersion were examined at 25°C in the laboratory condition. Third instar larvae desiccated in dry soils (0% field capacity) did not survive beyond 12 h, nor submerged under water beyond 3.5 d. The time to 50%, 90%, and 99% mortality (LT_{50}, LT_{90}, and LT_{99}) were 5.67, 8.60, and 10.99 h respectively in desiccation condition, and 1.81, 2.49, and 3.06 d in immersion test. The average eclosion time of survived flies was not significantly affected by the desiccation time, while that increased linearly with increasing immersion time. Cumulative weight loss of larvae increased curvilinearly with desiccation time, and the rate of weight loss was greatest during the first 2 h. The relationship between the mortality and the percentage of cumulative weight loss was described by Probit model in desiccation. It was estimated that when the percentage of cumulative weight loss was 29.1%, 36.7%, and 43.0% respectively, the mortality rates was 50%, 90%, and 99% correspondingly.

KEY WORDS *Bactrocera dorsalis* (Hendel), mortality, cumulative weight loss, eclosion time

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), was recorded for the first time in 1911 in Taiwan (Liu 1981). Over the next 90 years, this fly has been widely distributed in East Asia and around the Pacific Ocean, where it is a very destructive pest of tropical, subtropical and temperate fruits and vegetables (White & Elson-Harris 1992). Over one hundred species of plants are its hosts, including most types of commercial fruits, such as mango, citrus, guava, plum, papaya, banana, and a wide variety of other agriculture products such as coffee, chili peppers, watermelon, and also wild hosts (Steck 2003). It inflicts heavy economic losses not only on fruit and vegetable yields, but also on export markets. Hence, in many countries or regions, most commercial fruits are severely restricted by quarantine laws to prevent the spread of the oriental fruit fly. Nonetheless, this pest is expanding its distribution ranges unceasingly (Allwood & Drew 1997, Lux et al. 2003).

Tephritid distribution and abundance are markedly structured by several factors, such as temperature, humidity, light intensity, hosts, natural enemies

[1]Accepted for publication 29 October 2007.
[2]The College of Economics, Trade & Statistics, Guangdong University of Business Studies, Guangzhou 510320, P. R. China. E-mail: qiqixie@gmail.com.
[3]State Key Laboratory for Biocontrol & Institute of Entomology, Sun Yat-Sen University, Guangzhou 510275, P.R. China.

etc (White 1980, Boller & Remund 1989, Vargas et al. 1989, 1990, Ye & Liu 2005, 2007, Segura et al. 2006, Duyck et al. 2006). Among these factors, temperature and humidity are two of the most important abiotic factors. Many studies have been carried out to determine the minimum development thresholds and the effects of different temperatures (constant or alternating temperatures) on the development, survival and fertility of the oriental fruit fly (Vargas et al. 1984, Yang et al. 1994, Vargas et al. 1996, Vargas et al. 1997, Vargas et al. 2000). However, less attention is paid to the effects of humidity on the oriental fruit fly because its eggs and larvae develop within fruits and adults need little water to survive. Mature third instar larvae of the oriental fruit fly exit the fruit and burrowed into the ground to pupate, thus humidity is expected to be a major limiting factor for pupation. Some researches have demonstrated that soil humidity has a direct and strong effect on the depth of pupation and pupal development of the oriental fruit fly (Vargas et al. 1987, Jackson et al. 1998, Alyokhin et al. 2001, Hou et al. 2006). However, it is not well known about the tolerance of the oriental fruit fly to extreme humidity. In this study, the desiccation and immersion tolerance of the third instar larvae of the oriental fruit fly were examined in the laboratory condition. The weight loss of the third instar larvae in desiccation, and average eclosion time of the survived flies were also determined. And at last, the use of flooding to manage this pest is briefly discussed.

Materials and Methods

Insects. *B. dorsalis* used in experiments were taken from a stock colony maintained at the Research Institute of Entomology, Sun Yat-Sen University, Guangzhou, China. All larvae were reared with peeled banana (*Musa acuminata* Colla) at 25 ± 1°C, 75% RH and a photoperiod of 12:12 (light:dark) h. Late third-instars (characterized by their nonfeeding and wandering behavior) were collected and used in the experiments.

Desiccation conditions. Oven-dried soils (0% field capacity) were used for making desiccation conditions. Tested soils were sampled at 0–15 cm depths from Luntou Orchard in Guangzhou suburb (E113°17′, N23°8′). The soils consist of 40.7% silt, 32.5% sand and 26.8% clay. Prior to experimentation, soils were sifted through a 1-mm-mesh metal sieve and dried at 100°C for 48h in an oven, then kept in a desiccator containing activated silica gel.

Desiccation tolerance test. Eight durations (2, 4, 6, 8, 10, 12, 14, and 16 h) were designed in the desiccation experiment, each with 40 larvae for testing. The tested larvae were placed in plastic cups (12.0 × 7.5 × 5.0 cm, height × top diameter × bottom diameter) containing dried soil. The cup had some holes (ca. 0.8 mm diameter) drilled in a lid to provide adequate aeration. Before covering the cup with the lid, enough activated silica gel were placed on the surface of the soil to keep the inside air dry. Then, the cups were held in chamber at 25 ± 1°C, 50% RH, and a photoperiod of 12:12 (light:dark) h. Individual cups were removed from the chamber at specified time points. The larvae were screened from the soils, transferred to another plastic cup with moist (60% RH) and sterile sand, and kept at 25 ± 1°C, 75% RH, and a photoperiod of 12:12 (light:dark) h for emergence. Newly emerged flies were checked and removed daily until no emergence was observed. Control larvae were placed in moist sand at the

beginning (i.e., desiccation duration = 0 h). Four replicates were set up in this experiment.

Weight loss in desiccation. Cumulative weight loss of the third instar larvae in dried soils over time was determined gravimetrically.

Forty larvae were weighed as a group on an electronic balance (Mettler-Toledo, model AB104-S, Switzerland) and placed in desiccation conditions as described above. Following a defined desiccation time (2, 4, 6, 8, 10, 12, 14, and 16 h), the larvae were screened from the soils and reweighed rapidly. The cumulative percentage of weight loss for forty larvae was calculated using the following equation: $(M_0 - M_t) / M_0$, where M_0 was the initial fresh mass, M_t was the mass measured at specific time points as mentioned previously. Four replicates were performed.

Immersion tolerance test. Healthy and active mature larvae (N = 320) were immersed simultaneously in still water at 25 ± 1°C. Sodium benzoate was added to the tap water to prevent growth of fungi. To determine survivorship, forty larvae were removed randomly from the water and transferred to moist sand (60% RH) after immersion of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 d, respectively. All samples were held at 25 ± 1°C and 75% RH under a 12:12 (light:dark) h photoperiod for emergence. The number of newly emerged adults was recorded and removed daily. For control, larvae were placed directly into the moist sand, without any immersion. Four replicates were set for each immersion period.

Data analysis. The insects succeed in emerging from the puparia were counted as survivors. Probit analysis was adopted to calculate time to 50%, 90%, 99% mortality (LT_{50}, LT_{90}, LT_{99}) and 95% confidence intervals in desiccation and immersion test. Polynomial regression was used to estimate the relationship of cumulative weight loss of larvae to desiccation time. Probit model was used to analyze the relationship between mortality and cumulative weight loss. Data on adult eclosion rates at each observation time were calculated. The eclosion rates among different treatment time at the same observation were compared by Tukey-Kramer HSD tests. Arcsine square root transformation was applied to adult emergence rates prior to analysis, but untransformed means are presented. The average eclosion time of the survived flies in desiccation and immersion test was calculated using the formula as Hou et al. (2006) described. The relationship between average eclosion time and treatment time was analyzed by a one-way ANOVA (desiccation test) or a linear regression (immersion test). All analyses were conducted using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

Results

Larvae mortality and average eclosion time. The effects of desiccation and immersion on larvae mortality are shown in Figs 1 and 2. The mortality was increased with increasing treatment time. The larvae in the dried soils did not emerge beyond 12 h, nor submerged under water beyond 3.5 d. The LT_{50}, LT_{90}, and LT_{99} values were 5.67, 8.60, and 10.99 h in the desiccation test, and 1.81, 2.49, and 3.06 d in the immersion test, respectively (Table 1). The time to attain 50%, 90%, and 99% mortality in the desiccation condition was much shorter than those in the immersion.

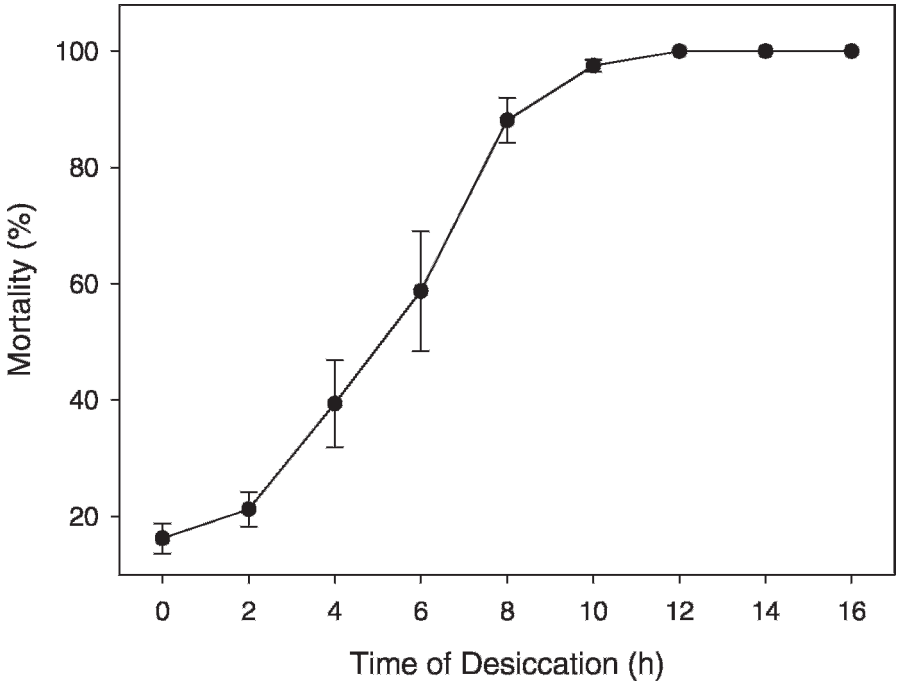

Fig. 1. The mortality rates of third instars of the oriental fruit fly (mean ± SEM) exposed to desiccation of different durations.

In the desiccation condition, adult emerged in 10–12 d, and the greatest number of adults emerged in 11 d, regardless of desiccation durations (Table 2). There were no significant differences in the average eclosion time among different desiccation durations ($F_{5,22} = 0.128$, $P = 0.984$). In the immersion test, however, adult eclosion time was related to the immersion durations. The eclosion time of the first adult was delayed with the increasing immersion duration. The similar trend was also observed in the day of peak emergence. Nevertheless, the total emergence period did not lengthen (i.e., all flies survived post immersion emerged within two or three days) (Table 3). The relationship between the average eclosion time (Y) and immersion time (X) was described as following equation: $Y = 10.953\ (\pm 0.097) + 1.496\ (\pm 0.054)\ X$, $F_{1,6} = 780.216$, $P < 0.001$, $R^2 = 0.994$ (Fig. 3). The equation indicated the average eclosion time increased about 18 h as the immersion time increased 12 h.

Weight loss in desiccation. Cumulative percentage of weight loss (Y) increased curvilinearly with desiccation time (X) (Fig. 4). The regression function was: $Y = 2.652\ (\pm 1.570) + 5.402\ (\pm 0.457)\ X - 0.156\ (\pm 0.028)\ X^2$, $F_{2,8} = 288.83$, $P < 0.001$, $R^2 = 0.990$. The highest rate of weight loss occurred between 0 and 2 h of desiccation, after which the rate of weight loss was gradual for the larvae. Throughout the 16 h desiccation, the cumulative weight loss was 50.7 ± 3.4%.

Relationship between weight loss and mortality in desiccation. Mortality was low (compared with the control) when the weight loss was less than 15%. However, the mortality increased sharply at the weight loss over 15%.

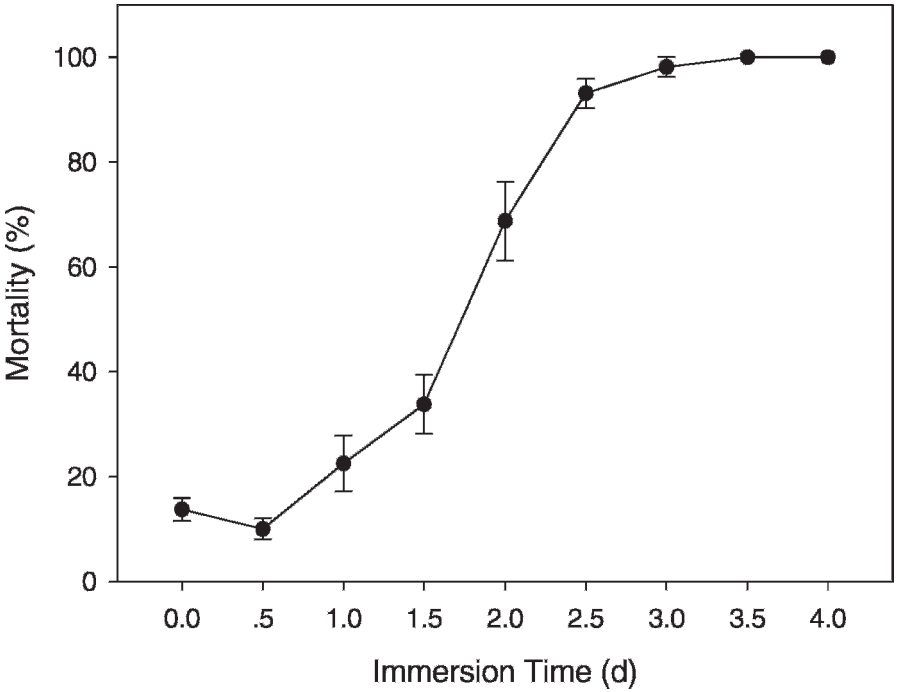

Fig. 2. The mortality rates of third instars of the oriental fruit fly (mean ± SEM) immersed in water of different durations.

Once a 40% of body weight lost, almost all larvae were dead (Fig. 5). The relationship between the weight loss and the mortality was well fitted by the Probit model (Intercept: −4.849 ± 0.529, Slope: 16.690 ± 1.546; χ^2 = 6.899, P = 0.330). It was estimated that when the percentage of cumulative weight loss was 29.1%, 36.7%, and 43.0% respectively, the mortality rates was 50%, 90%, and 99% correspondingly.

Table 1. Estimated lethal times (LT_{50}, LT_{90}, LT_{99}) and their approximate 95% CL (by Probit Analyses) for the oriental fruit fly third instars in different treatments.

Treatment	Intercept (SE)	Slope (SE)	LT_{50} (95% CL)	LT_{90} (95% CL)	LT_{99} (95% CL)
Dry soils[a]	−2.4790	0.4373	5.67	8.60	10.99
	(0.3037)	(0.0400)	(5.13–6.10)	(8.19–9.90)	(10.33–11.89)
Water[b]	−3.3657	1.8631	1.81	2.49	3.06
	(0.3251)	(0.1509)	(1.71–1.89)	(2.40–2.61)	(2.90–3.26)

[a]Lethal times are listed in hours with 95% CL.
[b]Lethal times are listed in days with 95% CL.

Table 2. Eclosion rate and average time to emergence of the oriental fruit fly third instars in desiccated soils with different times.

Desiccation time (h)	Eclosion rates (Mean ± SEM, %)				Average time to emergence (d)
	10d[a]	11d	12d	Total	
0	20.0±10.2a	61.3±7.3a	2.5±2.5a	83.8±2.6	10.8±0.1a
2	17.5±8.1a	56.3±5.5a	5.0±5.0a	78.8±3.0	10.9±0.2a
4	13.8±8.2a	40.0±1.8ab	6.9±6.1a	60.6±7.5	10.9±0.2a
6	9.4±6.0a	30.0±5.9b	1.9±1.9a	41.3±10.3	10.9±0.2a
8	1.3±0.7a	9.4±3.0c	1.3±1.3a	11.9±3.9	10.9±0.1a
10	0.6±0.6a	1.9±0.6c	0.0±0.0a	2.5±1.0	10.8±0.2a

[a]days after larvae treated.
Means within a column containing the same letter are not statistically different ($P > 0.05$) using Tukey test.

Discussion

The results indicated that the third instar larvae of the oriental fruit fly were more sensitive to desiccation (LT_{50} = 5.67 h) than to immersion (LT_{50} =1.81 d). The significant difference in tolerance of humidity may be related to its habitat

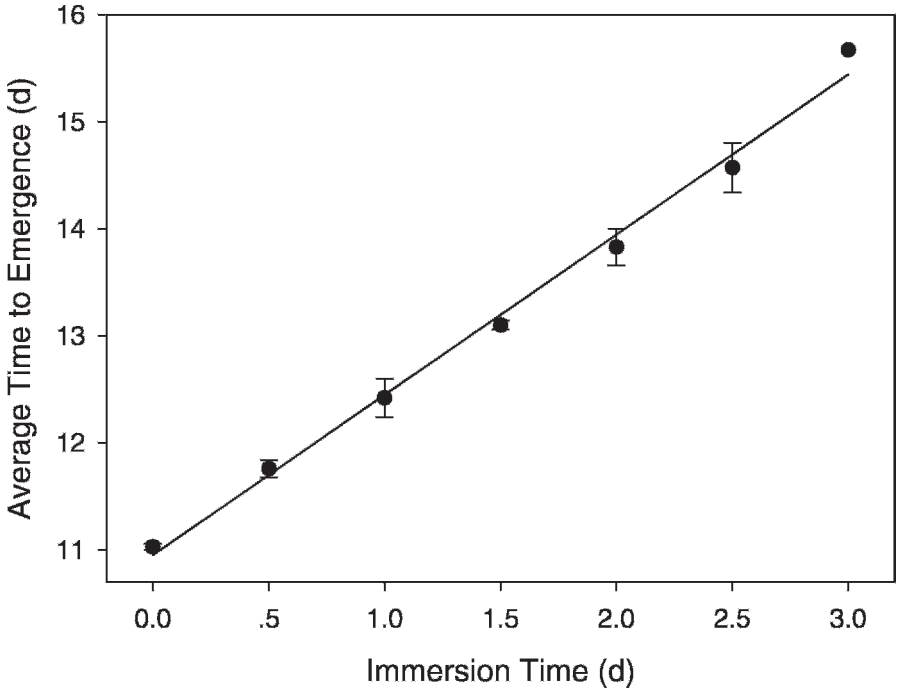

Fig. 3. The relationship between immersion time and average eclosion time of the oriental fruit fly third instars survived in water.

Table 3. Eclosion rate and average time to emergence of the oriental fruit fly third instars in water with different times.

Immersion time (d)	Eclosion rates (Mean ± SEM, %)									Average time to emergence (d)
	10d[a]	11d	12d	13d	14d	15d	16d	17d	Total	
0	0.6±0.6a	82.5±1.0a	3.1±2.4b	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0	0.0±0.0a	86.3±2.2	11.0±0.0
0.5	0.0±0.0a	21.3±6.9b	68.8±7.5a	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0	0.0±0.0a	90.0±2.0	11.8±0.1
1	0.0±0.0a	0.0±0.0c	45.6±15.4a	31.3±15.4ab	0.6±0.6bc	0.0±0.0b	0.0±0.0	0.0±0.0a	77.5±5.3	12.4±0.2
1.5	0.0±0.0a	0.0±0.0c	0.6±0.6b	59.4±6.2a	5.6±1.2b	0.6±0.6ab	0.0±0.0	0.0±0.0a	66.3±5.6	13.1±0.0
2	0.0±0.0a	0.0±0.0c	0.0±0.0b	7.5±7.5bc	23.8±6.6a	0.0±0.0b	0.0±0.0	0.0±0.0a	31.3±7.5	13.8±0.2
2.5	0.0±0.0a	0.0±0.0c	0.0±0.0b	0.0±0.0c	3.8±2.4bc	3.1±1.2a	0.0±0.0	0.0±0.0a	6.9±2.8	14.6±0.2
3	0.0±0.0a	0.0±0.0c	0.0±0.0b	0.0±0.0c	0.0±0.0c	1.3±1.3ab	0.0±0.0	0.6±0.6a	1.9±1.9	15.7

[a]days after larvae immersed.
Means within a column containing the same letter are not statistically different ($P > 0.05$) using Tukey test.

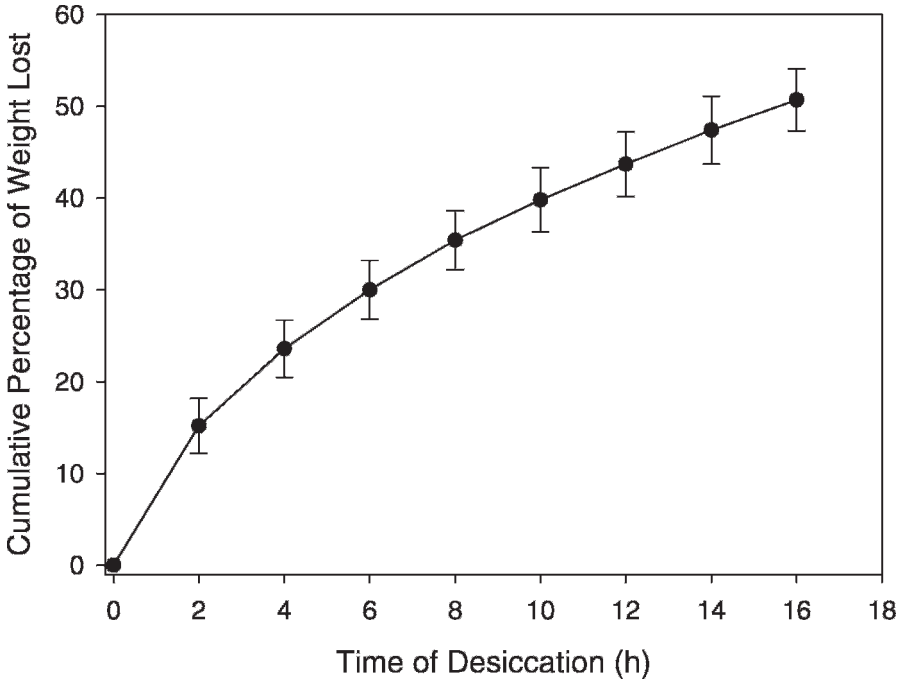

Fig. 4. Cumulative percentage of weight lost over time of the oriental fruit fly third instars during desiccation at 25 ± 1°C.

and physiological structure. The larvae of the oriental fruit fly develop within fruits with high water content. Before the puparium was formed, the cuticular structure and physiological characteristics of the mature larvae could still make them tolerate extreme humidity environment for a long period, but the ability to prevent water loss from the body may be relatively poor.

The tolerance to desiccation and immersion in different Tephritid species was quite different. For example, there were 19.5%–24.0% of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) mature larvae survived in different desiccated soils (0% water saturation soil) and only 3.5% mature larvae survived after a 2-d immersion (Eskafi & Fernandez 1990), while our results exhibited that no larvae of the oriental fruit fly survived in desiccated soils beyond 12 h and about 31% larvae emerged after a 2-d immersion. And for the pupae, the resistance to desiccation of the Mediterranean fruit fly was stronger than that of the oriental fruit fly (Vargas et al. 1987). This discrepancy in humidity tolerance may partially explain the phenomenon that though these two species have similar hosts and life history strategies, their ecological niches does not overlap completely in some zones, such as Hawaii.

In extreme desiccated soil, the rate of the larvae weight loss was greatest during the initial times of desiccation. The similar situation was also observed in the pupae of the oriental fruit fly and other two Tephritid species developed in low humidity (Vargas et al. 1987). Additionally, there was an obvious relationship between the weight loss and the mortality (Fig. 5). The results showed that

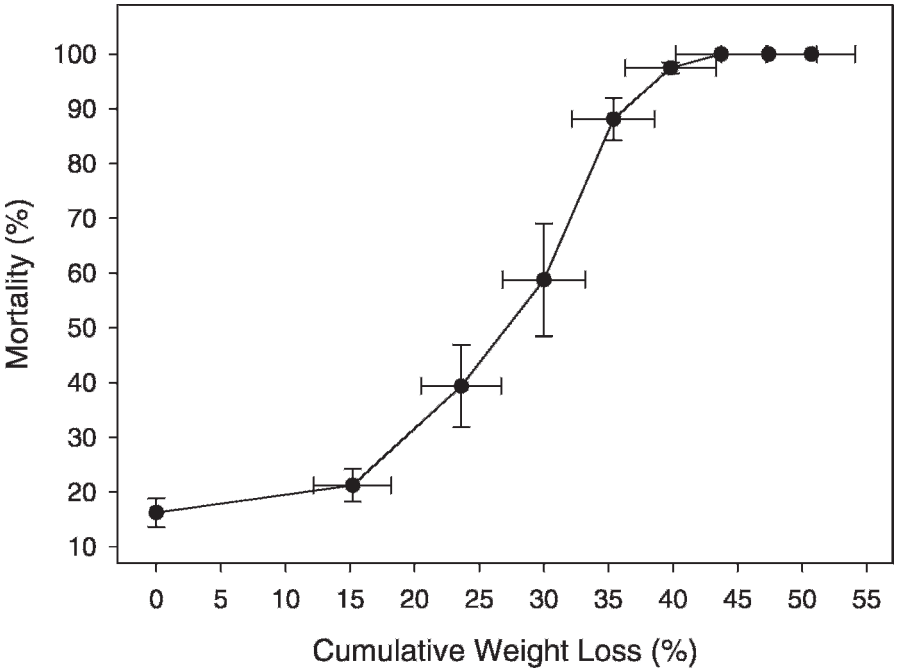

Fig. 5. The relationship between cumulative weight loss and mortality (mean ± SEM) of the oriental fruit fly third instars in desiccated soils.

cumulative weight loss of less than 15% had little effect on mortality, while losses exceeding about 29% caused a massive increase in mortality. It was suggested the extreme low relative soil moisture resulted in desiccation of the larvae, and the sharp increase in mortality may be attributed to the loss of weight reaching the critical level. This relationship between the weight loss and the mortality in our work was similar to that observed in eggs of the tick, *Rhipicephalus* (Boophilus) *microplus* (Canestrini) (Ixodidae) (Sutherst & Bourne 2006).

Immersion not only affected the survival, but also the development of the oriental fruit fly. The development time (i.e., the average eclosion time) was prolonged significantly with the immersion time. The reason was that in the water, the wandering phase larvae ceased movement, and the process of pupariation (puparium formation) was prevented or retarded by water. It was suggested that too much water may intensively interfere with the normal process of metamorphosis. This phenomenon was also observed in *Sarcophage peregrine* (Ohtaki 1966), *Anastrepha suspensa* (Taschenberg et al. 1974), and *Glossina morsitans* (Zdarek & Denlinger 1991). However, in extreme desiccation condition, the development of the oriental fruit fly was not influenced obviously by desiccation durations. The possible reason was that the larvae tolerance time to desiccation was too short to delay the development markedly.

The experiments indicated that although a large number of the larvae survived after 1.5 d of immersion, most of them died after longer immersion because of continuous hypoxia and water absorption by stigmata. This may in

part explain the sharp reduction of the oriental fruit fly population after continuous intensive precipitation (Ye & Liu 2005). On the basis of these information mentioned above, as well as the third instar larvae not moving deeply in the soil (Jackson et al. 1998, Alyokhin et al. 2001, Hou et al. 2006), we think that flooding would be an effective control method to depress population density in infested areas. However, it should require further study on immersion tolerance of pupae and the effects of flooding on crops.

Acknowledgments

This work was supported by the National Grand Fundamental Research "973" Program of China (grant No. 2002CB111405) and the National Nature Science Foundation of China (grant No. 30471162, 30671394).

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Qi Xie[2] and Runjie Zhang[3]

J. Agric. Urban Entomol. 24(1): 1–11 (January 2007)

ABSTRACT Responses of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), third instar larvae to desiccation and immersion were examined at 25°C in the laboratory condition. Third instar larvae desiccated in dry soils (0% field capacity) did not survive beyond 12 h, nor submerged under water beyond 3.5 d. The time to 50%, 90%, and 99% mortality (LT_{50}, LT_{90}, and LT_{99}) were 5.67, 8.60, and 10.99 h respectively in desiccation condition, and 1.81, 2.49, and 3.06 d in immersion test. The average eclosion time of survived flies was not significantly affected by the desiccation time, while that increased linearly with increasing immersion time. Cumulative weight loss of larvae increased curvilinearly with desiccation time, and the rate of weight loss was greatest during the first 2 h. The relationship between the mortality and the percentage of cumulative weight loss was described by Probit model in desiccation. It was estimated that when the percentage of cumulative weight loss was 29.1%, 36.7%, and 43.0% respectively, the mortality rates was 50%, 90%, and 99% correspondingly.

KEY WORDS *Bactrocera dorsalis* (Hendel), mortality, cumulative weight loss, eclosion time

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), was recorded for the first time in 1911 in Taiwan (Liu 1981). Over the next 90 years, this fly has been widely distributed in East Asia and around the Pacific Ocean, where it is a very destructive pest of tropical, subtropical and temperate fruits and vegetables (White & Elson-Harris 1992). Over one hundred species of plants are its hosts, including most types of commercial fruits, such as mango, citrus, guava, plum, papaya, banana, and a wide variety of other agriculture products such as coffee, chili peppers, watermelon, and also wild hosts (Steck 2003). It inflicts heavy economic losses not only on fruit and vegetable yields, but also on export markets. Hence, in many countries or regions, most commercial fruits are severely restricted by quarantine laws to prevent the spread of the oriental fruit fly. Nonetheless, this pest is expanding its distribution ranges unceasingly (Allwood & Drew 1997, Lux et al. 2003).

Tephritid distribution and abundance are markedly structured by several factors, such as temperature, humidity, light intensity, hosts, natural enemies

[1]Accepted for publication 29 October 2007.
[2]The College of Economics, Trade & Statistics, Guangdong University of Business Studies, Guangzhou 510320, P. R. China. E-mail: qiqixie@gmail.com.
[3]State Key Laboratory for Biocontrol & Institute of Entomology, Sun Yat-Sen University, Guangzhou 510275, P.R. China.

NOTE

Detection of Pathogen DNA from Filth Flies (Diptera: Muscidae) Using Filter Paper Spot Cards[1]

Sheri M. Brazil, C. Dayton Steelman, and Allen L. Szalanski

J. Agric. Urban Entomol. 24(1): 13–18 (January 2007)

ABSTRACT Filth flies play a major role in the transmission of microbial organisms that cause disease in animals and humans. A procedure was developed using filter paper to collect filth fly fecal/regurgitation droplets at dairies and turkey production facilities that could be used to detect pathogen DNA carried by filth flies. Weekly fly fecal/regurgitation droplets were collected on 11 cm diameter filter paper that was either tacked to beams or stapled to 30 cm wooden stakes. Molecular diagnostics using polymerase chain reaction (PCR) procedures detected the presence of *Escherichia coli* H7, *Campylobacter* sp., and *Cochlosoma anatis* DNA in fecal/regurgitation droplets deposited by filth flies at two dairy and one turkey farm in Arkansas. This procedure provided a rapid and effective method to monitor pathogen presence in filth fly populations.

KEY WORDS Filter paper, filth flies, fecal/regurgitation droplet, PCR

Campylobacter spp. (Doyle 1998), and *Escherichia coli* (Gyles 1993) have been reported to cause illnesses involving diarrhea, lethargy, severe abdominal pain, fever, vomiting, nausea, and kidney failure to occur in humans. Similar occurrences have been reported in livestock and poultry, and in various pathogen combinations may occur with more severity, resulting in economic losses. Rosef & Kapperud (1983) indicated that flies may contribute to the spread of *Campylobacter* by transmitting the bacteria from animals to human food. Alam & Zurek (2004) reported that house flies were a potential contributor to the transmission of *E. coli* O157:H7 in both farm and urban environments. Previous studies have shown that filth flies can disseminate viable pathogens such as *Helicobacter pylori* (Grubel et al. 1997), Salmonella (Greenburg 1965), and *E. coli* O157 (Kobayashi et al. 1999) to other substrates. Japan experienced outbreaks of *E. coli* O157:H7 during which students attending a nursery school located close to a farm became infected with the bacteria. House flies collected in the area were carrying the pathogen (Moriya et al. 1999), and fecal/regurgitation droplets on substrates within the school were suspected as a factor in the spread of the bacteria (Kobayashi et al. 1999). House flies have also been documented to carry the protozoan *Cochlosoma anatis*, which plays a role in turkey enteritis (McElroy et al. 2005). Filth flies such as the house fly, *Musca domestica* L., and black

[1]Accepted 27 November 2007.
Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.

garbage fly, *Hydrotaea aenescens* (Wiedemann), often disperse away from the areas where eggs, larvae, and pupae are located. Hogsette & Jacobs (1999) found that *H. aenescens* were capable of dispersing 0.3 km away from release points. Lysyk & Axtell (1986) stated that *M. domestica* can travel up to 20 km from points of release and some move from dairies and poultry houses to other farms or urban environments. Often different types of fly movement in and among the various components of the environment occur and pathogens that cause disease to both humans and animals are dispersed throughout the Agro-Ecosystem. Spot cards are used to collect fecal/regurgitation droplets as a monitoring device to determine the density of filth fly populations (Lysyk & Axtell 1985), and filter paper has been shown to work well for preserving insect and pathogen DNA (Owens & Szalanski 2005). This study was conducted to determine if filter paper could be used to collect fly fecal/regurgitation droplets under field conditions at dairies and turkey production facilities to identify pathogen DNA in adult filth flies.

Materials and Methods

Two dairy farms and two turkey facilities located in Carroll County, Arkansas were used in this study: two dairy farms and one turkey farm were located near Green Forrest, Arkansas, and one turkey farm was located near Berryville, Arkansas. At both dairy farms, the most common filth flies were *Musca domestica* and *Hydrotaea aenescens* (McElroy 2005). Whatman 11 cm filter paper (Aloe Scientific, St. Louis, MO) was placed at all farms to collect filth fly fecal and regurgitation droplets during August to October, 2004. Filter paper was stapled to a 7.6 cm $\times$ 12.7 cm index card and hung inside turkey production facilities with thumbtacks on wooden support beams 1 m above the floor along the side-curtain opening of the houses where flies were observed entering and exiting the facilities. At dairies, cards were attached to wooden surfaces where cows and calves could not reach (Fig. 1). In addition, filter paper was stapled to 30 cm wooden stakes placed in the ground with the filter paper 20 cm above the surface around calf hutches and milking parlors where fly activity was noted. After 1 wk, cards were collected and replaced. In the field, the cards were placed in 568 ml zip-lock bags, transported to the laboratory, and stored at room temperature.

Ten fly spots were chosen at random from each filter paper and 1.2 mm discs were punched from the filter paper using a Harris Micro Punch (Whatman). Each filter paper used in the field was tested separately by pooling 10 fly spots and extracting DNA using the Puregene DNA extraction kit (Gentra, Minneapolis, MN), which was resuspended in 50 ul Tris:EDTA pH 8.0 and frozen at -20°C. Contamination in the laboratory was avoided by performing all isolations in a Safety Class II hood cabinet. Extracted DNA was subjected to PCR in order to detect *E. coli* O157/H7, *Campylobacter* spp., and *Cochlosoma anatis*. PCR primers and PCR conditions for *E. coli* O157:H7, and *Campylobacter* sp. were from Szalanski et al. (2004), and the primers and PCR conditions for *Cochlosoma anatis* were from (McElroy et al. 2005). To visualize the presence of pathogens in all samples tested, 1% agarose gel electrophoresis was performed and recorded using a UVP biodoc-it system (UVP Inc., Upland, CA).

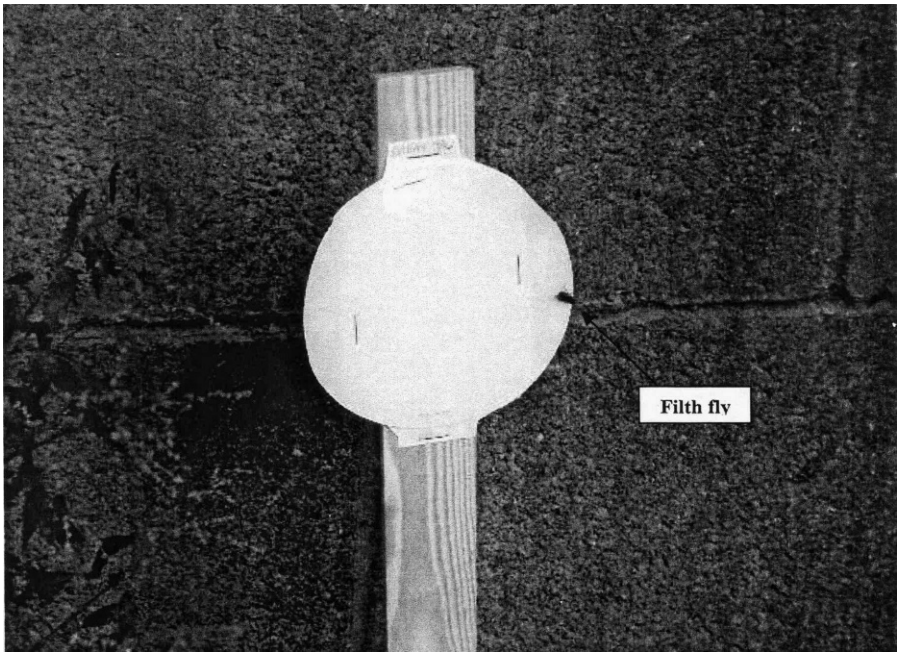

Fig. 1. Filter paper card and stake used for collecting filth fly fecal/regurgitation droplets.

Results and Discussion

A total of 118 pools of 10 fly droplets each were tested (Table 1) with 15 (12.7%) of the samples testing positive for a pathogen. *E. coli* serotype H7 was detected in two samples from dairy farm 1 and five from dairy farm 2, totaling seven, or 5.9%, of the pooled samples. *Campylobacter* spp. was found in seven, or 5.9%, of the pooled samples, with three being from dairy farm 1, two from dairy farm 2, and two from turkey farm 1. *Cochlosoma anatis* was found in one, or 0.8% of the pooled samples at dairy farm 2. There was no detection of *E. coli* serotype O157 at

Table 1. Total number of filth fly fecal/regurgitation droplet pools positive for pathogens.

Farm	*E. coli* H7	*Campylobacter* sp.	*Cochlosoma anatis*	Total fly pools
Dairy 1	2	3	0	27
Dairy 2	5	2	1	38
Turkey 1	0	2	0	19
Turkey 2	0	0	0	34
Total	7	7	1	118
Percent positive	5.9	5.9	0.8	12.7

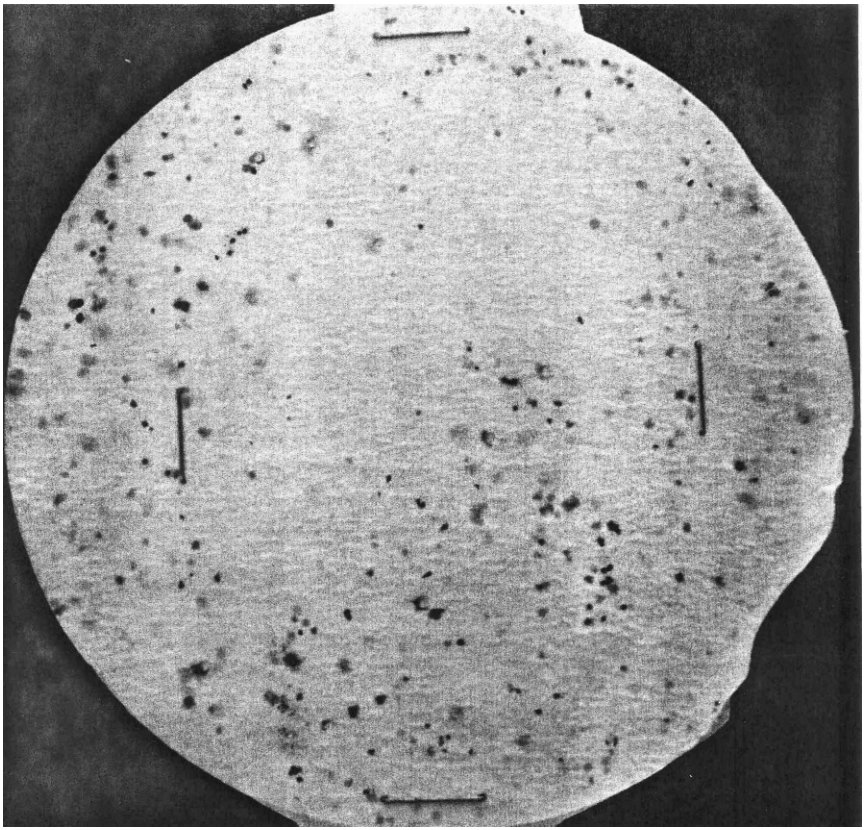

Fig. 2. Filth fly fecal/regurgitation droplets deposited on a filter paper card.

any farm. No pathogens were detected at turkey farm 2 which was located several km from other turkey production facilities and dairy farms.

Collecting filth fly fecal and regurgitation droplets on filter paper (Fig. 2) provided an effective way to monitor pathogen presence in filth fly populations at dairy and turkey production facilities. The filter paper placed at dairies and poultry facilities collected an abundance of fly fecal/regurgitation droplets. Filter paper attached to wooden stakes 20 cm above the surface collected greater numbers of droplets than filter paper hung by thumbtacks 1 m above the floor surface. This was likely due to the accessibility flies had to land on the stakes which were closer to manure on the ground. The method was also easier to place in areas where flies were congregating. Generally, more spots were deposited around the outer edges of the filter paper circles than towards the center area. There was a greater presence of pathogens in fly fecal/regurgitation spots collected on the filter paper from both dairies than from the turkey facilities. However, there was a greater abundance of spots on filter paper from dairies due to the easy access of placing the filter paper in areas where flies were congregating. Turkey farm 2 had no pathogens detected in fly spots, but also had no disease outbreaks occurring during times of collections, and this farm was

more isolated from other cattle or turkey production farms. In addition, the horse and beef cattle production on this farm created no concentrated area of manure necessary for filth fly breeding. In the case of an outbreak or epidemic it is vital that the source of pathogen spread be identified and controlled. Flies have been linked to the spread of many pathogens and currently, bio-terrorism has become a concern.

Studies that have indicated the presence of anthrax and cholera bacteria in flies (Fischer 1999) as well as the continuing discoveries in the vector competency of filth flies leads to a need for improved methods in pathogen detection, and the method of sample collection reported here offers quick, accurate results. This method of field sampling combined with molecular diagnostics using PCR procedures to detect pathogen DNA within 6 h after samples reach the laboratory allows rapid application of filth fly management tactics to aid in controlling disease outbreaks.

Acknowledgment

Research was supported in part by the University of Arkansas, Fayetteville, AR, Agricultural Experiment Station, and by a USDA-CSREES-PMAP grant to CDS and ALS.

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[1]Accepted 27 November 2007.
Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.

Susceptibility of Sunn Pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), to Various Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae)[1]

R. Canhilal,[2,3] W. Reid,[4] H. Kutuk,[5] and M. El-Bouhssini[4]

J. Agric. Urban Entomol. 24(1): 19–26 (January 2007)

ABSTRACT The susceptibility of adult sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), to five local Syrian isolates of *Heterorhabditis bacteriophora*, *Steinernema riobravae* Texas, *S. carpocapsae* C3B, and *H. bacteriophora* 8-14 strains was evaluated under laboratory conditions. Concentrations of 50, 100, 200, and 400 nematodes per adult were bioassayed; for each nematode concentration, four groups of five sunn pests in Petri dishes were used and mortality was recorded daily for five d. The mortality induced by nematodes increased, typically with increasing numbers of nematodes per adult and ranged from 30 to 90%. Overall, *Heterorhabditis bacteriophora* Musherphe strain produced the lowest mortality and *Steinernema riobravae* Texas strain produced the highest mortality. The LC_{50} values of *H. bacteriophora* Musherphe, *S. carpocapsae* C3B, and *S. riobrave* Texas were 107.1, 66.6, and 65.3 nematodes per adult sunn pest, respectively. LT_{50} values ranged from 3.6 to 7.2 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Musherphe strains at 50 nematodes per sunn pest, from 2.9 to 5.1 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Ariha strains at 100 nematodes per sunn pest, from 2.2 to 4.3 d for *S. carpocapsae* C3B and *H. bacteriophora* Ariha strains at 200 nematodes per sunn pest, and from 2.0 to 4.3 d for *S. riobrave* Texas and *H. bacteriophora* Musherphe strains at 400 nematodes per sunn pest, respectively. Our results suggest that *H. bacteriophora* Tabeh-gazira, *H. bacteriophora* El Ratla-1, *H. bacteriophora* El Ratla-2, *H. bacteriophora* 8-14, *S. carpocapsae* C3B, and *S. riobrave* Texas strains have potential as biocontrol agents against the sunn pest.

KEY WORDS Entomopathogenic nematodes, *Eurygaster integriceps*, *Heterorhabditis*, *Steinernema*, sunn pest, Syria

Entomopathogenic nematodes (EPNs) have been used for insect control since the 1930s (Smart 1995). These nematodes have been applied successfully against soil-inhabiting insects (Gaugler 1981, Georgis & Poinar 1984, Klein 1990), as well as against above-ground insects in cryptic habitats (Kaya 1990, Begley 1990). They have many ideal properties as biological control agents: wide host spectrum,

[1]Accepted for publication 11 March 2008.
[2]Corresponding author (r_canhilal@hotmail.com, ramazancanhilal@erciyes.edu.tr).
[3]Plant Protection Department, Seyrani Agricultural Faculty, Erciyes University, 38039 Melikgazi, Kayseri, Turkey.
[4]International Center for Agricultural Research in Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.
[5]Plant Protection Research Institute, P.O. Box 21, 01321, Adana, Turkey.

rapid kill the host within 48 h, easy commercial production *in vivo* or *in vitro*, active host seeking, long-term efficacy, easy application, compatibility with most chemicals, and environmental safety.

The sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), is a destructive insect pest of wheat and barley in Western and Central Asia, and Southeast Europe (El Bouhssini et al. 2002). Both nymphs and adults cause damage to plants by feeding on leaves, stems, and grains. Overwintered adults of the sunn pest attack the leaves and stems of young, succulent wheat and barley plants, causing them to wither and die prior to spike formation. They also suck the base of the spike during the early growing period, resulting in whitish spikes without kernels, called white spikes. Nymphs and new-generation adults feed on grains. Yield losses by this pest are estimated at 50–90% in wheat and 20–30% in barley. Apart from directly reducing yield, the insect injects digestive enzymes into kernels during feeding that reduce the baking quality of the dough. If as little as 2–3% of the grain has been fed on, the entire grain lot may be rendered unacceptable for baking purposes because of poor-quality flour (Canhilal et al. 2006a).

Sunn pest infestations affect about 15 million ha of cereal crops annually. For example, about 1.5 million ha in each of Turkey and Iran, 240,000 ha in Afghanistan, and 200,000 ha in Syria are sprayed for the pest (El Bouhssini et al. 2002). About US$15 million is spent each year on pesticides in Turkey alone (Canhilal et al. 2006b). In addition to the high cost of chemical control, insecticides pose a risk to human health, water quality, wildlife, and the environment as a whole. The present insecticide-based strategies must be replaced with multidimensional integrated pest management (IPM) approaches including the applications of environmentally friendly biological control agents against sunn pest. Considerable progress on fungal isolates has been made; based on laboratory and greenhouse bioassays and preliminary field works, several isolates have shown potential for use as biocontrol agents (El-Bouhssini et al. 2002). Another group of potential biocontrol agents for sunn pest may be EPNs. An effective nematode formulation can be used at the overwintering site of the sunn pest, which is a favorable cryptic habitat for EPNs.

A crucial element for sucsess in any biological control program with EPNs is pairing the most suitable nematode with the defined host, and relative pathogenicity among various nematodes is one of the important factors to consider in determining suitability (Georgis & Gaugler 1991, Shapiro-Ilan et al. 2002). Thus, in this investigation, our objective was to assess the susceptibility of sunn pest adults to several steinernematid and heterorhabditid nematodes under laboratory conditions.

Materials and Methods

The sunn pests used in the bioassays were collected from overwintering sites at Tel Hadya, ICARDA in March, 2003 before the sunn pest moved to fields. The Musherphe, Tabeh-gazira, El Rattla-1, El Rattla-2, and Ariha strains of *Heterorhabditis bacteriophora* were obtained from a survey in Syria (Canhilal et al. 2006c). *Steinernema carpocapsae* C3B, *S. riobravae* Texas, and *H. bacteriophora* 8-14 strains were provided by Dr. Khoung B. Ngyuen and Dr. Byron J. Adams of the University of Florida.

EPNs were produced on late-instar larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), obtained from the Department of Plant Protection of Aleppo University, following the standard rearing method described by Woodring & Kaya (1988). A modified White Trap (Canhilal & Carner 2006), consisting of a folded 11-cm filter paper (3 mm in depth after folding) in a Petri dish (100 × 15 mm) with 15–20 ml of distilled water, was used to collect the infective juveniles (IJs). These IJs were stored at 7–8°C in tissue culture flasks for 15–20 d (Kung et al. 1990). Before the assays, viability was confirmed by observing nematode activity (rapid wiggling) under a binocular microscope.

The Petri plate bioassay procedure was used to evaluate the susceptibility of overwintered sunn pest adults at concentrations of 50, 100, 200, and 400 IJs per adult in 1 ml of sterile distilled water, following the procedures described by Woodring & Kaya (1988). Petri dishes (100 × 15 mm) were lined with two Whatman No. 1 filter paper pieces (9 cm diameter). One hour before the beginning of the experiment, the IJs were applied and distributed evenly on the filter paper. For each treatment concentration, four groups of five sunn pests were placed per dish containing IJs. The Petri dishes were placed in a double plastic bag and put in a dark incubator at 25 ± 1°C (Glazer et al. 1991). Controls consisted of 1 ml of sterile distilled water without nematodes. The bioassay was repeated two times.

Sunn pest mortality was recorded every 24 h for 5 d (Epsky & Capinera 1994). Dead insects were incubated on modified White Traps at room temperature (25 ± 1°C) and examined to confirm the presence of nematodes. The mortalities were converted to percentages and adjusted for control mortality, using Abbott's correction formula. The data were analyzed as a completely randomized factorial design, using the Student-Neuman-Keuls means separation procedure to detect differences among treatments. Lethal concentration (LC_{50}) values were estimated by probit analysis for *H. bacteriophora* Musherphe, *S. carpocapsae* C3B, and *S. riobrave* Texas strains, excluding the 400-nematode concentration. Median lethal time (LT_{50}) values were estimated for all nematodes included in bioassays for 50, 100, 200, and 400 nematode concentrations; probit analysis was used with mortality data at 24-h intervals for each nematode. Log base 10 transformations were performed on the mortalities for LC_{50} and LT_{50} estimations (SPPS 2003).

Results

All nematodes were able to reproduce in the sunn pest adults. In all controls, low mortality (2.5–5.0%) was observed. Overwintered adults of *Eurygaster integriceps* differed in their susceptibility to steinernematid and heterorhabditid nematodes. After 24 hours, there was low mortality, which was not enough to differentiate the efficacy of nematode species and strains on sunn pest adults. On the second day, sunn pest mortalities increased and reached 5.0–62.5%. The mortalities were 15.0–75.0% and 22.5–85.0% on the third and fourth days, respectively. The *H. bacteriophora* Musherphe strain at 50 nematodes per sunn pest (lowest rate) produced the lowest rate of infection, and *S. riobravae* Texas strain at 400 nematodes per sunn pest (highest rate) produced the highest rate of infection on both days, as it was on the second day. The mortality ranged from 30 to 90% on the fifth day (final) count: *H. bacteriophora* Musherphe strain at the 50-nematode

concentration was the poorest and *S. riobrave* Texas strain at the 400-nematode concentration was the best performer, as for all other counts (Table 1).

In general, the sunn pest showed an associated increase in percent mortality with an increase in the concentration of nematodes. However, the differences in between different concentrations for the same nematode were not usually significant (Table 1).

When eight strains of entomopathogenic nematodes were compared at four different rates on the final count, the following were superior: *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* El Ratla-1 strains at the 50-nematode concentration with 60% mortality, *H. bacteriophora* Tabeh-gazira strain at 100 nematodes with 67.5% mortality, *S. carpocapsae* C3B strain at 200 nematodes with 82.5% mortality, and *S. riobrave* Texas strain at 400 nematodes with 90% mortality (Table 1). However, differences in mortalities between strains were mostly not significant. They were not significant for the 50, 100, and 200 nematode concentrations ($F = 1.65$, df = 7, $P = 0.14$; $F = 0.80$, df = 7, $P = 0.59$; $F = 1.76$, df = 7, $P = 0.14$, respectively). At the 400 nematode concentration, four different groups were observed based on fifth day mortalities: (1) *S. riobrave* Texas and *H. bacteriophora* 8-14 strains (2) *S. carpocapsae* C3B, *H. bacteriophora* El Ratla-1, *H. bacteriophora* El Ratla-2, and *H. bacteriophora* Tabeh-gazira strains (3) *H. bacteriophora* Ariha strain and (4) *H. bacteriophora* Musherphe ($F = 3.69$, df = 7, $P = 0.002$) (Table 1).

The LC_{50} values of *H. bacteriophora* Musherphe, *S. carpocapsae* C3B, and *S. riobrave* Texas were determined to be 107.1, 66.6, and 65.3 nematodes per adult sunn pest, respectively (Table 2). The LC_{50} values and associated statistics could not be estimated for the other nematodes because of high variance among concentrations. The LT_{50} values ranged from 3.6 to 7.2 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Musherphe at 50 nematodes per sun pest, from 2.9 to 5.1 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Ariha at 100 nematodes per sunn pest, from 2.2 to 4.3 d for *S. carpocapsae* C3B and *H. bacteriophora* Ariha at 200 nematodes per sunn pest, and from 2.0 to 4.3 d for *S. riobrave* Texas and *H. bacteriophora* Musherphe at 400 nematodes per sunn pest, respectively (Table 3).

Discussion

Our results showed that the sunn pest, *Eurygaster integriceps*, is susceptible to various heterorhabditids and steinernematids and their symbiotic bacteria. Variation in susceptibility among nematodes was detected. Usually heterorhabditids worked better at low concentrations (50–100 nematodes per sunn pest) and steinernematids worked better at high concentrations (200–400 nematodes per sunn pest). LT_{50} values also were usually smaller for heterorhabditids at low concentrations and greater at high concentrations than for steinernematids. Kepenekci (2004) found that *H. bacteriophora* Tur-H1 and Tur-H2 strains induced higher mortality than did *S. carpocapsae* on another scutellerid, *Eurygaster maura* L., contrary to our results. The mortalities caused by *S. carpocapsae* and *H. bacteriophora* Tur-H1 and Tur-H2 strains were 55, 69, and 99%, respectively.

Overall, *H. bacteriophora* Musherphe strain produced the lowest mortality and *S. riobravae* Texas strain produced the highest mortality against sunn pest

Table 1. Percent mortality of sunn pest adults to various entomopathogenic nematodes, ICARDA, Syria, 2003.

	Nematodes per sunn pest[a]							
	50	100	200	400	50	100	200	400
Nem[b]	2nd day reading				3rd day reading			
Mser	5.0Ab	27.5Aab	32.5Aa	27.5Aa	15.0Bb	40.0Aa	57.5Aa	35.0ABc
Tgzr	30.0Ba	50.0Aa	42.5ABa	55.0Aa	45.0Aa	57.5Aa	60.0Aa	65.0Aab
Rt-1	35.0Aa	32.5Aab	35.0Aa	55.0Aa	42.5Aa	45.0Aa	50.0Aa	57.5Aabc
Rt-2	27.5Aa	32.5Aab	45.0Aa	45.0Aa	40.0Bab	47.5ABa	55.0ABa	57.5Babc
Arha	12.5Aab	17.5Ab	30.0Aa	30.0Aa	32.5Aab	37.5Aa	40.0Aa	42.5Abc
C3B	30.0Aa	20.0Aab	47.5Aa	55.0Aa	42.5Aa	47.5Aa	72.5Aa	67.5Aab
8-14	35.0Ba	32.5Bab	37.5ABa	50.0Aa	47.5Ba	47.5Ba	52.5ABa	67.5Aab
Tex	35.0Aa	45.0Aab	42.5Aa	62.5Aa	37.5Bab	52.5ABa	65.0ABa	75.0Aa
Cont	0.0Ac	0.0Ac	0.0Ab	0.0Ab	2.5Ac	0.0Ab	0.0Ab	0.0Ad
	Nematodes per sunn pest[a]							
	50	100	200	400	50	100	200	400
Nem[b]	4th day reading				5th day reading			
Mser	22.5Bb	45.0Aa	62.5Aab	47.5Ab	30.0Ca	50.0Ba	65.0Aa	50.0ABc
Tgzr	57.5Aa	62.5Aa	67.5Aab	70.0Aab	60.0Aa	67.5Aa	75.0Aa	75.0Aab
Rt-1	50.0Aab	50.0Aa	55.0Aab	67.5Aab	60.0Aa	52.5Aa	62.5Aa	72.5Aab
Rt-2	50.0Aab	52.5Aa	57.5Aab	67.5Aab	55.0Ba	55.0ABa	60.0ABa	75.0Aab
Arha	32.5Aab	40.0Aa	45.0Ab	50.0Ab	40.0Aa	40.0Aa	50.0Aa	55.0Abc
C3B	42.5Bab	52.5ABa	82.5Aa	77.5Aab	45.0Aa	55.0Aa	82.5Aa	77.5Aab
8-14	47.5Bab	52.5Ba	57.5Bab	77.5Aab	47.5Ba	52.5Ba	57.5ABa	87.5Aa
Tex	40.0Bab	52.5ABa	67.5Aab	85.0Aa	45.0Ca	57.5BCa	67.5Ba	90.0Aa
Cont	2.5Ac	0.0Aa	0.0Ac	2.5Ac	2.5Ab	5.0Ab	2.5Ab	2.5Ad

[a]Means within the same columns followed by the same lower case letters are not significantly different; means within same rows followed by the same capital letters are not significantly different.

[b]Mser: *H. bacteriophora* Musherphe; Tgzr: *H. bacteriophora* Tabeh-gazira; Rt-1: *H. bacteriophora* El Rattla-1; Rt-2: *H. bacteriophora* El Rattla-2; Arha: *H. bacteriophora* Ariha; C3B: *S. carpocapsae* C3B; 8-14: *H. bacteriophora* 8-14; Tex: *S. carpocapsae* Texas; Cont: Control.

Table 2. LC_{50} of *H. bacteriophora* Musherphe, *S. carpocapsae* C3B, and *S. riobrave* Texas strains for overwintered *Eurygaster integriceps* adults, ICARDA, Syria, 2003.

Nematodes	No. adults	LC^a_{50}	95% CI	Slope	χ^2
H. bacteriophora Musherphe	40	107.1	70.7–175.7	1.51	0.08
S. carpocapsae C3B	40	66.6	34.6–91.4	1.71	1.243
S. riobrave Texas	40	65.3	18.8–97.7	1.32	0.119

[a]LC_{50} values expressed in number of nematodes per adult.

adults. Parallel to the mortality levels, LC_{50} values were highest for *H. bacteriophora* Musherphe strain and lowest for *S. riobravae* Texas strain (Table 2). Presumably, these results are due to the balance between pathogenicity of the nematode/bacterium complex and the insect defense mechanism (Gaugler et al. 1997, Gouge et al. 1999).

Table 3. LT_{50} for various entomopathogenic nematodes and strains at 50, 100, 200, and 400 nematodes per sunn pest for overwintered *Eurygaster integriceps* adults, ICARDA, Syria, 2003.

		50 nematodes				100 nematodes			
Nem[a]	No. adults	LT_{50}	95% CI	Slope	χ^{2b}	LT_{50}	95% CI	Slope	χ^{2b}
Mser	40	7.2	5.4–15.3	2.98	0.446	4.3	3.6–5.8	2.59	5.168
Tgzr	40	3.6	3.1–4.3	3.06	4.635	2.9	1.2–7.7	2.99	9.507[b]
Rt-1	40	3.7	3.1–4.7	2.54	4.020	3.9	2.5–51.1	2.60	6.925[b]
Rt-2	40	4.0	3.4–5.0	2.85	4.223	3.7	2.4–17.0	2.72	6.771[b]
Arha	40	5.6	4.5–8.7	2.69	3.274	5.1	4.2–7.6	2.55	4.886
C3B	40	4.7	3.7–7.2	2.08	5.243	3.9	3.3–4.8	2.85	3.063
8-14	40	3.7	2.3–32.1	2.36	6.372[b]	3.8	2.3–59.4	2.64	7.432[b]
Tex	40	5.1	3.8–9.5	1.74	4.692	3.3	2.6–4.6	1.73	3.595
		200 nematodes				400 nematodes			
Nem[a]	No. adults	LT_{50}	95% CI	Slope	χ^{2b}	LT_{50}	95% CI	Slope	χ^{2b}
Mser	40	3.2	2.0–5.4	3.24	6.564[b]	4.3	3.6–5.8	2.65	4.733
Tgzr	40	2.8	1.7–4.2	3.44	6.749[b]	2.5	0.8–4.5	3.03	9.758[b]
Rt-1	40	3.4	2.2–7.3	2.94	6.291[b]	2.8	1.5–4.7	3.27	7.932[b]
Rt-2	40	3.2	1.8–9.7	2.57	6.942[b]	2.8	1.6–4.3	3.37	7.364[b]
Arha	40	4.3	3.6–5.7	2.68	4.504	3.9	3.3–4.9	2.76	5.229
C3B	40	2.2	1.8–2.5	3.15	1.724	2.2	1.9–2.6	2.84	4.320
8-14	40	3.5	2.1–10.6	2.89	7.334[b]	2.4	2.1–2.7	3.86	4.061
Tex	40	2.6	2.2–3.2	2.48	3.861	2.0	1.3–2.7	3.87	5.664[b]

[a]Nem: Nematodes; Mser: *H. bacteriophora* Musherphe; Tgzr: *H. bacteriophora* Tabeh-gazira; Rt-1: *H. bacteriophora* El Rattla-1; Rt-2: *H. bacteriophora* El Rattla-2; Arha: *H. bacteriophora* Ariha; C3B: *S. carpocapsae* C3B; 8-14: *H. bacteriophora* 8-14; Tex: *S. carpocapsae* Texas.
[b]Chi-square test was significant at $P < 0.05$.

Our findings indicate that it may be possible to use *H. bacteriophora* Tabehgazira, *H. bacteriophora* El Ratla-1, *H. bacteriophora* El Ratla-2, *H. bacteriophora* 8-14, *S. carpocapsae* C3B, and *S. riobrave* Texas strains as biocontrol agents against the sunn pest. Sunn pests are known to aestivate in mountain areas around cereal fields during the hot and dry months of late summer and autumn and to hibernate in these same areas during the cold and often severe winter months. The adults of the sunn pest rest and hibernate 2–3 cm under soil and litter, which is a favorable habitat for EPNs (Canhilal et al. 2006a). An effective nematode can be used in these overwintering sites against the pest. *Steinernema riobrave* may have an advantage in controlling motionless sunn pest adults in the cryptic habitat of the overwintering sites because it has a high searching capacity. It was reported to be first in order of host-searching efficacy, compared with *H. bacteriophora* HP 88 strain, *H. bacteriophora* Bioenterprise strain, and *S. carpocapsae* Kapow, All, and UK strains (Lindegren et al. 1993). Further field studies with effective nematodes in various formulations and different application strategies and timing are needed.

Acknowledgments

We thank Ziyaad Sayyadi and Fawzi Rihawi of ICARDA for collecting the sunn pests and assistance in bioassays, and Abdulnasr Trissi of Aleppo University for providing Mediterranean flour moth larvae.

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Susceptibility of Sunn Pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), to Various Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae)[1]

R. Canhilal,[2,3] W. Reid,[4] H. Kutuk,[5] and M. El-Bouhssini[4]

J. Agric. Urban Entomol. 24(1): 19–26 (January 2007)

ABSTRACT The susceptibility of adult sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), to five local Syrian isolates of *Heterorhabditis bacteriophora*, *Steinernema riobravae* Texas, *S. carpocapsae* C3B, and *H. bacteriophora* 8-14 strains was evaluated under laboratory conditions. Concentrations of 50, 100, 200, and 400 nematodes per adult were bioassayed; for each nematode concentration, four groups of five sunn pests in Petri dishes were used and mortality was recorded daily for five d. The mortality induced by nematodes increased, typically with increasing numbers of nematodes per adult and ranged from 30 to 90%. Overall, *Heterorhabditis bacteriophora* Musherphe strain produced the lowest mortality and *Steinernema riobravae* Texas strain produced the highest mortality. The LC_{50} values of *H. bacteriophora* Musherphe, *S. carpocapsae* C3B, and *S. riobrave* Texas were 107.1, 66.6, and 65.3 nematodes per adult sunn pest, respectively. LT_{50} values ranged from 3.6 to 7.2 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Musherphe strains at 50 nematodes per sunn pest, from 2.9 to 5.1 d for *H. bacteriophora* Tabeh-gazira and *H. bacteriophora* Ariha strains at 100 nematodes per sunn pest, from 2.2 to 4.3 d for *S. carpocapsae* C3B and *H. bacteriophora* Ariha strains at 200 nematodes per sunn pest, and from 2.0 to 4.3 d for *S. riobrave* Texas and *H. bacteriophora* Musherphe strains at 400 nematodes per sunn pest, respectively. Our results suggest that *H. bacteriophora* Tabeh-gazira, *H. bacteriophora* El Ratla-1, *H. bacteriophora* El Ratla-2, *H. bacteriophora* 8-14, *S. carpocapsae* C3B, and *S. riobrave* Texas strains have potential as biocontrol agents against the sunn pest.

KEY WORDS Entomopathogenic nematodes, *Eurygaster integriceps*, *Heterorhabditis*, *Steinernema*, sunn pest, Syria

Entomopathogenic nematodes (EPNs) have been used for insect control since the 1930s (Smart 1995). These nematodes have been applied successfully against soil-inhabiting insects (Gaugler 1981, Georgis & Poinar 1984, Klein 1990), as well as against above-ground insects in cryptic habitats (Kaya 1990, Begley 1990). They have many ideal properties as biological control agents: wide host spectrum,

[1]Accepted for publication 11 March 2008.
[2]Corresponding author (r_canhilal@hotmail.com, ramazancanhilal@erciyes.edu.tr).
[3]Plant Protection Department, Seyrani Agricultural Faculty, Erciyes University, 38039 Melikgazi, Kayseri, Turkey.
[4]International Center for Agricultural Research in Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.
[5]Plant Protection Research Institute, P.O. Box 21, 01321, Adana, Turkey.

Bradysia Odoriphaga Copulatory Behavior and Evidence of a Female Sex Pheromone[1]

Hong-Jun Li, Xiong-Kui He, Ai-Jun Zeng, Ya-Jia Liu, and Shu-Ren Jiang

J. Agric. Urban Entomol. 24(1): 27–34 (January 2007)

ABSTRACT *Bradysia odoriphaga* Yang and Zhang is the most serious pest of Chinese chive, *Allium tuberosum* Rottle ex Spreng. (Liliaceae) in North China. Pesticide residues in this vegetable are very high following excessive use of organophosphorate insecticides. Because there have been no reports on the sex pheromones of *B. odoriphaga*, sex pheromone and mating behavior of *B. odoriphaga* were investigated as a possibility of developing semiochemical-based monitoring and control of this pest. In laboratory bioassays, live *B. odoriphaga* virgin females stimulated 78% of males to vibrate their wings and 67% of males to attempt to mate. Methylene dichloride washes of female whole bodies and excised ovipositors also attracted males. In field test, many *B. odoriphaga* males were attracted to the traps containing live *B. odoriphaga* virgin females or methylene dichloride washes of female whole bodies. Most flies mated only once, while a few mated as many as six times. After mating, females were still attractive to males. Flies' sexual behavior showed a daily rhythm. The higher mating activity was from 2200 to 0600 h and the lower from 1200 to 1800 h. These results indicate the presence of a female sex pheromone in *B. odoriphaga* with the ovipostor as the most likely source of pheromone production. It is possible to collect the maximum amount of sex pheromone between 2200 and 0600 h. There is the possibility that this sex pheromone may be used to monitor and control of *B. odoriphaga* in the future.

KEY WORDS Diptera, Sciaridae, *Bradysia*, *odoriphaga*, sex pheromone, mating behavior

Introduction

Many sex pheromones have been identified within the order Diptera, especially in the families Cecidomyiidae (Hillbur et al. 1999, McKay & Hatchett 1984), Drosophilidae (Nemoto et al. 1994), Glossinidae (McDowell et al. 1981), and Muscidae (Uebel et al. 1975). However, only three species of Sciaridae have been reported to have sex pheromones. Casartelli et al. (1971) reported a sex pheromone in *Bradysia tritici* (Coquillett). Heptadecane was identified as the major component of a sex pheromone of *Lycoriella mali* (Fitch) (Kostelc et al. 1980). Gotoh et al. (1999) reported that heptadecane did not attract unmated adult males *L. mali* in Japan. *Bradysia impatiens* (Johannsen) was also shown to have a sex pheromone by Alberts et al. (1981) and Liu et al. (2002), but the

[1]Accepted 4 November 2007.
College of Science, China Agricultural University, Beijing 100094, China.

chemical structure has not been identified. Overall, little is known about the sex pheromones of sciarids, probably because of their short lifespan and tiny bodies makes them difficult to work with.

The sciarid fly, *B. odoriphaga*, is a serious pest of Chinese chive, *Allium tuberosum* Rottle ex Spreng. The larvae live in the roots and stems of Chinese chive, making it difficult to control with common strategies. It is common practice for farmers to add organophosphate insecticides into the soil to kill larvae. Thus, pesticide residues are very high, and there have been many reports of pesticide poisoning from consuming the Chinese chive in recent years (Wang et al. 2006a, Wang et al. 2006b). Therefore, new and less poisonous alternative control methods have been developed, including an insecticidal-engineered bacterium (Wu et al. 2003), botanical secondary metabolites (Yu et al. 2003), a pesticide-degrading bacterium (Jiang et al. 2004), and an entomopathogenic nematode (Sun et al. 2004).

However, these new substances do not provide adequate control, so better methods to prevent this fly are required. This paper describes the research on whether or not there is a sex pheromone in the female *B. odoriphaga*, and some factors that affect sexual behavior of the adults.

Materials and methods

Test insects and rearing methods. Sciarid flies, *B. odoriphaga*, were collected from a glasshouse in a Beijing suburb (40°13′N, 116°12′E) in China and were reared in an incubator. Rearing methods were similar to those described by Mu et al. (2003). The culture was maintained at 20±1°C, 50±5% RH, with a 12-h photoperiod from 0600 to 1800 h CST.

Effect of time-of-day on copulation and other mating behavior. From 0000 to 0500h, newly emerged flies in plastic pot were placed in plastic tubes (4 cm long and 0.5 cm diam, one fly per tube). The tubes were labeled with date and stored in the incubator. One virgin male and one virgin female were placed together in a clean plastic tube before 0600 h on 0 day (day of emergence), 1 day and 2 day, and then the tubes were placed in the incubator. Mating activities of all flies were observed for 20 min every 2 h from 0600 h of 0 day to 1200 h of 2 day. If flies died during an experiment, they were replaced by flies of the same age. There were 4 replications and 40 couples per replication for each day. A dim red light was used in night during observation.

Bioassay procedure. Sexual attraction of sciarid flies was studied using a glass Y-tube olfactometer similar to the one described by Chaudhury et al. (1972). The two arms were 10 cm long and the base was 20 cm long, both had a diameter of 1.8 cm (Fig. 1). The two arms and the base of one Y-tube were closed by a glass stopper (3 cm long) with a cone hole (the diameters of two bottom of the hole were 1 cm and 0.4 cm, respectively). All holes were blocked with a thin layer of glass wool to prevent the flies from escaping. Two arms of this Y-tube were connected to a bottle filled with distilled water through another Y-tube, such that humidified air could pass through the arms. A charcoal filter was put before the bottle to purify the air. The velocity of air in the arms was about 22 cm sec^{-1} and the test temperature was 20 ± 2°C.

Attractiveness of live flies. The attractiveness of virgin females, mated females, mated males, and virgin males to virgin males and the attractiveness of

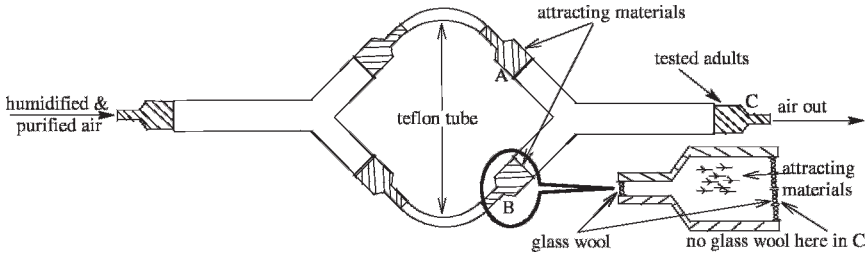

Fig. 1. The sketch map of main parts of Y-tube device.

virgin and mated males to virgin females were tested separately. Virgin flies were collected every 2 h following removal of all the flies to ensure that all flies were unmated. One fly was held in a plastic tube (4 cm long and 0.5 cm diam) with two flies of the opposite sex for 2 h and was observed the mating activities at intervals. The flies would not be further tested if no copula was observed. Groups of five attracting flies or other designed attracting materials as mentioned in Table 1 were placed in the hole of the stopper of two arms. Four responding flies were introduced to the Y-tube from the base following 2 min of air flow. Each group of responding flies was monitored for 8 min, and three activities, orientation, wing vibration and attempted copulation, were recorded (Landolt et al. 1985). If one fly entered half length of one arm, this would be recorded as orientation; hastily fly and frequently wing-flap of a fly would be considered as the activity of wing vibration; when a fly curved its abdomen and sticked its clasper to the glass wool, this indicated that the responding fly attempted to copulate. If one fly responded to both arms, each arm was recorded as one response. The live male fly's respond to both arms without any flies in them were also observed to provide information about bias in the setup. Each attraction test was replicated 12 to 26 times using new flies. For a new replication, the Y-tube was reversed 180° to eliminate the unclear asymmetry, and the stoppers were rinsed with acetone to remove possible trace chemicals of flies. All observations were made between 0600 and 1000 h. The results were analyzed using chi-square tests (version 12.0, SPSS Inc) to compare the difference of two numbers.

Attractiveness of methylene dichloride washes of female whole bodies and excised ovipositors. Methylene dichloride washes of whole bodies, excised ovipositors, and whole bodies without ovipositors were bioassayed to compare their attractiveness to that of the virgin females. The preparation of methylene dichloride washes was similar to McKay & Hatchett (1984). One-hundred virgin females were collected and cooled at 0°C for 5 min. Females with extended ovipositors were placed on clean glass slides. Ovipositors were fully extended by gently pressing the abdomens, excised with a sharp scalpel, and pulled away from the bodies. The ovipositors and bodies of 100 females were soaked separately in 1 ml methylene dichloride for 30 min at 0°C. A 50-µl (5 FE) wash was pipetted onto filter paper (1 cm long and 0.5 cm wide) and the solvent was evaporated for about 20 s in air. The filter paper was then placed in the hole of stopper of one arm. Three groups of material, methylene dichloride solvent versus air, whole body wash versus methylene dichloride solvent, and ovipositor wash versus whole body without ovipositor wash were selected in this experiment. Observations and data recorded were the same as the previous test.

Table 1. Response of virgin *Bradysia odoriphaga* males to virgin females, mated females, mated males, and virgin males[1].

Attraction test	Total number of ♂	Orienting and vibrating wings: Percentage of ♂ to A	vs. B	P[2]	Attempted copulation: Percentage of ♂ to A	vs. B	P[2]
control (A)[3] vs. control (B)[3]	56	1	0	–	0	0	–
Virgin ♀ (A) vs. control (B)[3]	104	78	27	<0.005	67	2	<0.005
Mated ♀ (A) vs. control (B)[3]	48	54	8	<0.005	38	0	–
Virgin ♀ (A) vs. Mated ♀ (B)	56	77	77	>0.05	71	54	>0.05
Mated ♂ (A) vs. control (B)[3]	52	0	4	–	0	0	–
Virgin ♂ (A) vs. control (B)[3]	52	0	0	–	0	0	–

[1]There were 12 to 26 replications for each test, with four males for each replication.
[2]Means for each two-choice situation were compared using χ^2-test, except when means were not available (0%).
[3]Control was air without flies.

The test was replicated 14 to 16 times, using a new filter paper and flies for each replication. The results were analyzed using chi-square tests.

Field test. Self-made △-shaped sticky traps (10 cm length of triangle side and 30 cm length of trap) were used in greenhouse in a Beijing suburb in China. Ten virgin females, a filter paper with 10FE methylene dichloride wash, or a filter paper with same volume methylene dichloride solvent was placed in a cage of 1 mm mesh, and the cage was put in center of the △-shaped trap. An empty cage was used as control test. Traps were set 30 cm above the ground at ca. 5 m intervals. Twenty four hours later, numbers of trapped males were accounted. Each treatment was replicated five times. The results were analyzed using Duncan's tests (version 12.0, SPSS Inc) to compare the difference of four treatments.

Results

Effect of time-of-day on copulation and other mating behavior. *B. odoriphaga* mating activity had a W-shape fluctuation (Fig. 2). The most active period was in the late at night, and the peak copulation was at 0200 h of 1 day (28.8%). In contrast, the lower period of copulation was between noon and evening. For example, the lowest copulation time of 0 day and 1 day were at 1200 h (0.0%) and 1800 h (1.3%), respectively.

In the 0-day mating test with 160 pairs of *B. odoriphaga*, 104 couples mated. Among the mated flies, 52 pairs copulated once, 25 pairs mated twice, and one pair mated up to 6 times.

Attractiveness of live flies. In most cases, responding flies walked in both arms, while they vibrated their wings and attempted to copulate to a certain arm. Thus, numbers of orienting and wing-vibrating flies were combined as one data, and numbers of attempted copulation flies were as another data (Table 1). The

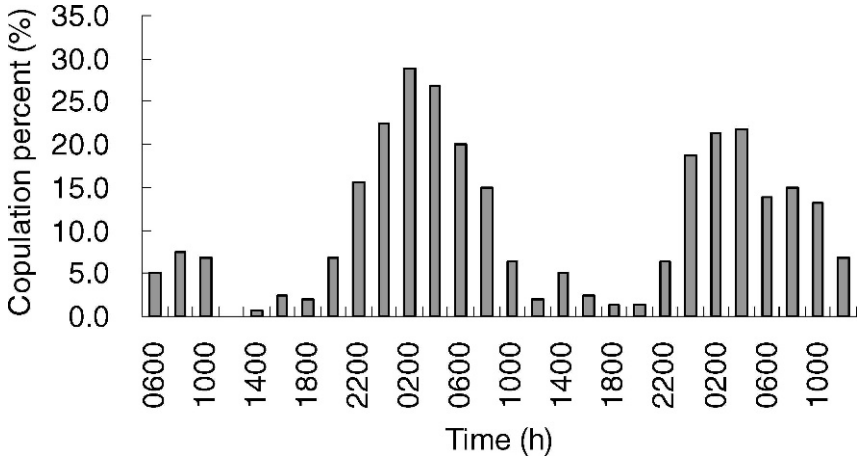

Fig. 2. *B. odoriphaga* male copulated with live female during a 54-h period. There were 4 replications and 40 couples per replication.

sum of A and B sometimes was lager than 100%, for one fly was recorded twice responses if it responded to both arms.

In blank control vs. blank control test, virgin *B. odoriphaga* males did not respond to either arm. Virgin *B. odoriphaga* males responded to virgin females, with 78% of males orienting and vibrating wings in response to virgin females, whereas, only 27% of males responded to the control (Table 1). Attempted copulation by virgin males was 67% in the presence of virgin females, but only 2% in the presence of the control. Virgin males also vibrated their wings to 77% of both unmated and mated females, but attempted copulation to mated females was less than that to virgin females (54% vs. 71%). Neither virgin males nor mated males attracted virgin males and females.

The mean time for males to orient, vibrate their wings, and to mate with females in the Y-tube test was 1.5 min, 2.0 min, and 2.8 min, respectively. The shortest time for males from being released to copulation was only 10 s.

Attractiveness of methylene dichloride washes of female whole bodies and excised ovipositors. Methylene dichloride alone was not any more attractive to virgin *B. odoriphaga* males than the control (Table 2). The methylene dichloride wash of female whole bodies was highly attractive to virgin males with 64% of males orienting and vibrating their wings and 36% of males attempting copulation. In comparison, the methylene chloride solvent alone only caused 13% of males to vibrate their wings and only 4% of males attempted mating. Also, virgin males showed a much greater response to the ovipositor wash than the wash of the whole body without ovipositor, with 58% of males exposed to the ovipositor wash vibrating their wings and 33% attempting copulation while the whole-body-without-ovipositor wash only stimulated 13% male wing vibrations and 5% attempted copulation.

Field test. In 24 h period in field test, 10 virgin females or 10FE CH_2Cl_2 wash attracted about 74 or 57 *B. odoriphaga* males, respectively (Table 3). While only 11 or 8 males were attracted by an empty cage or a paper with CH_2Cl_2 alone.

Table 2. Response of virgin *Bradysia odoriphaga* males to methylene dichloride (CH_2Cl_2) washes of whole bodies and excised ovipositors of virgin females[1].

Attraction test	Total number of ♂	Orienting and vibrating wings: Percentage of ♂ to A vs. B		P	Attempted copulation: Percentage of ♂ to A vs. B		P
CH_2Cl_2 solvent (A) vs. control (B)	56	2	2	>0.05	0	0	–
Whole body wash (A) vs. CH_2Cl_2 solvent (B)	56	64	13	<0.005	36	4	<0.005
Ovipositor wash (A) vs. whole body w/o ovipositor wash (B)	64	58	13	<0.005	33	5	<0.005

[1]There were 14 to 16 replications for each test. Other parameters and statistical analyses were as described in Table 1.

The differences among the numbers of caught males by live females, female wash, and CH_2Cl_2 are significant ($P<0.01$).

Discussion

Test of effect of time-of-day on copulation of *B. odoriphaga* showed that during the imago period of 3 days, most flies mated in night from midnight to dawn, and in daytime copulation frequency reduced significantly. Compared with *B. odoriphaga*, the highest sexual activity time of *B. impatiens* was from 4 h before the scotophase to 1 h after the scotophase (Alberts et al. 1981).The lowest sexual activity time was at noon, which was about 12 hours after the highest activity time. And in the next 12 hours, the mating frequency increased stably. Therefore the mating behavior of *B. odoriphaga* is greatly affected by the time-of-day, and the copulation cycle is about 24 hours.

The non-significance between male's responses to blank control vs. blank control indicated the glass Y-tube olfactometer had little bias in this test. Heterogeneity between males to virgin females and the control suggested that virgin females had attracted the males. In contrast, virgin males did not respond

Table 3. Catches *Bradysia odoriphaga* males in traps containing virgin females, CH_2Cl_2 solvent, or female body wash of CH_2Cl_2.

Test material	Control[1]	10 virgin ♀	CH_2Cl_2 solvent	10 FE CH_2Cl_2 wash
Catches ♂(mean±SE)	10.8 ± 1.6 c[2]	74.4 ± 2.7 a[2]	8.0 ± 1.3 c[2]	57.4 ± 2.6 b[2]

[1]Control was empty cage without flies or filter paper.
[2]Means followed by different letter are significantly different at $P < 0.01$.

to virgin males. Furthermore, the whole female body wash had much more attraction to virgin males than that of the solvent alone. Also, males ran to females and mated with females only in 10 s after being released. Thus, it was virgin females, not virgin males that attracted males. These results strongly indicate that *B. odoriphaga* females produce a sex pheromone that attracts males.

Orienting and wing-vibrating by virgin *B. odoriphaga* males to mated females were more than that to the control, which indicated that mated females also attracted virgin males. In the comparison of the attraction of unmated and mated females, males were strongly attracted to both arms. Mating times showed females had attraction to males; even the females were mated for several times. The multi-time copulation of the same fly suggested that the attraction of females to males was not affected too much by copulation behavior. Therefore, there was no difference in the attraction of virgin males to mated and unmated females. This response differed from Hessian fly, *Mayetiola destructor* (Say), which belongs to the same suborder of Nematocera, because its female sex pheromone was transferred to males during mating (McKay & Hatchett 1984). Gotoh et al. (1999) also reported that copulatory behavior of unmated sciarid males of *L. mali* decreased quickly after the female had mated once. It is presumed that the sex pheromone in female *B. odoriphaga* is not lost or transferred to the male during mating.

More males responded to ovipositor wash than that of whole female body without ovipositor wash. The whole female body wash also had attraction for males. Thus, the inference from these results is that the ovipositor is the site of pheromone release and possibly the source of pheromone production. By contrast, it was presumed that the female sex pheromone of *L. mali* was presented on all parts of female flies (Gotoh et al. 1999). The male sciarid fly, *B. paupera* also responded to all parts of female flies (Liu et al. 2002).

In field test, 10 virgin females had more attractiveness to males than 10FE CH_2Cl_2 wash, which was similar to the test in laboratory. It could be explained that the live females released sex pheromone all through 24 h period, while the sex pheromone in CH_2Cl_2 faded away in the air as time passed.

Overall, the results clearly demonstrated that in laboratory and in field female sciarid fly, *B. odoriphaga* produced a sex pheromone and that the ovipositor was the probable site of pheromone production and release. Mating behavior was found to follow a daily rhythm, with male flies most responsive between 2200 to 0600 h. Both males and females could mate more than one time.

Acknowledgments

This research was supported by National Science Project (No. 30671388). We thank Prof. Zhang Zhongning of the Institute of Zoology, Chinese Academy of Sciences for guidance in our experiments.

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Bradysia Odoriphaga Copulatory Behavior and Evidence of a Female Sex Pheromone[1]

Hong-Jun Li, Xiong-Kui He, Ai-Jun Zeng, Ya-Jia Liu, and Shu-Ren Jiang

J. Agric. Urban Entomol. 24(1): 27–34 (January 2007)

ABSTRACT *Bradysia odoriphaga* Yang and Zhang is the most serious pest of Chinese chive, *Allium tuberosum* Rottle ex Spreng. (Liliaceae) in North China. Pesticide residues in this vegetable are very high following excessive use of organophosphorate insecticides. Because there have been no reports on the sex pheromones of *B. odoriphaga*, sex pheromone and mating behavior of *B. odoriphaga* were investigated as a possibility of developing semiochemical-based monitoring and control of this pest. In laboratory bioassays, live *B. odoriphaga* virgin females stimulated 78% of males to vibrate their wings and 67% of males to attempt to mate. Methylene dichloride washes of female whole bodies and excised ovipositors also attracted males. In field test, many *B. odoriphaga* males were attracted to the traps containing live *B. odoriphaga* virgin females or methylene dichloride washes of female whole bodies. Most flies mated only once, while a few mated as many as six times. After mating, females were still attractive to males. Flies' sexual behavior showed a daily rhythm. The higher mating activity was from 2200 to 0600 h and the lower from 1200 to 1800 h. These results indicate the presence of a female sex pheromone in *B. odoriphaga* with the ovipostor as the most likely source of pheromone production. It is possible to collect the maximum amount of sex pheromone between 2200 and 0600 h. There is the possibility that this sex pheromone may be used to monitor and control of *B. odoriphaga* in the future.

KEY WORDS Diptera, Sciaridae, *Bradysia*, *odoriphaga*, sex pheromone, mating behavior

Introduction

Many sex pheromones have been identified within the order Diptera, especially in the families Cecidomyiidae (Hillbur et al. 1999, McKay & Hatchett 1984), Drosophilidae (Nemoto et al. 1994), Glossinidae (McDowell et al. 1981), and Muscidae (Uebel et al. 1975). However, only three species of Sciaridae have been reported to have sex pheromones. Casartelli et al. (1971) reported a sex pheromone in *Bradysia tritici* (Coquillett). Heptadecane was identified as the major component of a sex pheromone of *Lycoriella mali* (Fitch) (Kostelc et al. 1980). Gotoh et al. (1999) reported that heptadecane did not attract unmated adult males *L. mali* in Japan. *Bradysia impatiens* (Johannsen) was also shown to have a sex pheromone by Alberts et al. (1981) and Liu et al. (2002), but the

[1]Accepted 4 November 2007.
College of Science, China Agricultural University, Beijing 100094, China.

Survey for Hymenopteran and Dipteran Parasitoids of the Fall Armyworm (Lepidoptera: Noctuidae) in Chiapas, Mexico[1]

Ramiro Eleázar Ruíz-Nájera,[3] Jaime Molina-Ochoa,[2,4,7] James E. Carpenter,[5] Jorge A. Espinosa-Moreno,[3] José Alfredo Ruíz-Nájera,[6] Roberto Lezama-Gutiérrez,[4] and John E. Foster[7]

J. Agric. Urban Entomol. 24(1): 35–42 (January 2007)

ABSTRACT A survey of hymenopteran and dipteran parasitoids of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) larvae was conducted to determine their occurrence and parasitism rates in Western Chiapas, Mexico. 1247 FAW larvae were collected from whorl-stage corn cornfields in 21 locations in the region called "La Frailesca" in Chiapas, Mexico during the summer of 2002; 251 larvae produced parasitoids for an overall parasitism rate of 20.1%. Five braconids were recovered from FAW larvae, *Rogas vaughani* Muesebeck, *R. laphygmae* Viereck, *Chelonus insularis* Cresson, *C. cautus* Cresson, and *Glyptapanteles militaris* Walsh. Two ichneumonids, *Neotheronia* sp., and *Ophion flavidus* Brulle, and one eulophid, *Euplectrus plathypenae* Howard were recovered. Dipteran parasitoids were also recovered from last instars. These were the tachinids *Archytas marmoratus* Towsend, *Lespesia archippivora* Riley, *Archytas* sp., and *Winthemia* sp. *Megaselia scalaris* Low was a unique phorid recovered. Dipteran parasitoids produced a parasitism rate of 6.3%, and were mostly recovered from 5th and 6th FAW instars. Most of the parasitoid species were recovered from FAW larvae that were collected from corn plants in the V3 growth stage. In this survey, *O. flavidus*, *E. plathypenae*, *Chelonus* spp., and species of *Rogas* (Syn: *Aleiodes*) were the most frequently recovered species in "La Frailesca".

KEY WORDS Fall armyworm, survey of parasitoids, corn growth stages, parasitism rate, occurrence, Hymenoptera, Diptera, Chiapas, Mexico

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a highly polyphagous agricultural insect pest. Host plant species for FAW come from a broad diversity of families and include important agricultural

[1]Accepted for publication.
[2]Corresponding author (jmolina@ucol.mx, jmolina18@hotmail.com).
[3]Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Departamento de Producción vegetal, Apartado postal 78, Villaflores, Chiapas 30470, México.
[4]Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Apartado postal 36, Tecomán, Colima 28100, México.
[5]United States Department of Agriculture, Agricultural Research Service, Crop Protection and Management Research Unit, 2747 Davis Road, Tifton, GA 31793-0748, USA.
[6]Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Agropecuarias, Carretera Villahermosa-Teapa, Km. 25, Villahermosa, Tabasco, México.
[7]University of Nebraska Lincoln, Department of Entomology, 312F Plant Industry Building, Lincoln, NE 68583-0816, USA.

crops (Andrews 1980). FAW is distributed throughout the Americas and has a tropical-subtropical origin in the western Hemisphere (Sparks 1979). Chemical control of FAW is a common practice using granular and spray insecticide applications; however, currently in agricultural pest control, the adverse effects of the use of insecticides are leading scientists to search for alternatives to chemical control of insect pests based on health, environmental, wild life, and economic concerns (Mattson *et al.* 2000). Biological control has been used for pest management for many years and it has gained renewed interest because of problems caused by the intensive use of pesticides. Most concentrated efforts for biological control appear to be directed towards the "rear and release" augmentation, followed by importation and thirdly by conservation; Lewis et al. (1997) suggested a reversed order of priorities. They maintained that there is a need to understand, promote and maximize the effectiveness of indigenous populations of natural enemies, then based on the knowledge and results of these actions, we should supplement any gaps by importation. In accordance with Lewis et al. (1997), and as a result of economic and environmental concerns, surveys for natural enemies of the FAW, particularly, pathogens, parasitoids and parasites, occurring in the Americas, and the Caribbean Basin have been conducted to develop a better understanding of this complex (Molina-Ochoa et al. 2001, 2003a, 2004).

The occurrence and parasitism rate of FAW larval parasitoids varies considerably between localities, regions, crop practices, plant stage, and years. This information is needed to assess the potential value of the existing larval parasitoid fauna in controlling FAW on corn. In Western Chiapas, Mexico, particularly in the region called "La Frailesca" documentation of the occurrence, diversity and parasitism rates of FAW parasitoids recovered from whorl-stage corn is incomplete or unknown. In this study, we surveyed whorl-stage corn in "La Frailesca" and recorded the occurrence, diversity and parasitism rates of hymenopteran and dipteran parasitoids of FAW larvae, as well as the corn growth stages from which FAW larvae were recovered.

Materials and Methods

This survey was conducted from June–August 2001 in Chiapas, Mexico in the region known as "La Frailesca" which includes the municipalities of Villacorzo, and Villaflores. This region has a tropical wet and dry climate (García 1987).

Twenty one localities were sampled in the municipalities of Villaflores and Villacorzo Chiapas, Mexico; twelve, and nine localities, respectively. The municipality of Villacorzo is located at the coordinates of 16°11′ Latitude North and 93°17′ Longitude West, has an elevation of 580 m, and has a annual mean temperature and mean rainfall of 48.0°C, and 1200 mm, respectively. Coordinates for Villaflores are 16°14′ Latitude North, 93°45 Longitude West. Villaflores has an elevation of 540 m, and has an annual mean temperature and rainfall of 22°C and 1200 mm, respectively. We used a Garmin eTrex (Olathe, KS) GPS to obtain the coordinates, and altitude.

For each locality, 43 larvae were sampled. Each sample consisted of different FAW instars, however the numbers of each instars were not classified. The larvae were collected from whorl-stage cornfields, and they were individually placed into glass flasks of 4.6 × 10.0 cm (diameter and height, respectively), covered on top

with fine screen from which the parasitoids did not go through the mesh. The larvae were fed with pieces of fresh corn leaves about 20 cm^2 which were replaced every 36 hours until pupation, and held at 25°C, 80% RH, and a photoperiod of 12:12 (L:D) h in the laboratory until parasitoid emergence. Coconut fiber was used as a substrate for pupation when the larvae reached the prepupal stage. For each larva collected, the corn growth stage was recorded according to Ritchie et al. (1992).

The parasitoids that emerged from the larvae and pupae were recorded every 24 h. For the dead larvae or pupae where nothing emerged, no dissections were made to examine for dead parasitoids. Percent of parasitism was calculated according Pair et al. (1986). Parasitoids were identified using the Manual for Identification of Parasitoids of Agricultural Pests in Central America (Cave 1995) and later submitted to the USDA-ARS Systematic Entomology Laboratory, Beltsville MD for confirmation of the identification.

Results and Discussion

In this study, out of the 1247 FAW larvae collected from whorl-stage corn, 251 larvae produced parasitoids, for a percent parasitism of 20.1%. One hundred fifty three larvae died from unknown causes, possibly unidentified pathogens or injury during handling.

Nine species of hymnopterous parasitoids were identified. Five species belonged to the family Braconidae: *Rogas vaughani* (Muesebeck), *R. laphymae* (Viereck), *Chelonus cautus* (Cresson), *Chelonus insularis* (Cresson), and *Glyptapanteles militaris* (Walsh.); three species belonged to the family Ichneumonidae: *Neotheronia* sp., *Pristomerus* sp., and *Ophion flavidus* (Brulle); and only one species belonged to the family Eulophidae: *Euplectrus plathypenae* (Howard) (Table 1).

Five species of dipteran parasitoids were recovered from FAW last instars and pupae; they belonged to the families Tachinidae and Phoridae. Tachinid parasitoids identified were *Archytas marmoratus* (Townsend), *Lespesia archippivora* (Riley), *Archytas* sp., and *Winthemia* sp. The unique phorid parasitoid recovered was *Megaselia scalaris* (Loew.) (Table 1).

According to the occurrence and percent of parasitism, the most prevalent parasitoids were *O. flavidus*, *E. plathypenae*, *R. vaughani*, and *A. marmoratus*. They occurred in 19, 18, 11, and 6 of the 21 localities sampled in this survey, and caused 5.77, 3.53, 1.36, and 1.60% of parasitism, respectively (Table 1). However, *L. archippivora* played an important role in the parasitism caused by tachinids (1.52%). The ichneumonid *Neotheronia* sp. occurred in four localities causing low mortality rates less than 0.32%. This is the first report of *Neotheronia* sp. as a larval parasitoid of *S. frugiperda*. A phorid parasitoid, *M. scalaris*, emerged from pupae of FAW from five localities, and caused a percent parasitism of 1.04% (Table 1). Something to highlight is that the complex of the genus *Archytas* totalized 3.04% of parasitism, almost comparable to the percent parasitism caused by *E. plathypenae* (3.53%) a eulophid parasitoid frequently reported in previous surveys conducted in Mexico (Molina-Ochoa et al. 2001, 2004). The braconids were more frequently recovered from young instars of FAW collected from corn plants in the V2–V3 stage of growth; most ichneumonids were recovered from third to fifth instars parasitizing corn plants in the growth stages

Table 1. Occurrence and percent parasitism of hymenopteran and dipteran parasitoids of Fall Armyworm larvae collected in cornfields in whorl-stage in "La Frailesca", Chiapas, Mexico.

Family and species	NLO*	Instar attacked	% parasitism	Corn growth stage
Braconidae				
Rogas vauganhi Muesebeck	11	1st, 2nd	1.36	V2, V3
Rogas laphygmae Viereck	5	1st, 2nd	0.56	V2, V3
Chelonus cautus Cresson	5	1st	0.48	V3
Chelonus insularis Cresson	8	1st	1.20	V2, V3
Glyptapanteles militaris Wash.	4	3rd, 5th	0.32	V2, V3
Ichneumonidae				
Neotheronia sp.	4	5th **	0.32	V2, V3
Pristomerus sp.	3	3rd, 4th	0.24	V3, V4
Ophion flavidus Brulle	19	3rd, 4th, 5th	5.77	V2, V3, V4
Eulophidae				
Euplectrus plathypenae Howard	18	1st, 2nd	3.53	V2, V3, V4
Tachinidae				
Archytas marmoratus Townsend	6	Pupae£	1.60	V3, V4
Archytas sp.	6	Pupae£	1.44	V3, V4, V5
Lespesia archippivora Riley	5	5th, Pupae£	1.52	V4
Winthemia sp.	5	Pupae£	0.72	V3, V4
Phoridae				
Megaselia scalaris Low.	5	Pupae£	1.04	V4

*Number of localities of occurrence.
**Collected from FAW last instar, Pupae£ parasite recovered from FAW pupae.

from V2–V4, and the dipteran parasitoids were recovered from last instars and pupae obtained from corn plants in V3–V5 (Table 1).

The most important parasitoid in this survey was *O. flavidus* due to its widespread occurrence and high larval parasitism rates (≈5.8%) (Table 1). It has been reported from Argentina, Brazil, Honduras, Mexico, Nicaragua, and the US (Molina-Ochoa et al. 2003b). Previous reports of percent of parasitism of *O. flavidus* against FAW larvae ranged from 4.8% to 9.6% (Molina-Ochoa et al. 2001). Most of our results are between those ranges, with only three exceptions. *O. flavidus* has been reported attacking the fourth, fifth, and sixth instars of FAW with equal success (Gross & Pair 1991). We most frequently collected larvae parasitized by *O. flavidus* from corn plants in the V2, V3, and V4 growth stages in both municipalities.

The second most frequently recovered and important parasitoid in this survey was *E. plathypenae*. Its rate of parasitization was about 3.5%. Molina-Ochoa et al. (2001) found a similar percent parasitism in a survey conducted in the western coast of Mexico. It was more frequently recovered from FAW larvae collected from corn plants in the growth stages V2, V3, and V4. This parasitoid has been reported from Barbados, Brazil, Chile, Colombia, Cuba, Guyana, Lesser Antilles, Mexico, Nicaragua, Puerto Rico, Trinidad, and the US (Molina-Ochoa et al. 2003b).

Rogas vaughani caused 1.36% of parasitism most in first and second FAW instars. It has been reported to attack FAW larvae in Honduras, and Nicaragua (Maes 1989, Wheeler et al. 1989, Cave 1993) and other lepidopterous pests such as *Helicoverpa zea* (Boddie), *Peridroma saucia* (Hbn), *P. margaritosa* (Haworth), *Trichoplusia ni* (Hbn), *Spodoptera exigua* (Hbn), *Alabama argillacea* (Hbn), and *Prodenia ornithogalli* (Guenee) in North America, Tapachula Chiapas, Mexico, and Central America (DeCoss et al. 1977, Marsh 1978, Butler et al. 1982, King & Saunders, 1984).

Rogas laphygmae ≈ *Aleiodes laphygmae* (Viereck), has been reported to occur in Brazil, Chile, Honduras, Mexico, Nicaragua, Puerto Rico, and US (Molina-Ochoa et al. 2003b). It was reported to attack lepidopterous pests such as *Autographa* sp., *Feltia subterranean* (F.), *H. zea*, *P. saucia*, *Pseudaletia unipuncta* (Haw.), *Spodoptera eridania* (Cram.), *S. exigua*, *S. frugiperda*, *Agrotis subterranean* (Cram), and *Colias eurytheme* (Boisduval). It was well distributed in North, South, and Central America (Marsh 1978, King & Saunders 1984). This species was reported also to attack L1–L3 stages of *S. exigua* in Georgia, US (Ruberson et al. 1993, Ruberson et al. 1994), and it was found on first, and second instars collected from corn plants in the V1 stage; however, in this survey it occurred more in V2, and V3, causing 0.56% of parasitism.

The braconid, *C. insularis* caused low parasitism rate about 1.2%. Similar percent parasitism was reported by Molina-Ochoa et al. (2001). Luginbill (1928), and Vickery (1929) indicated that *C. insularis* was an important parasitoid controlling FAW populations in its overwintering habitats of Florida and Southern Texas. This parasitoid is reported to attack eggs and larvae of noctuids such as *F. subterranean*, *P. saucia*, *T. ni*, *H. zea*, *S. eridania*, *S. exigua*, *S. frugiperda*, *S. ornithogalli*, *S. praefica*, and the pyralids, *Loxostege stictidalis* (L.), *Ephestia eleutella* (Hbn), and it is distributed in the US (Ashley et al. 1980, 1982, 1983; Ashley 1986, Butler et al. 1982, Pair et al. 1986, Andrews 1988), Africa, and Hawaii (Marsh 1978), and Venezuela (Notz 1972). In this survey, *C. insularis* was found in FAW larvae collected from V2, and V3 corn. *C. cautus* also was found in V3 corn in five localities, but its distribution in this survey was less frequent than *C. insularis*, and its percent parasitism was never higher than 0.48% (Table 1). Molina-Ochoa et al. (2001) reported percents parasitism of *Chelonus* (probably *cautus*) of 11% and 10%, in one sample conducted in Colima and another in Jalisco, respectively. This braconid has been reported to occur in Honduras, and Mexico attacking FAW eggs and larvae (Cave 1993, Canas & O'Neil 1998, Molina-Ochoa et al. 2001). In this survey, the parasitoid was recovered from third instars collected from corn plants in the V3 growth stage (Table 1).

Glyptapanteles militaris and *Pristomerus* sp. were the braconid and ichneumonid parasitoids with the lowest occurrence with 0.4% and 0.24%, recovered from V2 and V3, and V3, and V4 corn growth stages, respectively (Table 1). *Neotheronia* sp. an ichneumonid occurred in four localities causing mortality rates less than 2.5%. This is the first report of *Neotheronia* sp as a parasitoid of FAW.

The results of this survey suggest that the parasitoid complex functioning in cornfields of Chiapas has the capacity to cause a significant reduction of the FAW larval populations. The results of this survey sustain that the parasitoid complex is a valuable component of the tropical agroecosystems, even though the rate of parasitism exhibited by each parasitoid species is variable among localities and

collections. In our survey parasitoid species diversity was greatest when FAW larvae were collected from cornfields in the growth stage V3.

In this survey, *O. flavidus*, *E. plathypenae*, *Chelonus* spp., and species of *Rogas* (Syn: *Aleiodes*) played a low suppressing role on the FAW larval populations in both municipalities of Chiapas. However, these parasitoids are well adapted to the environmental conditions of Western Chiapas and frequently occurred in most of the localities sampled. Something important to consider in this survey is that we did not dissect dead larvae and pupae to examine for dead parasitoids. Therefore, the actual percent parasitism could have been higher than what we report in this study. Because percent parasitism was usually less than 6%, there is potential to increase parasitism by mass releasing parasitoids. Additional studies of the behavior and ecology of these parasitoids are needed.

Acknowledgments

We thank S. R. Shaw, P. M. Marsh, N. E. Woodley, R. F. Schroeder, and K. Lloyd, USDA-ARS Systematic Entomology Laboratory, Beltsville MD for the specimen identification and John J. Hamm (USDA-ARS Crop Protection and Management Research Unit, Tifton, GA, retired) for his critical review of the manuscript. This paper is a contribution of the Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Departamento de Producción Vegetal, Apartado postal 78, Villaflores, Chiapas 30470, México, the Universidad de Colima-Facultad de Ciencias Biológicas y Agropecuarias, Tecomán, Colima, México, the USDA-ARS CPMRU Tifton, GA, and the Department of Entomology, University of Nebraska Lincoln, Lincoln, NE, USA.

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Survey for Hymenopteran and Dipteran Parasitoids of the Fall Armyworm (Lepidoptera: Noctuidae) in Chiapas, Mexico[1]

Ramiro Eleázar Ruíz-Nájera,[3] Jaime Molina-Ochoa,[2,4,7] James E. Carpenter,[5] Jorge A. Espinosa-Moreno,[3] José Alfredo Ruíz-Nájera,[6] Roberto Lezama-Gutiérrez,[4] and John E. Foster[7]

J. Agric. Urban Entomol. 24(1): 35–42 (January 2007)

ABSTRACT A survey of hymenopteran and dipteran parasitoids of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) larvae was conducted to determine their occurrence and parasitism rates in Western Chiapas, Mexico. 1247 FAW larvae were collected from whorl-stage corn cornfields in 21 locations in the region called "La Frailesca" in Chiapas, Mexico during the summer of 2002; 251 larvae produced parasitoids for an overall parasitism rate of 20.1%. Five braconids were recovered from FAW larvae, *Rogas vaughani* Muesebeck, *R. laphygmae* Viereck, *Chelonus insularis* Cresson, *C. cautus* Cresson, and *Glyptapanteles militaris* Walsh. Two ichneumonids, *Neotheronia* sp., and *Ophion flavidus* Brulle, and one eulophid, *Euplectrus plathypenae* Howard were recovered. Dipteran parasitoids were also recovered from last instars. These were the tachinids *Archytas marmoratus* Towsend, *Lespesia archippivora* Riley, *Archytas* sp., and *Winthemia* sp. *Megaselia scalaris* Low was a unique phorid recovered. Dipteran parasitoids produced a parasitism rate of 6.3%, and were mostly recovered from 5th and 6th FAW instars. Most of the parasitoid species were recovered from FAW larvae that were collected from corn plants in the V3 growth stage. In this survey, *O. flavidus*, *E. plathypenae*, *Chelonus* spp., and species of *Rogas* (Syn: *Aleiodes*) were the most frequently recovered species in "La Frailesca".

KEY WORDS Fall armyworm, survey of parasitoids, corn growth stages, parasitism rate, occurrence, Hymenoptera, Diptera, Chiapas, Mexico

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a highly polyphagous agricultural insect pest. Host plant species for FAW come from a broad diversity of families and include important agricultural

[1]Accepted for publication.
[2]Corresponding author (jmolina@ucol.mx, jmolina18@hotmail.com).
[3]Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Departamento de Producción vegetal, Apartado postal 78, Villaflores, Chiapas 30470, México.
[4]Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Apartado postal 36, Tecomán, Colima 28100, México.
[5]United States Department of Agriculture, Agricultural Research Service, Crop Protection and Management Research Unit, 2747 Davis Road, Tifton, GA 31793-0748, USA.
[6]Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Agropecuarias, Carretera Villahermosa-Teapa, Km. 25, Villahermosa, Tabasco, México.
[7]University of Nebraska Lincoln, Department of Entomology, 312F Plant Industry Building, Lincoln, NE 68583-0816, USA.

NOTE

A Comparison of the Activity of Soil Fungal Isolates Against Three Insect Pests[1]

Sergio R. Sánchez-Peña,[2,3] Elisa Casas-De-Hoyo,[3] Rogelio Hernandez-Zul,[3] and Kristin M. Wall[4]

J. Agric. Urban Entomol. 24(1): 43–47 (January 2007)

Entomopathogenic fungi (e.f.) are important agents of biological control of insects. Two aspects, among others, are important for the use of e.f. against insect pests: the logistics of e.f. strain acquisition, and the activity of individual strains against multiple insects. Soil is the natural reservoir for many e.f., but only few strains used against insect pests originate from soil; most have been isolated from insects. Also, there are few reports that simultaneously compare the activity of individual strains of e.f. (of insect or soil origin) against more than one insect species. This is important for the deployment of e.f. in agroecosystems, where simultaneous control of several insect pests is very often desirable. In this work we determined and compared the simultaneous activity of local strains of e.f. (isolated from soil or insects at Saltillo, Mexico) against important regional pests: fall armyworm, *Spodoptera frugiperda*, greenhouse whitefly, *Trialeurodes vaporariorum*, and potato psyllid, *Bactericera cockerelli*. Against all three insects, one *Beauveria bassiana* strain from soil was as active as or more active than other *B. bassiana* and *Metarhizium anisopliae* strains from insect or soil origin. In some localities, native individual strains of e.f. from soil might have good activity against multiple local insect pests. We recommend the isolation and testing of local soil strains of e.f. for use in local biocontrol projects.

KEY WORDS Entomopathogenic fungi, Insecta, biological control, isolate

Introduction

Entomopathogenic fungi (e.f.) are natural enemies with attributes of desirable biocontrol agents. Two aspects are crucial for the deployment of e.f. in pest control: the long-term availability of strains and the virulence of individual strains. Regarding the former, soil is the reservoir for important e.f. like *Beauveria*, *Metarhizium*, and *Paecilomyces* (Vanninen et al. 1989; Milner 1992; Bidochka et al. 2002), though most fungal strains utilized for insect biocontrol have been isolated from infected insects, not soil (Poprawski et al. 1999; Lui et al.

[1]Accepted for publication 1 August 2008.
[2]Corresponding author (elcheco@usa.net).
[3]Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico.
[4]School of Public Health, University of Texas Health Science Center, Houston, Texas, 77030.

1999; Liu and Bauer 2006). Soil strains of e.f. were more active than many insect-derived strains against the brown-winged green bug, *Plautia stali* Scott (Hemiptera: Pentatomidae) (Ihara et al. 2001), and against the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) (Quesada-Moraga et al. 2006). It is more feasible to isolate e.f. from local soils than attempting to collect infected insect specimens, which are unpredictable to locate (Milner 1992). Regarding strain virulence, it is often desirable to possess strains that are highly pathogenic against several insect species, considering that in agricultural ecosystems the simultaneous management of several insects is often a requirement. However, few studies have compared the simultaneous activity of individual soil strains of e.f. against two or more insect species. Another point to consider is that the use of e.f. isolated from local soils avoids legal restrictions and concerns about the field application of exotic strains. It is therefore advisable to evaluate the insecticidal activity of individual strains of e.f., isolated from soils at the localities where biocontrol of different insect pest species is required. The present report evaluated the activity of e.f from soil against the following insects, of economic importance at the same locality that the fungi were isolated from in Mexico: larvae of fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae); nymphs of the potato psyllid, *Bactericera* (=*Paratrioza*) *cockerelli* (Sulc.) (Homoptera: Psyllidae); and nymphs of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). These three insects are primary pests of numerous crops (Poprawski et al. 2000; Heppner 1998; S.R. Sánchez-Peña, unpublished). In the American continent, the fall armyworm and other *Spodoptera* species like the beet armyworm, *S. exigua* (Hübner), the southern armyworm, *S. eridania* (Cramer), and the yellowstriped armyworm, *S. ornithogalli* (Guenée) can occur simultaneously with *B. cockerelli* and *T. vaporariorum* in the same field and plant; for example, infesting solanaceous crops (tomato, *Solanum lycopersicum* L.; pepper, *Capsicum annum* L.; potato, *Solanum tuberosum* L.; and eggplant, *Solanum melongena* L.) (S.R. Sánchez-Peña, unpublished).

This work was carried out at the Universidad Autónoma Agraria Antonio Narro, (UAAAN), in Saltillo, Coahuila, México (Long. 101°40′W, Lat. 25°05′N., alt. 1600 m). Fall armyworm larvae were obtained from egg masses collected on field corn plants, *Zea mays* L. Masses were incubated in petri dishes in the laboratory until eclosion. Newborn larvae were placed individually in plastic cups (30 ml) with lids and were fed fresh foliage of Bermuda grass, *Cynodon dactylon* L. For tests with psyllids, plants infested with *B. cockerelli* ("Serrano" pepper cv. "Tampiqueno 74" and "Huaje" tomato) were established both in the field and in the greenhouse. For field applications, plots of tomato and pepper plants were established in the locality of Venado, San Luis Potosí state, México (Lat. 22° 56′00″N., Long. 101°05′34″W., alt. 1790 m) on irrigated plots using no pesticides and standard local agronomic practices. Potato psyllid nymphs were also established in a greenhouse at the University on tomato and pepper plants. Whitefly (*T. vaporariorum*) colonies were established on potted lantana plants (*Lantana camara* L.) in a greenhouse at the University for greenhouse and laboratory tests.

Fungi were isolated as detailed in Zimmerman (1986). Briefly, soil samples (0.9 L) were separately collected at a depth of 10 cm in 1 L plastic containers from experimental vegetable plots at the UAAAN on 10 May 2005. In the

laboratory, three full-grown mealworm larvae, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) from a laboratory colony were added to each soil sample in containers. Larvae were covered with soil, and containers were incubated at room temperature for three weeks. Spores from mealworm larvae with external fungal growth were transferred to Potato Dextrose Agar (PDA) (Bioxon, Mexico) and pure cultures were obtained. Fungal morphospecies were identified (Humber 1998). Three out of eighteen *Beauveria bassiana* (Bals.) strains and two out of twelve *Metarhizium anisopliae* (Metch.) strains were randomly chosen for analysis. One *B. bassiana* insect isolate (from a banded-winged grasshopper, Acrididae: Oedipodinae, from the UAAAN fields) was also included in bioassays (Table 1).

Fungi were grown on autoclaved rice grains in polypropylene bags as described by Mendonca (1992). After three weeks of incubation, conidia were harvested from inoculated rice in bags for use in bioassays against insects. Spores produced as described normally have >80% spore germination when conidia are plated on PDA plus 1% yeast extract for 24 hours and examined microscopically. Fifty grams of fungal-colonized rice grains were vigorously shaken in 100 ml of 0.025% Tween 20 (Sigma-Aldrich, Mexico) in water. Resulting spore suspensions were filtered through cotton cloth and the filtrate was placed in a blender (Osterizer 6694-015, Jarden Corp., Rye, NY) at maximum speed for 30 seconds to separate spores. Spores counts were made using an improved Neubauer hemocytometer (Caprette 2004), and all fungi were applied at 1×10^8 conidia ml^{-1}.

The combination of individual fungal strains and insect species constituted the treatments. The insects and the fungi applied to them are listed on Table 1. Fall armyworms were exposed to fungi in two ways: by submerging larvae (3rd instar larvae, n = 15/fungal strain) in spore suspensions for five seconds, and by immersing fresh cut bermudagrass foliage into spore suspensions then feeding this foliage to 1st and 2nd instar larvae (n = 18 larvae/fungal strain). The foliage was dried, and placed as the only food for individual larvae in 30 ml plastic cups. Mortality was registered daily.

Fungi were applied to psyllid nymphs in both tomato and pepper plants arranged in separate (field and greenhouse) randomized block experimental designs with ten replicates (plants) per treatment (see Table 1 for treatments). Greenhouse treatments were applied on 6 and 24 October 2005. Psyllid nymphs on tomato and pepper leaves were sprayed with fungal suspensions using hand atomizers. All treated leaves were sprayed to runoff. Sprayed plants in the greenhouse were maintained at 21–33°C. In the field in Venado, fungi were applied 12 September 2005 and 25 June 2006 on both pepper and tomato. After 48 hours under either field or greenhouse conditions, ten sprayed pepper leaves and ten tomato leaflets were randomly collected from each of the treatments, placed in moist chambers (Petri dishes with disks of moist filter paper) and incubated at room temperature for 72 hours. Leaves were then examined under the dissecting microscope and all nymphs on leaves were scored as alive or dead.

For whitefly tests, three lantana plants were used for each fungal strain. Infested plants were sprayed to runoff with the spore suspensions using hand atomizers and then placed either in laboratory (23–25°C) or greenhouse (21–33°C) conditions. Sprayed *Lantana* leaves were collected from plants 72 hours after spraying and examined under the dissecting microscope; these leaves were not incubated in moist chambers. Nymphs were counted on three leaves/fungus strain and mortality was determined for a minimum of 25 nymphs per leaf.

Table 1. Insect mortality after inoculation with conidia of entomopathogenic fungal strains from soil at Saltillo, Mexico

	Fungus strain and percent insect mortality					
Inoculated insect	Control[a]	*B. bassiana* UA-3 Host: Grasshopper (Oedipodinae)	*B. bassiana* UA-21 Soil isolate	*B. bassiana* UA-15 Soil isolate	*M. anisopliae* UA-12 Soil isolate	*M. anisopliae* UA-11 Soil isolate
Psyllid, greenhouse tomato[b]	2.0x	85.1z	85.0z	83.1z	45.1y	39.8y
Psyllid, field tomato	3.5x	86.5z	86.1z	89.8z	50.1y	43.2y
Psyllid, greenhouse pepper	6.6x	84.4z	87.4z	86.1z	54.2y	57.4y
Psyllid, field pepper	3.1x	88.5z	87.7z	83.7z	43.7y	41.5y
Whitefly lantana greenhouse	30.2x[e]	39.5x	42.9x	not tested	49.5x	62.6y
Whitefly lantana laboratory	8.3x	48.2y	49.5y	not tested	62.3y	not tested
[c] Fall armyworm, immersion	33.0x[f]	80.0x	100y	not tested	90.0y	60.0x
[d] Fall armyworm, aspersion	30.4x	72.0x	83.3y	not tested	100y	not tested

[a]Insects treated with a solution of 0.025% Tween 20 in water.
[b]Each psyllid mortality value shown is the average of two applications; these were analyzed separately and showed the same significance patterns.
[c]Fall armyworm larvae exposed to fungal suspension by immersion.
[d]Fall armyworm larvae exposed to fungal suspension by aspersion to foliage.
[e]For the psyllid data, means followed by the same letter within rows were not significantly different, Tukey's test ($p < 0.01$).
[f]For the whitefly and fall armyworm data, within rows, means significantly different from the control are followed by a letter different from that of the control according to Fisher's Exact Test ($p < 0.01$).

For each application date, mean percent mortality of psyllid nymphs was arcsine transformed and subjected to Analysis of Variance (ANOVA); significant differences among the means were detected using the Tukey test ($P = 0.01$) (SAS Institute 1998). For the fall armyworm and whitefly tests, treatment mortalities were compared pair-wise with the control mortality using Fishers Exact Test (Pezzullo 2006). To guard against an inflated Type-1 error, a $p < 0.0125$ (0.05/4 contrasts) was required to conclude significant differences between treatments and control (Bonferroni adjustment for multiple comparisons) (J. Pezzullo, Georgetown University, personal communication).

Insect mortality levels (untransformed) after fungal application are listed on Table 1. For psyllids, the incubation of infested leaves in moist chambers after application provided an optimal environment for fungi that probably accounts for the generally high infection rates observed. The whiteflies were not incubated in moist chambers and likewise infection levels were lower in general than those of the psyllids. We observed that overall, all isolates were moderately to highly pathogenic against at least one insect. The *M. anisopliae* strains from soil were less active against psyllids, and as active or more active against fall armyworm and whiteflies compared to the *B. bassiana* strains (Table 1). Also, the two *B. bassiana* strains from soil were similarly or more active than the *B. bassiana* insect-derived strain. The *B. bassiana* strain UA-21 from soil attained the highest mortality levels against all three insects. Few individual strains of e.f. of any origin have been tested for their activity against different insect species. The *B. bassiana* strain GHA, the active ingredient in the product Mycotrol (Emerald BioAgriculture Corp., Lansing, Michigan), is often considered an activity standard, and has shown moderate to high activity against multiple insects in different families and orders (Poprawski et al. 1999, Poprawski et al. 2000, Liu et al. 2002, Liu and Bauer 2006). This strain was originally isolated from the grasshopper *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae) (Liu et al. 2002). *Beauveria bassiana* strain ARSEF 252, originally isolated from the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), has also been considered a standard to compare fungal activity (Jackson et al. 1997). However, both of these are insect-derived strains, and the individual soil-derived strains have not been tested against multiple insects. The relatively high activity of the fungi tested in this work might be due to the fact that all strains had been kept less that a few months on artificial media, thus reducing potential loss of pathogenicity (Poprawski et al. 1999). These results suggest that biocontrol projects where multiple pest control is required should promote the isolation of e.f. from local soils as described, and the individual, indigenous strains should be screened against different insect pests. Local soils might be a source of e.f. strains promising for use in biocontrol projects.

Acknowledgment

We are thankful for the comments and statistical advice of J. C. Pezzullo. Project UAA-412, Dirección de Investigación, UAAAN.

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NOTE

A Comparison of the Activity of Soil Fungal Isolates Against Three Insect Pests[1]

Sergio R. Sánchez-Peña,[2,3] Elisa Casas-De-Hoyo,[3] Rogelio Hernandez-Zul,[3] and Kristin M. Wall[4]

J. Agric. Urban Entomol. 24(1): 43–47 (January 2007)

Entomopathogenic fungi (e.f.) are important agents of biological control of insects. Two aspects, among others, are important for the use of e.f. against insect pests: the logistics of e.f. strain acquisition, and the activity of individual strains against multiple insects. Soil is the natural reservoir for many e.f., but only few strains used against insect pests originate from soil; most have been isolated from insects. Also, there are few reports that simultaneously compare the activity of individual strains of e.f. (of insect or soil origin) against more than one insect species. This is important for the deployment of e.f. in agroecosystems, where simultaneous control of several insect pests is very often desirable. In this work we determined and compared the simultaneous activity of local strains of e.f. (isolated from soil or insects at Saltillo, Mexico) against important regional pests: fall armyworm, *Spodoptera frugiperda*, greenhouse whitefly, *Trialeurodes vaporariorum*, and potato psyllid, *Bactericera cockerelli*. Against all three insects, one *Beauveria bassiana* strain from soil was as active as or more active than other *B. bassiana* and *Metarhizium anisopliae* strains from insect or soil origin. In some localities, native individual strains of e.f. from soil might have good activity against multiple local insect pests. We recommend the isolation and testing of local soil strains of e.f. for use in local biocontrol projects.

KEY WORDS Entomopathogenic fungi, Insecta, biological control, isolate

Introduction

Entomopathogenic fungi (e.f.) are natural enemies with attributes of desirable biocontrol agents. Two aspects are crucial for the deployment of e.f. in pest control: the long-term availability of strains and the virulence of individual strains. Regarding the former, soil is the reservoir for important e.f. like *Beauveria*, *Metarhizium*, and *Paecilomyces* (Vanninen et al. 1989; Milner 1992; Bidochka et al. 2002), though most fungal strains utilized for insect biocontrol have been isolated from infected insects, not soil (Poprawski et al. 1999; Lui et al.

[1]Accepted for publication 1 August 2008.
[2]Corresponding author (elcheco@usa.net).
[3]Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico.
[4]School of Public Health, University of Texas Health Science Center, Houston, Texas, 77030.

ISSN 1523-5475

Journal of Agricultural and Urban Entomology

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Volume 24, No. 2 April 2007

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Arthropod Fauna of *Brassica Napus and Brassica Juncea* from Southern Punjab (Pakistan)[1]

Muhammad Aslam and Muhammad Razaq[2]

J. Agric. Urban Entomol. 24(2): 49–50 (April 2007)

Cultivation of oilseed brassicas in the Indian Sub-continent date back from 2000 to 1500 B.C. These crops are an important source of edible oil, with current local production of edible oil in Pakistan accounting for 31% with the remaining 69% met through imports. Damage due to insect pests is a major constraint to production of these crops. In Pakistan 70–80% losses in yield have been reportedly due to aphids in these crops in other parts of country (Rustamani et al., 1988). We have been surveying these crops since the mid 1990s. Insect pests and natural enemies were recorded on a weekly basis from the end of November to the end of March at Multan, Bahawalpur and Dera Ghazi Khan are 100 km away from Multan. Populations of aphids were recorded by beating the top 10 cm of the central inflorescence of plants gently 10 times with a 15 cm stick (of pencil thickness). Aphids were collected on a piece of white sheet and counted. Populations of predators were noted on a per plant basis. No arthropods other than insects were observed during the study. Specimens occasionally sampled from time to time have been deposited in the Canola Laboratory at the University College of Agriculture, Bahauddin Zakariya University, Multan (Pakistan).

Cabbage aphid, *Brevicoryne brassicae* (L.) and turnip aphid, *Lipaphis erysimi* (Kaltenbach) were continuously observed as the most abundant among all insects observed. Only a few plants of *B. napus* were found to be infested by *Myzus persicae* (Sulzer) during 2003 at Multan. Population of the cabbage aphid were always greater in abundance than that of turnip aphid on *B. napus* and *B. juncea*. Other insects recorded were the sawfly, *Athalia lugens* (Klug) (Hymenoptera: Tenthredinidae), *Spodoptera litura* L., *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *A. lugens* feeds upon the leaves by making holes. No insect other than *B. brassicae* and *L. erysimi* were observed to cause economic damage on crops sown timely, i.e., last week of October and onward. However, *S. litura* and *B. tabaci* severely damaged the crops sown early, i.e., mid-October in years when outbreaks occurred on cotton. Cotton is an important source to these two species of insects, and both of these insects are a threat to very early sown oilseed

[1]Received 12 February 2008; Accepted 22 April 2008.
[2]Corresponding author (mrazaq_2000@yahoo.com).
University College of Agriculture, Bahauddin Zakariya University, Multan-60800 (Pakistan). E-mail: aslamuca@yahoo.com

brassicas. Moreover, *A. lugens* and *M. persicae* have been reported as major insect pests of oil seed brassica crops in the past (Hashmi & Hassal 1988).

Other major insect pests reported previously, i.e., pea leafminer, *Phytomyza horticola* Goureau (Diptera: Phytomyzidae) and painted bugs, *Bagrada hilaris* F. and *Bagrada cruciferarum* (F.) (Hemiptera: Pentatomidae) were not recorded. Minor insect pests, i.e., *Agromyza* sp. (Diptera: Agromyzidae), *Nezara viridula* L. (Hemiptera: Pentatomidae), *Euporctis* sp. (Lepidoptera: Lymantriidae), *Pieris brassicae* L. (Lepidoptera: Pieridae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and two species of crickets, *Gryllus bimaculatus* DeGeer and *Acheta domesticus* L. (Orthoptera: Gryllidae) that have been reported in literature (Hashmi 1994) were not recorded during this study.

The ladybird beetle *Cocinella septempunctata* L. (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephen) (Neuroptera: Chrysopidae) were the only natural enemies observed at both locations in all years. Populations of *C. septempunctata* were always greater in abundance than that of *C. carnea*. Populations of both the natural enemies were insufficient to control aphid populations. *Diaretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) was observed parasitizing aphids in some years near maturity of crop. At this stage aphids have damaged the crop.

Acknowledgments

Funds for research were provided by Pakistan Agricultural Research Council Islamabad, Pakistan and Bahauddin Zakariya University, Multan, Pakistan. The identification of different species of insects was confirmed by Dr. M. Amjad, Senior Scientific Officer, Oilseed Program, National Agricultural Research Centre, Islamabad (Pakistan). G. David Buntin Georgia Experiment Station Entomology and Francisco R. Badenes-Perez, Department of Entomology, Cornell University reviewed the earlier draft of the manuscript.

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NOTE

Arthropod Fauna of *Brassica Napus and Brassica Juncea* from Southern Punjab (Pakistan)[1]

Muhammad Aslam and Muhammad Razaq[2]

J. Agric. Urban Entomol. 24(2): 49–50 (April 2007)

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[1]Received 12 February 2008; Accepted 22 April 2008.
[2]Corresponding author (mrazaq_2000@yahoo.com).
University College of Agriculture, Bahauddin Zakariya University, Multan-60800 (Pakistan). E-mail: aslamuca@yahoo.com

Molecular Identification of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Using PCR-RFLP[1]

Jennifer A. Lewter and Allen L. Szalanski

J. Agric. Urban Entomol. 24(2): 51–57 (April 2007)

ABSTRACT The fall armyworm, *Spodoptera frugiperda*, as with many noctuid moths, is a serious agricultural pest in the United States. Researchers often use pheromone traps to monitor for the presence of economically important noctuid pests. Pheromone traps may attract more than one species and samples often degrade, making morphological identification of some adult noctuid species nearly impossible. A molecular diagnostics protocol using polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) was developed in order to distinguish the fall armyworm from six other noctuid species commonly found in Arkansas. A 611-bp region of the mtDNA COI, COII genes was amplified using PCR and then sequenced. The restriction enzymes *Dra* I, *Alu* I and *Nla* III had specific restriction sites that distinguished the seven noctuid species. This proved to be a reliable, quick and economical technique for identifying the fall armyworm as well as six other noctuid species.

KEY WORDS Fall armyworm, Noctuidae, PCR-RFLP, molecular diagnostics

Noctuid moths, including the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), are well-known agricultural pests of economical importance. Fall armyworms are known to attack at least 60 varieties of crops, most notably corn, rice, peanuts, cotton, soybeans, alfalfa and forage grasses (Knipling 1980, Pashley 1986, Lu & Adang 1996). In Arkansas, estimates of cotton losses alone from fall armyworm damage were $2.7 million for insecticide treatments in 2003 and $2.6 million in cotton bale losses (Williams 2003). On the national level, the FAW ranked 8th as the most important insect pest of cotton in 2003 and it was the 3rd most important pest to cotton in Arkansas (Williams 2003). The preferred host plants of FAW came under scrutiny in 1986 when Pashley proposed that the fall armyworm consists of two morphologically undistinguishable strains, a corn strain that prefers corn, cotton and sorghum, and a rice strain that prefers rice and bermudagrass (Pashley 1986, 1988). Recent studies on *S. frugiperda* genetic variation have revealed that populations can be divided two morphologically identical, but genetically distinct strains using mtDNA cytochrome oxidase sequences (Lewter et al. 2006, Nagoshi et al. 2007).

Pheromone traps are a very easy and popular method for collecting adult male noctuid moths in areas where it is prudent to monitor for destructive insects.

[1]Received 18 February 2008; Accepted 6 May 2008.
Dept. of Entomology, University of Arkansas, 319 Agriculture Building, Fayetteville, Arkansas 72701, Phone: (479) 575-4342, Fax: (479) 575-2452, E-mail: aszalan@uark.edu

Although it is relatively easy to use pheromone traps, the insects captured in this manner can survive for several days and adult moths typically remove a large portion of their scales while fluttering around inside the metal traps. Unless a pheromone trap is emptied on a daily basis, the moths trapped inside may become difficult to identify to species. FAW and yellow striped armyworm, *S. ornithogalli* (Guenee), species are particularly difficult to distinguish, even with properly preserved specimens. A molecular diagnostics protocol may be very useful in distinguishing the fall armyworm from other noctuids commonly found in northwest Arkansas, particularly when dealing with specimens that are in poor condition.

The polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) technique offers a very affordable and accurate method for the identification of insect species, and it can be used on the insect during any developmental stage from egg to adult (Taylor & Szalanski 1999). The technique can verify proper identification of moths before subjection to costly DNA sequencing analysis. Although PCR-RFLP has already been used on the fall armyworm (Levy et al. 2002), Levy's research was focused on distinguishing between the two strains of FAW. The objective of this study was to apply PCR-RFLP to distinguish the fall armyworm from other noctuid moths.

Materials and Methods

Adult and larval FAW, beet armyworm *S. exigua* (Hubner), yellow striped armyworm *S. ornithogalli* (Guenee), true armyworm *Mythimna unipuncta* (Halworth), corn earworm *Helicoverpa zea* (Brodie), soybean looper *Pseudoplusia includens* (Walker), bent-line dart *Choephora fungorum* (Grote and Robinson) and tobacco budworm *Heliothis virescens* (F.) were obtained from lab colonies maintained at the University of Arkansas, lab colonies at the University of Mississippi, lab colonies from the USDA-ARS CMAVE lab in Gainesville, FL and from pheromone traps operated at the University of Arkansas Agricultural Research Farm (Table 1). Larval species identification was confirmed with morphological keys of Peterson (1962), and voucher specimens have been deposited in the Arthropods Museum, University of Arkansas, Fayetteville, AR.

DNA was extracted from the thorax using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). PCR was conducted using 2 µl of extracted genomic DNA and the primers C1-J-2797 (5′- CCTCGACGTTATTCAGATTACC-3′) (Simon et al. 1994) and C2-N-3400 (5′-TCAATATCATTGATGACCAAT-3′) (Taylor et al. 1997) with the following thermalcycler profile: 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 45 s per Szalanski et al (2000). These primers amplify a 3′ portion of COI, tRNA-leu and a 5′ portion of COII. The mitochondrial DNA amplicon (PCR product) was sequenced at the University of Arkansas Medical School DNA Sequencing Facility in Little Rock, AR. Accession numbers for DNA sequences submitted to GenBank from this study are provided in Table 1, as well as accession numbers from Lewter et al. (2006).

The DNA sequence data were used to predict restriction sites with Neb Cutter 2.0 (www.tools.neb.com/NEBcutter2). The digest was performed using the protocol outlined by Cherry et al. (1997) using 3 µl of the PCR product and the restriction enzymes *Dra I*, *Alu I* and *Nla III*. An overnight digest was performed at 37°C for each of the enzymes, and the fragments were visualized using

Table 1. Noctuid moths used in polymerase chain reaction - restriction fragment length polymorphism analysis (PCR-RFLP).

Species/strain	number (n)	GenBank
S. frugiperda corn 1 haplotype	32	AY714298
S. frugiperda corn 2 haplotype	2	AY714299
S. frugiperda corn 3 haplotype	1	AY714300
S. frugiperda rice 1 haplotype	4	AY714301
S. frugiperda rice 2 haplotype	29	AY714302
S. frugiperda rice 3 haplotype	1	AY714303
S. frugiperda rice 4 haplotype	1	AY714304
S. ornithogalli	15	EU918931
S. exigua	25	EU812749-EU812751
Mythimna unipuncta	5	EU918926
Choephora fungorum	5	EU918927
Helicoverpa virescens	5	EU918928
Heliothis zea	5	EU918929
Pseudoplusia includens	5	EU918930

electrophoresis with a 2% agarose gel stained with ethidium bromide and a 50 base pair DNA size marker. The agarose gel was photographed in a UVP BioDoc-it system (Upland, CA).

Results and Discussion

The mtDNA region amplified by PCR was 611 base pairs long. A total of four rice and three corn strain haplotypes were observed for *S. frugiperda* (Lewter et al. 2006). No genetic variation was observed for the other species used for the study, with the exception of *S. exigua* which had three distinct haplotypes. The *Alu* I digest distinguished 5 patterns among the seven noctuid moths (Table 2, Fig. 1). The *Dra* I digest yielded 4 different patterns (Table 3, Fig. 2), and the *Nla*

Table 2. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Alu* I.

Species / Strain	Restriction site	Fragments	Pattern
*Spodoptera frugiperda**	182, 420	238, 191, 182	A
S. frugiperda Rice 3 haplotype	182	429, 182	B
S. ornithogalli	182	429, 182	B
S. exigua	182	429, 182	B
Mythimna unipuncta	71, 182, 496	314, 115, 111, 71	C
Choephora fungorum	71, 182, 328	280, 146, 111, 71	D
Helicoverpa virescens	71	540, 71	E
Heliothis zea	71	540, 71	E
Pesudoplusia includens	182	429, 182	B

*All FAW haplotypes from Lewter et al. (2006) except for haplotype Rice 3.

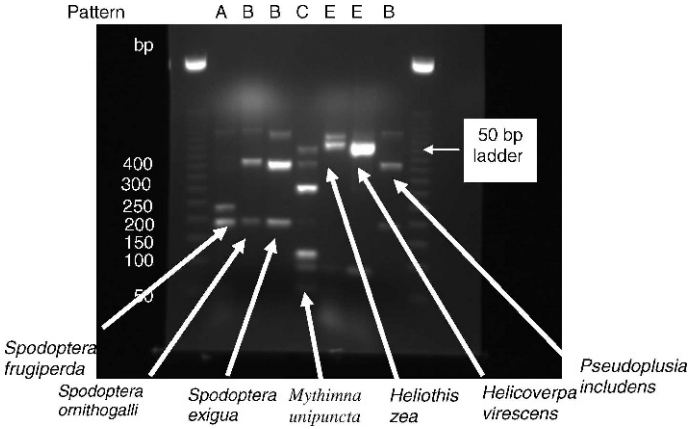

Fig. 1. PCR-RFLP agarose gel for fall armyworm and other noctuids using *Alu* I.

III digest yielded 3 patterns (Table 3). By combining the patterns from all 3 digests, each of the seven species of noctuids were distinguished from one another (Tables 4 and 5) as well as the two FAW strains. The rice R3 haplotype of the fall armyworm could also be distinguished.

This is the first PCR-RFLP technique to distinguish the fall armyworm from other noctuid species commonly found in Arkansas. This technique is quick, reliable and costs less than two dollars per sample. It is also particularly useful when trying to identify adults that have been caught in pheromone traps, where specimens often have a large portion of their scales missing.

One advantage to using the COI, COII mtDNA region for the PCR-RFLP technique is that the DNA fragments created by cleaving this area are relatively large and clearly separate during electrophoresis. It is not necessary to use high percentages of agarose gels in order to detect size differences in the cleaved DNA, so this could be cost-efficient when dealing with large numbers of samples (Roehrdanz 1997). The COI, COII region is also a useful PCR-RFLP marker because it is not as hypervariable as some noncoding regions are (ie, nuclear

Table 3. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Dra* I.

Species	Restriction site	Fragments	Pattern
*Spodoptera frugiperda**	138, 252	359, 138, 114	A
S. ornithogalli	138, 252	359, 138, 114	A
S. exigua	252	359, 252	B
Mythimna unipuncta	252, 578	326, 252, 33	C
Choephora fungorum	138, 252	359, 138, 114	A
Helicoverpa virescens	252	359, 252	B
Heliothis zea	138, 252	359, 138, 114	A
Pseudoplusia includens	252, 309	299, 252, 57	D

*All *S. frugiperda* haplotypes from Lewter et al. (2006).

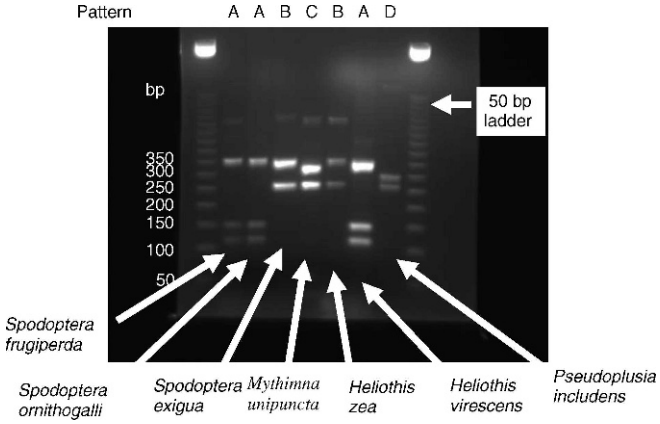

Fig. 2. PCR-RFLP agarose gel for fall armyworm and other noctuids using *Dra* I.

rDNA intergenic spacer region.) When intraspecific polymorphisms are at a minimum, there is a lesser likelihood that the restriction enzymes will cleave DNA at the same place in different species. In other words, the patterns designated to each species are truly species-specific.

Another major advantage of the PCR-RFLP technique is that there are no false negatives; the restriction patterns are based on DNA sequences and are specific (Roehrdanz 1997). Also, results can be obtained from PCR-RFLP within one single working day. Other researchers have published RFLP protocols for use on the fall armyworm, but they have been aimed at distinguishing between the two strains of the fall armyworm and not at distinguishing the FAW from other noctuids. The *Nla* III digest is able to identify the R3 haplotype from the other six FAW haplotypes that we have described. This technique is useful for identifying samples that will undergo DNA sequencing and it works as well at identifying the six other noctuid species as it does for the FAW.

Table 4. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Nla* III.

Species / Strain	Restriction site	Fragments	Pattern
Spodoptera frugiperda corn*	31, 301, 362	270, 246, 61, 31	A
S. frugiperda rice*	31, 301	307, 270, 31	B
S. ornithogalli	31, 301, 362	270, 246, 61, 31	A
S. exigua	31, 301, 362	270, 246, 61, 31	A
Mythimna unipuncta	31, 301, 362	270, 246, 61, 31	A
Choephora fungorum	301, 362	307, 270, 31	B
Helicoverpa virescens	301, 362	307, 270, 31	B
Heliothis zea	301	307, 301	C
Pseudoplusia includens	31, 301, 362	270, 246, 61, 31	A

*All *S. frugiperda* haplotypes from Lewter et al. (2006).

Table 5. Patterns for noctuid PCR-RFLP using *Alu* I, *Dra* I, *Nla* III.

Species / Strain	Pattern*
Spodoptera frugiperda corn 1,2,3 haplotypes	AAA
S. frugiperda rice 1,2 haplotypes	AAB
S. frugiperda rice 3 haplotype	BAB
S. ornithogalli	BAA
S. exigua	BBA
Mythimna unipuncta	CCA
Choephora fungorum	DAB
Heliocoverpa virescens	EBB
Heliothis zea	EAC
Pseudoplusia includens	BDA

**Alu* I, *Dra* I, *Nla* III digest patterns.

Acknowledgment

We thank C. Allen, R. Bagwell, D. Parker, R. N. Nagoshi, R. L. Meagher, and R. G. Luttrell for providing samples. Research was supported in part by a University of Arkansas, Agricultural Experiment Station Research Initiation Grant to ALS.

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Molecular Identification of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Using PCR-RFLP[1]

Jennifer A. Lewter and Allen L. Szalanski

J. Agric. Urban Entomol. 24(2): 51–57 (April 2007)

ABSTRACT The fall armyworm, *Spodoptera frugiperda*, as with many noctuid moths, is a serious agricultural pest in the United States. Researchers often use pheromone traps to monitor for the presence of economically important noctuid pests. Pheromone traps may attract more than one species and samples often degrade, making morphological identification of some adult noctuid species nearly impossible. A molecular diagnostics protocol using polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) was developed in order to distinguish the fall armyworm from six other noctuid species commonly found in Arkansas. A 611-bp region of the mtDNA COI, COII genes was amplified using PCR and then sequenced. The restriction enzymes *Dra* I, *Alu* I and *Nla* III had specific restriction sites that distinguished the seven noctuid species. This proved to be a reliable, quick and economical technique for identifying the fall armyworm as well as six other noctuid species.

KEY WORDS Fall armyworm, Noctuidae, PCR-RFLP, molecular diagnostics

Noctuid moths, including the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), are well-known agricultural pests of economical importance. Fall armyworms are known to attack at least 60 varieties of crops, most notably corn, rice, peanuts, cotton, soybeans, alfalfa and forage grasses (Knipling 1980, Pashley 1986, Lu & Adang 1996). In Arkansas, estimates of cotton losses alone from fall armyworm damage were $2.7 million for insecticide treatments in 2003 and $2.6 million in cotton bale losses (Williams 2003). On the national level, the FAW ranked 8th as the most important insect pest of cotton in 2003 and it was the 3rd most important pest to cotton in Arkansas (Williams 2003). The preferred host plants of FAW came under scrutiny in 1986 when Pashley proposed that the fall armyworm consists of two morphologically undistinguishable strains, a corn strain that prefers corn, cotton and sorghum, and a rice strain that prefers rice and bermudagrass (Pashley 1986, 1988). Recent studies on *S. frugiperda* genetic variation have revealed that populations can be divided two morphologically identical, but genetically distinct strains using mtDNA cytochrome oxidase sequences (Lewter et al. 2006, Nagoshi et al. 2007).

Pheromone traps are a very easy and popular method for collecting adult male noctuid moths in areas where it is prudent to monitor for destructive insects.

[1]Received 18 February 2008; Accepted 6 May 2008.
Dept. of Entomology, University of Arkansas, 319 Agriculture Building, Fayetteville, Arkansas 72701, Phone: (479) 575-4342, Fax: (479) 575-2452, E-mail: aszalan@uark.edu

Diallel Analysis of Corn Earworm (Lepidoptera: Noctuidae) Resistance in Maize[1]

Gerald A. Matthews, W. Paul Williams, and Christopher A. Daves[a]

J. Agric. Urban Entomol. 24(2): 59–66 (April 2007)

ABSTRACT Corn earworm, *Helicoverpa zea* Boddie, is a major pest of maize in the United States. Host plant resistance is widely considered a desirable method for reducing losses to this pest. Nine inbred lines were evaluated for resistance to ear damage, larval survival, and weight of larvae. Mp313E and Mp717 sustained less damage than the other lines, and larval survival and growth on these lines was reduced. Analysis of a diallel cross among the nine lines indicated that both general combining ability (GCA) and specific combining ability (SCA) were significant sources of variation in the inheritance of resistance to ear damage. GCA was also a significant source of variation in the inheritance of resistance to larval growth. GCA effects for reduced larval weight were significant for Mp313E and Mp717. These lines could be useful in the development of maize hybrids with corn earworm resistance.

KEY WORDS *Helicoverpa zea*, Lepidoptera, Noctuidae, corn earworm, diallel cross, host plant resistance, maize, *Zea mays*

Corn earworm, *Helicoverpa zea* Boddie, is a major pest of maize, *Zea mays* L., in the United States. Moths lay eggs on fresh silks where the neonates initiate feeding before moving into the ear where they feed on the developing kernels. Because corn earworm larvae feed primarily within the husks, they are difficult to control with insecticides (Lynch et al. 1999). The limited effectiveness of insecticides as well as the cost and potentially adverse environmental impacts associated with their use make host plant resistance an attractive alternative for controlling corn earworm.

Maize germplasm with genetic resistance to corn earworm has been identified and released (Wiseman & Widstrom 1992, Widstrom & Snook 2001). Tight husks and high maysin content in the silks have been linked to corn earworm resistance (Wiseman et al. 1992, Archer et al. 1994, Rector et al. 2002). Transgenic maize hybrids expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have also exhibited resistance to corn earworm damage (Chilcutt et al. 2006, Ni et al. 2007). The expression of this resistance and its effectiveness in reducing corn earworm damage has, however, varied across environments.

Development of corn earworm populations with resistance to the Bt insecticidal proteins is another concern (Gahan et al. 2001). Combining genetic

[1]Received 18 February 2008; Accepted for publication 14 June 2008.
USDA-ARS Corn Host Plant Resistance Research Unit, Box 9555, Mississippi State, Mississippi 39762, Phone: 662-325-2771, FAX: 662-325-8441, E-mail: Boo.Matthews@ars.usda.gov
[a]Current address: Central Mississippi Research and Extension Center, 1320 Seven Springs Road, Raymond, MS 39154.

resistance to corn earworm with resistance from Bt insecticidal proteins could prevent or at least delay the development of corn earworm populations with resistance to Bt. Multiple sources and different mechanisms of resistance should be most effective in preventing the buildup of resistance to Bt.

This investigation was undertaken to evaluate nine inbred lines for resistance to corn earworm. Some of these lines exhibit resistance to southwestern corn borer, *Diatraea grandiosella* Dyar, and fall armyworm, *Spodoptera frugiperda* (J.E. Smith). A second objective was to evaluate a diallel set of crosses among the nine lines for resistance to corn earworm damage and to determine the importance of general combining ability (GCA) and specific combining ability (SCA) in the inheritance of resistance.

Materials and Methods

Nine maize inbred lines were planted at the R.R. Foil Plant Science Research Center, Mississippi State, Mississippi on 18 April 2003, 23 April 2004, and 25 April 2005. Four of the inbred lines, Mp496, Mp707, Mp714, and Mp716, were developed and released as sources of resistance to leaf feeding by fall armyworm and southwestern corn borer (Scott & Davis 1981, Williams & Davis 1984, 2000, 2002). Other inbred lines included Mp305, Mp313E, Mp339, Mp420, and Mp717 were developed and released for traits other than insect resistance. A diallel cross of 36 F_1 hybrids produced from crossing the nine inbred lines was planted in adjacent field plots on the same dates. The inbred lines and F_1 hybrids were planted as separate experiments because of differences in growth and vigor.

The inbred lines or F_1 hybrids were assigned to single-row plots that were 4 m long, spaced 0.97 m apart, and thinned to 20 plants. Within each experiment, plots were arranged in a randomized complete block design with three replications. Standard production practices for the area were followed. No insecticides were applied.

Seven days after silks emerged from 50% of the plants in a plot, the primary ear of each plant was infested with 30 corn earworm larvae. Fifteen neonates each were placed on the silks and in the leaf axil of the primary ear (Davis & Williams 1994) using a portable plastic dispensing device (Mihm 1981). Corn earworm larvae were obtained from a colony maintained by USDA-ARS at the Mississippi State Crop Science Research Center. Ten days after infestation, the primary ear was removed from five plants per plot. Corn earworm larvae recovered from each ear were counted and weighed. Twenty-one days after infestation, the ears from 10 additional plants in each plot were removed. Damage was measured as centimeters of penetration into the ear using the revised scale developed by Widstrom (1967).

Plot means were calculated for each character for both the parental inbred lines and the F_1 hybrids. Data were analyzed using SAS (SAS Institute, Cary, NC). The data for the inbred lines were analyzed using the SAS general linear models (GLM) procedure. Means were compared using Fisher's Protected Least Significant Difference (LSD) at $P = 0.05$ (Steel and Torrie 1980). Data for the F_1 hybrids were analyzed using the DIALLEL-SAS procedure developed by Zhang & Kang (1997, 2003), according to Griffing's (1956) Method 4, Model 1, which includes F_1 progeny, but neither parents nor reciprocals. Variation among hybrids was partitioned into GCA and SCA, and the GCA and SCA effects were calculated. Hybrids and years were considered fixed effects.

Table 1. Corn earworm damage and larval survival and growth on maize inbred lines grown at Mississippi State, MS in 2003, 2004, and 2005.

Inbred Line	Ear damage[a]	Larval no.[b]	Larval wt. (g)[c]
Mp339	9.0 ± 1.9	1.2 ± 0.8	110 ± 97
Mp714	6.7 ± 1.8	1.0 ± 0.5	155 ± 110
Mp420	6.6 ± 1.2	0.7 ± 0.5	198 ± 187
Mp496	5.6 ± 1.8	0.7 ± 0.5	148 ± 137
Mp716	5.4 ± 0.6	0.9 ± 0.3	150 ±116
Mp305	5.1 ± 1.5	1.4 ± 1.0	135 ± 103
Mp707	4.8 ± 1.7	0.6 ± 0.5	44 ± 62
Mp313E	4.4 ± 1.7	0.2 ± 0.2	32 ± 77
Mp717	4.3 ± 1.2	0.4 ± 0.3	43 ± 84
$LSD_{(0.05)}$	2.0	0.5	108
$F_{8,35df}$	4.25	—	—
$F_{8,68df}$	—	4.79	3.82
P	0.01	0.01	0.01

[a]Ear damage ratings, based on depth (cm) of kernel penetration, were rated 21 days after infestation with 30 corn earworm larvae.
[b]Mean number of larvae recovered from the top an ear 10 days after infestation with corn earworm larvae.
[c]Mean weight of larvae recovered from an ear 10 days after infestation.

Results

The analysis of variance for the inbred lines indicated significant differences among lines for ear damage, number of surviving larvae, and mean larval weight. Years was a significant ($P < 0.05$) source of variation for only number of larvae. The interactions of years and inbred lines were not significant for any of the three traits. Among the inbred lines, Mp339 sustained the heaviest ear damage (Table 1). Mp714 and Mp420 sustained the second highest levels of ear damage, but did not differ significantly from four other lines. Mp717 and Mp313E sustained the least damage. The greatest number of larvae were recovered from Mp305 and Mp339. The fewest larvae were recovered from Mp313E and Mp717 with only 0.2 larvae per plant recovered from Mp313E. The mean weight of larvae recovered from Mp420 was 198 g while the mean weight of larvae recovered from Mp313E was only 32 g.

Heavy winds in 2005 caused severe lodging of the F_1 plants so data from only 2003 and 2004 were available for analysis. Differences between years were significant for ear damage and larval weight, but not number of surviving larvae (Table 2). Likewise, differences for hybrids were significant only for ear damage and mean larval weight. The interaction of years and hybrids was significant for ear damage only. Both GCA and SCA were highly significant ($P < 0.01$) for ear damage, but only GCA was significant ($P < 0.01$) for mean larval weight. Ear damage for the F_1 hybrids varied from a high of 10.1 for Mp313E × Mp707 to a low of 3.6 for Mp305 × Mp707 (Table 3). Mean larval weights varied from 140 g for Mp420 × Mp496 to 15 g for Mp714 × Mp717.

Table 2. Analysis of variance of ear damage, number of surviving larvae, and larval weights for a diallel cross of maize infested with corn earworm larvae evaluated at Mississippi State, MS in 2003 and 2004.

		Mean square		
Source	df	Ear damage	Larval no.	Larval wt.
Years	1	466.63**	0.54	350659**
Replications (years)	4	2.30	0.36	1653
Hybrids	35	8.62**	0.17	7196*
GCA	8	5.04**	0.34	12036**
SCA	27	9.69**	0.13	5762
Years × hybrids	35	9.75**	0.17	4182
GCA × years	8	10.70**	0.29	7079
SCA × years	27	9.47**	0.14	3324
Error	140	1.05	0.20	4061

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.

Estimates of GCA effects associated with ear damage ratings were significant and negative for Mp339, Mp420, and Mp496 (Table 4). GCA effects estimated for Mp707, Mp313E, and Mp717 were significant and positive. Estimates of GCA effects for mean larval weights were significant and negative for Mp313E and Mp717 and significant and positive for Mp420. SCA effects for ear damage ratings were significant for 16 of the F_1 hybrids (Table 3). Ten of these were negative indicating that these hybrids sustained less ear damage than expected based on estimates of GCA effects for these lines.

Discussion

Ear damage ratings are indicative of the level of injury inflicted on the developing ear by corn earworm larvae. The number of larvae recovered and the mean weight of larvae reflect the plant's effect on larvae. The results of the evaluation of inbred lines indicated that Mp717, Mp313E, and Mp707 sustained the lowest levels of damage (Table 1). Fewer larvae with lower mean weights were also recovered from these lines. It appears that these lines adversely affected larval survival and growth resulting in reduced damage to the ears.

The performance of the lines in crosses is indicated by estimates of SCA and GCA effects is not as easily explained (Tables 3, 4). Estimates of GCA effects for Mp717, Mp313E, and Mp707 were significant and positive indicating that the single cross hybrids with these lines tended to sustain heavier ear damage than other hybrids (Table 4). The F_1 hybrid Mp313E × Mp707, with a rating of 10.1 exhibited the highest level of ear damage and highly significant SCA effect for increased ear damage (Table 3). The estimated SCA effect for Mp339, which sustained the heaviest ear damage as an inbred, was significant and negative as were the SCA effects for Mp420 and Mp496.

Table 3. Corn earworm damage and associated SCA effects and weights of larvae recovered from a diallel set of crosses among nine inbred lines grown at Mississippi State, MS in 2003 and 2004.

	Ear damage[a]		
Single cross	Rating	SCA effect	Larval wt. (g)[b]
Mp305 × Mp313E	7.0 ± 2.7	0.36	21 ± 18
Mp305 × Mp339	7.0 ± 2.4	1.30**	82 ± 67
Mp305 × Mp420	6.8 ± 3.9	1.19**	94 ± 97
Mp305 × Mp496	5.8 ± 1.8	0.06	127 ± 125
Mp305 × Mp707	3.6 ± 1.0	−2.80**	50 ± 56
Mp305 × Mp714	4.7 ± 1.4	−1.38**	128 ± 103
Mp305 × Mp716	7.3 ± 3.8	1.26**	61 ± 60
Mp305 × Mp717	6.4 ± 2.9	0.02	69 ± 116
Mp313E × Mp339	4.7 ± 0.6	−1.64**	49 ± 82
Mp313E × Mp420	5.9 ± 0.7	−0.39	30 ± 25
Mp313E × Mp496	5.1 ± 1.9	−1.24**	59 ± 37
Mp313E × Mp707	10.1 ± 5.5	3.08**	31 ± 30
Mp313E × Mp714	7.8 ± 3.8	1.18**	79 ± 97
Mp313E × Mp716	5.6 ± 2.3	−1.07**	26 ± 18
Mp313E × Mp717	6.7 ± 2.5	−0.27	89 ± 101
Mp339 × Mp420	5.0 ± 1.3	−0.33	120 ± 148
Mp339 × Mp496	4.1 ± 0.9	−1.31**	118 ± 98
Mp339 × Mp707	6.3 ± 1.5	0.15	56 ± 59
Mp339 × Mp714	6.4 ± 1.1	0.65	44 ± 49
Mp339 × Mp716	5.3 ± 1.6	−0.38	63 ± 72
Mp339 × Mp717	7.7 ± 2.1	0.73*	39 ± 34
Mp420 × Mp496	6.1 ± 2.2	−0.57	140 ± 130
Mp420 × Mp707	5.5 ± 1.8	−0.07	135 ± 98
Mp420 × Mp714	5.6 ± 1.3	−0.07	82 ± 58
Mp420 × Mp716	4.8 ± 1.0	−0.85*	80 ± 83
Mp420 × Mp717	6.3 ± 1.9	0.28	25 ± 25
Mp496 × Mp707	5.7 ± 2.0	−0.39	63 ± 72
Mp496 × Mp714	5.7 ± 1.4	−0.07	67 ± 79
Mp496 × Mp716	7.6 ± 4.3	−1.90**	32 ± 25
Mp496 × Mp717	6.4 ± 2.4	0.32	32 ± 22
Mp707 × Mp714	7.3 ± 1.7	0.84*	63 ± 106
Mp707 × Mp716	6.5 ± 1.6	0.13	80 ± 80
Mp707 × Mp717	6.3 ± 1.7	−0.44	36 ± 63
Mp714 × Mp716	5.7 ± 0.6	−0.33	80 ± 82
Mp714 × Mp717	5.6 ± 0.7	−0.83*	15 ± 17
Mp716 × Mp717	5.7 ± 2.6	−0.64	48 ± 36
$LSD_{(0.05)}$	1.2		73

[a]Ear damage as indicated by depth (cm) of kernel penetration was rated 21 days after infestation with 30 corn earworm larvae.
[b]Mean weight of larvae recovered from an ear 10 days after infestation.
*Significantly different from 0 at $P < 0.05$.
**Significantly different from 0 at $P < 0.01$.

Table 4. Estimates of GCA effects for corn earworm damage and larval weights on a diallel cross evaluated at Mississippi State, MS in 2003 and 2004.

Inbred line	Ear damage[a]	Larval No.[b]
Mp339	−0.33*	5
Mp714	−0.03	3
Mp420	−0.42**	24**
Mp496	−0.35*	15
Mp716	−0.05	−9
Mp305	−0.04	14
Mp707	0.33*	−3
Mp313E	0.58**	−22*
Mp717	0.32*	−26*

*Significantly different from 0 at $P < 0.05$.
**Significantly different from 0 at $P < 0.01$.

Although differences among hybrids for number of surviving larvae were not significant, differences among hybrids for larval weight were significant (Table 2). Performance of inbred lines per se and in hybrid combinations were more consistent for mean larval weights than for ear damage. Mean larval weights were lowest for the inbred lines Mp313E and Mp717. Estimated GCA effects were significant and negative for these lines indicating that their resistance to larval growth was expressed not only in the inbreds, but also in their hybrids.

Neither Mp313E nor Mp717 was selected for resistance to insect damage: both inbred lines were developed and released as sources of resistance to infection by *Aspergillus flavus* Link ex Fries and the subsequent accumulation of aflatoxin (Scott & Zummo 1990, Williams & Windham 2006). It is possible that the resistance they exhibited to corn earworm may account, at least in part, for their resistance to *A. flavus* infection and the accumulation of aflatoxin. It is also possible that characteristics of the ear that impede fungal infection also impede establishment of corn earworm. Another line included in this investigation, Mp420, was also developed as a source of resistance to *A. flavus* infection and aflatoxin; however, its level of resistance is generally lower than that of Mp313E and Mp717 (Scott & Zummo 1992, Williams 2006, Gardner et al. 2007).

The nine inbred lines differed in ear damage as well as number of surviving larvae and mean larval weight. The inbred lines Mp313E and Mp717, which had been developed and released as sources of resistance to *A. flavus* infection and aflatoxin accumulation, exhibited the highest levels of resistance to corn earworm as inbred lines. This was reflected in the amount of ear damage and number and weight of surviving larvae. The significant GCA effects associated with reduced larval weights for these lines indicate that they could be useful in developing maize hybrids with resistance to corn earworm although the GCA effects associated with higher levels of ear damage are contradictory. Additional evaluations of these lines in crosses with other lines should provide better evidence of their value in developing corn earworm resistant maize hybrids.

Acknowledgment

This paper is a joint contribution of USDA-ARS and the Mississippi Agricultural and Forestry Experiment Station and is published as journal no. J-11242 of the Miss. Agric. and For. Exp. Stn. The research reported herein was conducted in partial fulfillment of the requirement for the degree of M.S. in Genetics by the senior author. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA.

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Diallel Analysis of Corn Earworm (Lepidoptera: Noctuidae) Resistance in Maize[1]

Gerald A. Matthews, W. Paul Williams, and Christopher A. Daves[a]

J. Agric. Urban Entomol. 24(2): 59–66 (April 2007)

ABSTRACT Corn earworm, *Helicoverpa zea* Boddie, is a major pest of maize in the United States. Host plant resistance is widely considered a desirable method for reducing losses to this pest. Nine inbred lines were evaluated for resistance to ear damage, larval survival, and weight of larvae. Mp313E and Mp717 sustained less damage than the other lines, and larval survival and growth on these lines was reduced. Analysis of a diallel cross among the nine lines indicated that both general combining ability (GCA) and specific combining ability (SCA) were significant sources of variation in the inheritance of resistance to ear damage. GCA was also a significant source of variation in the inheritance of resistance to larval growth. GCA effects for reduced larval weight were significant for Mp313E and Mp717. These lines could be useful in the development of maize hybrids with corn earworm resistance.

KEY WORDS *Helicoverpa zea*, Lepidoptera, Noctuidae, corn earworm, diallel cross, host plant resistance, maize, *Zea mays*

Corn earworm, *Helicoverpa zea* Boddie, is a major pest of maize, *Zea mays* L., in the United States. Moths lay eggs on fresh silks where the neonates initiate feeding before moving into the ear where they feed on the developing kernels. Because corn earworm larvae feed primarily within the husks, they are difficult to control with insecticides (Lynch et al. 1999). The limited effectiveness of insecticides as well as the cost and potentially adverse environmental impacts associated with their use make host plant resistance an attractive alternative for controlling corn earworm.

Maize germplasm with genetic resistance to corn earworm has been identified and released (Wiseman & Widstrom 1992, Widstrom & Snook 2001). Tight husks and high maysin content in the silks have been linked to corn earworm resistance (Wiseman et al. 1992, Archer et al. 1994, Rector et al. 2002). Transgenic maize hybrids expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have also exhibited resistance to corn earworm damage (Chilcutt et al. 2006, Ni et al. 2007). The expression of this resistance and its effectiveness in reducing corn earworm damage has, however, varied across environments.

Development of corn earworm populations with resistance to the Bt insecticidal proteins is another concern (Gahan et al. 2001). Combining genetic

[1]Received 18 February 2008; Accepted for publication 14 June 2008.
USDA-ARS Corn Host Plant Resistance Research Unit, Box 9555, Mississippi State, Mississippi 39762, Phone: 662-325-2771, FAX: 662-325-8441, E-mail: Boo.Matthews@ars.usda.gov
[a]Current address: Central Mississippi Research and Extension Center, 1320 Seven Springs Road, Raymond, MS 39154.

Sustained Mass Release of Pupal Parasitoids (Hymenoptera: Pteromalidae) for Control of *Hydrotaea aenescens* and *Musca domestica* (Diptera: Muscidae) in Broiler-Breeder Poultry Houses in Arkansas[1]

Tanja McKay,[2] C. Dayton Steelman,[3] Sheri M. Brazil,[3] and Allen L. Szalanski[3]

J. Agric. Urban Entomol. 24(2): 67–85 (April 2007)

ABSRACT *Muscidifurax zaraptor* Kogan and Legner, *M. raptorellus* Kogan and Legner, and *Spalangia cameroni* Perkins were released bi-weekly in two facilities at a broiler-breeder egg production farm in Arkansas during 2003 and 2004. Of the recovered house fly, *Musca domestica* L., sentinel pupae, 18.8% were parasitized in 2003, with *M. zaraptor* being the dominant species (66.8%) and *M. raptorellus* contributing 6.9% of the parasitism. The release of *M. raptorellus* did not result in substantial parasitism in sentinel house fly pupae until the second year of study when *M. raptorellus* was the most dominant species, contributing approximately 61.9% parasitism. At the non-release farm, 13.9% of the sentinel house fly pupae were parasitized, with *S. cameroni* and *M. zaraptor* dominating in 2003. Parasitism at the control farm decreased to 3.4% in 2004. It appeared that sustained releases of parasitoids at the release farm over two years provided a significant increase in house fly pupal parasitism when compared to the percentage of pupae parasitized at the non-release farm. Of the sentinel *Hydrotaea aenescens* (Wiedemann) pupae recovered from the release farm, 9.3% were parasitized in 2003. Filth fly data indicated that the combined predator activity of *H. aenescens* and sustained parasitoid releases reduced *M. domestica* to a level well below the treatment threshold. In addition, the sustained release of parasitoids reduced *H. aenescens* numbers to below the treatment threshold of 100 filth flies per sticky ribbon per week by 9 wk during the latter part of the filth fly season during 2003 and 2004.

KEY WORDS Parasitoids, *Musca domestica*, house fly, *Hydrotaea aenescens*, biological control, poultry

The house fly, *Musca domestica* (L.) and *Hydrotaea aenescens* (Wiedemann) (formerly *Ophyra aenescens*) (Diptera: Muscidae) are common filth flies found in broiler-breeder houses (Davis 1997, Axtell 1999). The house fly is the primary target of most filth fly management programs (Wilhoit et al. 1991). When present in large numbers, house flies not only annoy employees working in these facilities (Thomas & Skoda 1993), but can also reduce aesthetics by leaving fecal and regurgitation spots on structures, light fixtures, and eggs (Axtell 1999). Best

[1]Received 18 January 2008; Accepted 8 May 2008.
Dr. C. Dayton Steelman, Dept. of Entomology, Rm 320, Agric. Bldg., University of Arkansas, Fayetteville, AR, 72701, Phone: (479) 575-2510, Fax: (479) 575-2452, E-mail: dsteelm@uark.edu
[2]Department of Biological Sciences, Arkansas State University – Jonesboro, Arkansas USA.
[3]Department of Entomology, University of Arkansas, Fayetteville, Arkansas USA.

known for the transmission of many organisms that cause disease in humans, livestock, and poultry (Greenburg 1971), house flies have been reported to disperse into the environment, resulting in lawsuits as urban encroachment expands into rural areas (Thomas & Skoda 1993). Excessive numbers of *H. aenescens* can be as annoying as house flies (Axtell 1986), and can become problematic to neighbors living close to broiler-breeder facilities (Axtell 1999). *Hydrotaea aenescens* is not always considered a pest. The larvae of *H. aenescens* are predators and have been used to control house flies in the United States and in various parts of Europe (Nolan & Kissam 1985, Turner et al. 1992, Hogsette & Jacobs 1999).

There has been considerable interest in using hymenopterous parasitoids to control house flies associated with poultry manure throughout the United States (Olton & Legner 1975, Rutz & Axtell 1979, 1981, Morgan & Patterson 1990). However, there has been little research on the pteromalids associated with filth flies associated with poultry production in Arkansas. Yust (1980) studied the parasitoid fauna of filth flies occurring in caged-layer houses in northwest Arkansas and also evaluated the efficacy of releasing *Spalangia endius* Walker and *Pachycrepoideus vindemiae* (Rondani). Not all parasitoids in that study were identified to species and accurate rates of released parasitoids were not calculated. Dry et al. (2007) examined the occurrence and seasonal distribution of parasitoids in broiler-breeder poultry houses in Arkansas. However, the producers at some of the facilities used in that study released *Spalangia* spp. and *Muscidifurax* spp. without accurate release rate estimates.

With no accurate data on the efficacy of releasing parasitoids in broiler-breeder houses in Arkansas, the objective of our study was to evaluate the effectiveness of sustained mass releases of parasitoids to control *H. aenescens* and *M. domestica* in broiler-breeder facilities.

Materials and Methods

Two broiler-breeder farms, where no parasitoids had previously been released, were selected for this study. One farm, used for the release of commercially available parasitoids, was ≈5.0 km southeast of Mountainburg, Crawford County, AR. This farm had two separately enclosed houses, attached to one another by a common egg room. The second farm, located ≈10 km south of Hackett, Sebastian County, AR, was used as a non-release site and had three enclosed houses that were separated from one another. Only two houses from the non-release farm were used for this study. Each poultry house measured ≈12 × 121 m and contained ≈9,000 hens and 800 roosters. Roosters were provided food and water in a central social area that contained wood shavings and comprised one-half of the house (≈726 m^2). Feeders and waterers were provided for hens on the elevated wood-slat floors (0.8 m above the floor) that ran along both walls the entire length of the house that comprised the other half of the house. The wood slatted floor allowed manure to fall through to accumulate below. Nest boxes, were placed along the entire length of the house, and were positioned along the edge of the slatted floor closest to the central social area on both sides of the house.

A three species mix containing *Muscidifurax zaraptor* Kogan & Legner, *M. raptorellus* Kogan & Legner and *Spalangia cameroni* Perkins were purchased

from Beneficial Insectary (Redding, CA). Parasitized pupae were shipped every 2 wk in plastic bags containing wood shavings. Immediately upon receipt of the shipment (2 bags per shipment), bags were weighed and 5, 5.0 g sub-samples were taken from each bag. The mean number of pupae from all sub-samples was calculated to allow the estimated number of house fly pupae to be determined for each bag. All sub-samples were returned to the bags. To assess the quality of the purchased hosts/parasitoids, 96 house fly pupae were removed from each bag and placed in BD Falcon® PVC, 96-well tissue culture plates (VWR International, Irving, Texas) before bags were resealed. Parasitoids were held at room temperature (18°C) before being released in the field 2 d after receiving the shipment.

Tissue culture plates, covered with 2 layers of Parafilm® and 1 layer of packing tape to prevent emerged parasitoids from moving between wells, were stored at room temperature (18°C) for a minimum of 60 d. Species, sex, percent parasitism, and number of parasitoids emerging from each pupa were recorded for each plate. All intact puparia were dissected and examined for dead parasitoids. The number of parasitized pupae, percent parasitism, percent emergence and sex ratios for each species was determined for each bag for each shipment.

Permission to use the farms from the poultry company-integrator was received in June 2003 and May 2004. Therefore, parasitoids were released every second week from 9 June to 31 October, 2003. In 2004, releases were conducted every second week from 13 May to 22 July. Parasitized pupae and wood shavings included in the shipments were distributed by hand between the wooden slats adjacent to the nest boxes (≈2 m from the site where sentinel bags were located). One bag of parasitized pupae was distributed along both sides of the entire length of each house.

In previous studies, poultry facilities containing caged chickens (Rutz & Axtell 1979, Crespo et al. 1998, Tobin & Pitts 1999, Geden & Hogsette 2006) were used to determine the efficacy of using pupal parasitoids to manage house flies. The pupal parasites generally consisted *of Muscidifurax raptor* Girault and Sanders, *M. raptorellus*, *Spalangia endis* Walker and *S. cameroni* and the numbers of pupal parasites released ranged from 40,000 (Rutz & Axtell 1979) to >100,000 per week (Geden & Hogsette 2006). In the present study, the poultry structures were close-walled and tunnel-air houses in which the mature hens and roosters were free to move throughout the house. Two bags of commercially purchased parasitized house fly pupae were released in each house on 2 wk intervals. Each bag contained ≈14,582 to 33,900 potentially parasitized pupae and ≈559,589 and 254,000 pupae were released in 2003 and 2004, respectively.

PROC GLM (SAS Institute 1999) was used to determine differences in number of parasitized pupae released, the number of parasitized pupae with parasitoid emergence and the average number of *M. raptorellus* emerging per pupa: 1) between each house and, 2) among shipments. The PROC GLM procedure in SAS was also used to determine if there were differences in the number of female *M. raptorellus* and *M. zaraptor* released in each house. All values presented as means (SE).

The pupal bag technique (Rutz & Axtell 1980) was used to monitor parasitoids of *M. domestica* and *H. aenescens*. House flies, purchased from Beneficial Insectary (Redding, CA), were shipped as third instars from the insectary to

ensure that pupae were <1-d-old when placed in the field. Ten house fly sentinel bags were placed weekly from 9 June until 7 November 2003 and from 13 May until 22 July 2004. To determine if parasitoids readily parasitize *H. aenescens*, ten sentinel bags, containing 30 1-d-old pupae of *H. aenescens*, were also placed in each house at the release farm from 19 September until 7 November 2003. *Hydrotaea aenescens* were reared as per Hogsette et al. (2002) at the Center for Medical, Agricultural, and Veterinary Entomology USDA-ARS, Gainesville, FL. Pupal bags (9 × 14 cm), made of standard aluminum window screening (≈8 openings per cm), each contained 30 *M. domestica* or 30 *H. aenescens* pupae. Bags were hung at regular intervals below the slatted floor using a wire to ensure that the bags were placed on the surface of the manure. After one week, sentinel bags were returned to the laboratory where the numbers of emerged house flies were counted and the intact pupae isolated into tissue culture plates. Plates were stored at room temperature (18°C) for a minimum of 60 d. Species, sex, and numbers of parasitoids emerging from each pupa were recorded for each sentinel bag. All intact puparia were dissected and examined for dead parasitoids. The number of parasitized pupae, percent parasitism, percent emergence and sex ratios for each species was determined for each sentinel bag. Percent pupal mortality (the percent of placed pupae that had either emerged parasitoids, unemerged parasitoids, or unemerged house flies) was determined for both release and control farms and transformed using arcsine before analyzing using a one way analysis of variance (Minitab 2004). No naturally occurring pupae were collected due to the difficulty in removing slats and finding ample numbers of pupae. Parasitoids that emerged from the sentinel pupae were identified by keys published by Rueda & Axtell (1985a).

Two CatchMaster® sticky fly ribbons (Atlantic Paste & Glue Co., Inc., Brooklyn, NY) were placed in each house to monitor fly populations at both farms. In 2003, fly populations were monitored from 9 June to 31 October 2003. In 2004, the fly populations were monitored from 31 May through 12 October although parasitoid releases ended on 22 July, fly numbers were monitored until 12 October.

One ribbon (one on each side of the house) was hung from the rafter above the slatted floor half way down the length of the house on each side. The ribbons were replaced weekly and the species and number of flies counted. The mean number of house flies and *H. aenescens* collected per ribbon was used to determine the abundance of each fly species. Several authors have proposed economic or injury thresholds using jugtraps, sticky ribbons and cards and spot cards (Axtell 1970, Rutz 1981, Lysyk & Axtell 1986, Kaufman et al. 2000). However, all agreed that the treatment threshold for fly activity was set at 100 flies per ribbon per week. Mean adult fly numbers obtained weekly from the two sticky ribbons placed in the parasite release and non-release farm were used to calculate the percentage reduction in fly numbers caused by the parasitoids.

Results

In 2003, an estimated total of 559,600 pupae were purchased, of which 391,600 were assumed to be parasitized. The weight of each commercial bag of parasitized pupae (n = 22) were 235.0 (12.4) g and contained ≈25,436 (1,074) pupae (range: 16,200 to 33,900 pupae). Total percentage parasitism per bag was 69.6 (2.5) %

(range: 38 to 90%). There were no differences in the number of parasitized pupae released in each house (F = 0.08; df = 1, 21; P = 0.7810). Although the mean number of parasitized pupae per shipment varied (range: 7,587 to 24,714 pupae) (F = 8.64; df = 9, 21; P < 0.0008), the number of pupae released that had parasitoid emergence did not vary between houses (F = 0.13; df = 1, 21; P = 0.7235) (Table 1) or among shipments (F = 0.79; df = 9, 21; P = 0.6347). An estimated total of 283,554 pupae released had parasite emergence, of which 120,208, 126,149 and 37,197 were parasitized by *M. raptorellus*, *M. zaraptor* and *S. cameroni*, respectively (Table 1). *Muscidifurax raptorellus* and *M. zaraptor* were released throughout the summer. However, *S. cameroni* was only present in the commercial shipments and released from 9 June to 10 July, 2003. The insectary failed to notify us on this change. The sex ratios for released *M. raptorellus*, *M. zaraptor* and *S. cameroni* were estimated to be 1.7♀♀:1♂, 1.5♀♀:1♂ and 2.7♀♀:1♂, respectively.

The number of *M. raptorellus* per pupa was 6.1 (0.2) (n = 505) and ranged from 1–18. The average number of *M. raptorellus* per pupa did not vary among shipments (F = 2.43; df = 9, 21; P = 0.0835). There were no significant differences in the number of *M. raptorellus* per pupa released in each house (F = 1.25; df = 1, 21; P = 0.2868) (Table 2) nor did the number of female *M. raptorellus* released in each house vary (F = 0.54; df = 1, 21; P = 0.4821) [House 1: 22,604 (3,997); House 2: 20,082 (4,323)]. It was estimated that 738,400 adults of *M. raptorellus* were released, of which 469,500 were females (Table 2). Equal numbers of female *M. zaraptor* were released in each house per release period (F = 0.57; df = 1, 21; P = 0.4729) [House 1: 4,805 (2,368); House 2: 3,035 (797)]. The estimated number of female parasitoids (all three species) released was ~573,000.

In 2004, an estimated total of 254,000 pupae were purchased, of which 174,000 were assumed to be parasitized. The numbers of parasitized pupae received per shipment in 2004 were similar to shipments received in 2003. The weight of each commercial bag of parasitized pupae (n = 12) was 262.9 (11.7) g and contained ~ 21,169 (1,071) pupae (range: 14,582 to 26,471 pupae). Total percentage parasitism per bag was 69.0 (3.2) % (range: 56 to 84%). There were no differences in the number of parasitized pupae released in each house (F = 0.33; df = 1, 11; P = 0.5923). The number of pupae released that had parasitoid emergence did not vary between houses (F = 0.00; df = 1, 11; P = 0.9772) or among shipments (F = 2.21; df = 5, 11; P = 0.2017). Unlike in 2003, there were no differences in the number of parasitized pupae per shipment (F = 1.67; df = 5, 11; P = 0.2928). *Muscidifurax raptorellus* and *M. zaraptor* made up the majority of parasitoids released (Table 1). *Spalangia cameroni* was only present in the first commercial shipment (13 May 2004). Similar to 2003, the insectary failed to notify us of this change.

Considerably fewer *M. raptorellus* emerged per pupa as compared to pupae sampled in 2003. The average number of *M. raptorellus* per pupa was 3.2 (0.2) (n = 210) and ranged from 1–7. The average number of *M. raptorellus* per pupa did not vary among shipments (F = 0.20; df = 4, 8; P = 0.9204) nor did the number of *M. raptorellus* per pupa released in each house vary (F = 0.0; df = 4, 8; P = 0.9931). Equal numbers of female *M. zaraptor* (F = 0.21; df = 1, 5; P = 0.6669) [House 1: 2,462 (503); House 2: 2,704 (718)] and *M. raptorellus* (F = 0.16; df = 1, 5; P = 0.7086) [House 1: 8,184 (3,359); House 2: 8,966 (2,619)] were released in each house per release period.

Table 1. Estimated numbers of parasitized pupae, numbers of pupae with parasite emergence, and numbers of pupae parasitized by *M. raptorellus*, *M. zaraptor* and *S. cameroni* purchased from an insectary and released in two broiler-breeder houses in Arkansas over two years (9 June to 31 October 2003 and 13 May to 22 July 2004).

Year	House	No. of shipments	No. of parasitized pupae	Estimated no. pupae with parasite emergence *n* (%)	Estimated no. of house fly pupae parasitized by		
					M. raptorellus *n* (%)	*M. zaraptor* *n* (%)	*S. cameroni*[a] *n* (%)
2003	1	10	197,400	149,656 (75.8)	63,249 (42.3)	67,087 (44.8)	19,320 (12.9)
	2	10	194,248	133,898 (68.9)	56,959 (42.5)	59,062 (44.1)	17,877 (13.4)
	Total		**391,648**	**283,554 (72.4)**	**120,208 (42.4)**	**126,149 (44.5)**	**37,197 (13.1)**
2004	1	6	89,726	60,083 (65.6)	31,405 (51.7)	24,416 (42.5)	4,262 (5.8)
	2	6	84,271	60,368 (72.3)	31,305 (50.0)	27,291 (46.5)	1,772 (3.5)
	Total		**173,997**	**120,451 (68.9)**	**62,710 (50.9)**	**51,707 (44.5)**	**6,034 (4.7)**

[a]*S. cameroni* were only released from 9 June to 10 July 2003 and on 13 May 2004. After these dates, *S. cameroni* were not present in the commercially purchased house fly pupae (determined by our evaluation of the purchased shipments received bi-weekly).

Table 2. Mean (± SE) number of *M. raptorellus* per parasitized *M. domestica* pupa, number of adult *M. raptorellus*, number of females of *M. raptorellus*, *M. zaraptor*, and *Spalangia cameroni*, and total number of females released in two broiler-breeder houses in Arkansas over two years (9 June to 31 October 2003 and 13 May to 22 July 2004).

		M. raptorellus		No. of females released			
Year	House	Mean (± SE) no. per pupa [a]	No. of adults	*M. raptorellus*	*M. zaraptor*	*S. cameroni*[b]	Total no. females released
2003	1	6.2 ± 0.3a	402,097	248,650	39,996	14,454	303,100
	2	5.9 ± 0.2a	336,309	220,902	36,286	12,692	269,880
	Total	**6.1 ± 0.2**	**738,406**	**469,552**	**76,282**	**27,146**	**572,980**
2004	1	3.2 ± 0.2a	97,357	49,104	14,771	4,262	68,137
	2	3.2 ± 0.3a	118,594	53,796	16,226	1,772	71,794
	Total	**3.2 ± 0.2**	**215,951**	**102,900**	**30,997**	**6,034**	**139,931**

[a]Means within the same year followed by a different letter are significantly different (LSMEANS, SAS Institute 1996) at $\alpha = 0.05$.
[b]*S. cameroni* were only released from 9 June to 10 July 2003 and on 13 May 2004.

Table 3. Number of sentinel pupae of *M. domestica* parasitized by parasitoids on two broiler-breeder farms in Arkansas over two years (9 June to 7 November 2003 and 13 May to 22 July 2004).

House	Mraptls[a]	Mz	Sc	Se	S	Unk	Total[b]	No. of pupae	% parasitism
				Release farm 2003					
1	57	677	153	37	7	145	1,076	5,537	19.4
2	82	662	78	21	1	86	930	5,148	18.1
Total[c]	**139**	**1,339**	**231**	**58**	**8**	**231**	**2,006**	**10,685**	**18.8**
Percent	6.9	66.8	11.5	2.9	0.4	11.5	100		
				Non-release farm 2003					
1	0	125	315	87	12	101	640	4,793	13.4
2	0	272	150	151	23	92	688	4,731	14.5
Total[c]	**0**	**397**	**465**	**238**	**35**	**193**	**1,328**	**9,524**	**13.9**
Percent	0	29.9	35.0	18.0	2.6	14.5	100		
				Release farm 2004					
1	573	307	5	0	1	112	998	2,909	34.3
2	410	126	0	3	0	51	590	2,381	24.8
Total[c]	**983**	**433**	**5**	**3**	**1**	**163**	**1,588**	**5,290**	**30.0**
Percent	61.9	27.3	0.3	0.1	0.06	10.3			
				Non-release farm 2004					
1	0	0	14	2	2	1	19	3,038	0.6
2	0	23	51	62	27	19	182	2,908	6.3
Total[c]	**0**	**23**	**65**	**64**	**29**	**20**	**201**	**5,946**	**3.4**
Percent	0	11.4	32.3	31.8	14.5	10			

[a]Mraptls, *Muscidifurax raptorellus*; Mz, *Muscidifurax zaraptor*; Sc, *Spalangia cameroni*; Se, *Spalangia endius*; S, *Spalangia* that could not be identified to species; Unk., dissected pupae where immature parasitoids could not be identified.
[b]Total number of sentinel pupae parasitized for each house and farm.
[c]Total number of parasitoids and pupae, including total prevalence for the release and non-release farms.

Four species of parasitoids were collected from house fly sentinel pupae at the release farm. Of the 10,685 sentinel house fly pupae recovered, 2,006 (18.8%) were parasitized by *M. zaraptor* (66.8%), *S. cameroni* (11.5%), *S. endius* (2.9%), and *M. raptorellus* (6.9%) (Table 3). Dissected immature parasitoids and *Spalangia* spp. that could not be identified to species accounted for 11.5% and 0.4% of the parasitism, respectively. The number of *M. raptorellus* per sentinel house fly pupa was 7.2 (0.3) ($n = 134$) and ranged from 1–19.

Of the 3,166 sentinel *H. aenescens* pupae recovered from the release farm, 299 (9.4%) were parasitized by *M. zaraptor* (92.0%), *M. raptorellus* (2.0%), and *S. cameroni* (1.0%). Those parasitoids that could not be identified to species made up 5.0% of the parasitism.

Of the 9,524 sentinel house fly pupae recovered at the non-release farm, 1,328 (13.9%) were parasitized by three species of parasitoids (Table 3). *Spalangia cameroni* was the most abundant, accounting for 35.0% of the parasitism.

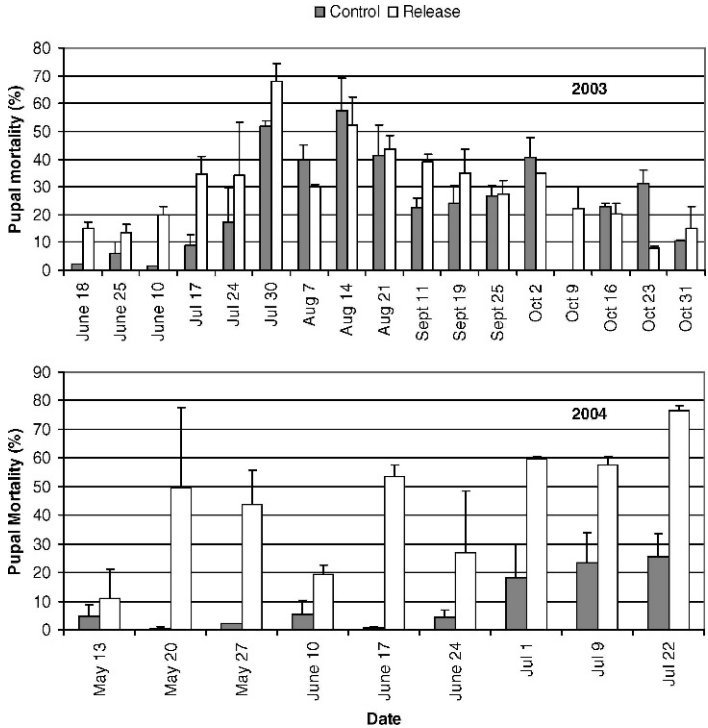


Fig. 1. Mean (± SE) percent mortality of sentinel pupae collected in control and release houses in 2003 and 2004.

Muscidifurax zaraptor and *S. endius* occurred in smaller numbers and represented 29.9% and 18.0% of the parasitism, respectively. Mean (±SE) pupal mortality was significantly different between release (30.7 ± 3.1%) and non-release (24.3 ± 2.9%) farms (F = 4.48; df = 64, 1; P <0.038) (Fig. 1).

Although the same parasitoid species were recovered, parasitism was higher in comparison to 2003 at the release farm. Of the 5,290 sentinel pupae recovered, 1,588 (30%) were parasitized by *M. raptorellus* (61.9%), *M. zaraptor* (27.3%), *S. cameroni* (0.3%) and *S. endius* (0.1%) (Table 3). The number of *M. raptorellus* per sentinel house fly pupa was 3.1 (0.11) (n = 103) and ranged from 2–7.

Parasitism at the non-release farm was very low in comparison to 2003. Of the 5,946 sentinel pupae recovered, 201 (3.4%) were parasitized by *Spalangia* spp. (78.6%) and *M. zaraptor* (11.4%) (Table 3). Mean (±SE) pupal mortality between release (47.2 ± 5.2%) and non-release (9.4 ± 2.7%) farms was significantly different (F = 20.58; df = 35, 1; P < 0.0001) (Fig. 1).

The flock production cycle at the parasite release farm was initiated on 25 March 2003 when the birds were 20-wk-old while the flock production cycle at the non-release farm was started with chickens of the same age on 9 May. Thus, when filth fly monitoring was initiated at both farms on 9 June, manure accumulation and fly breeding had started some 6 wk earlier at the parasite release farm than at the non-release farm. The effect of this 6 wk difference in

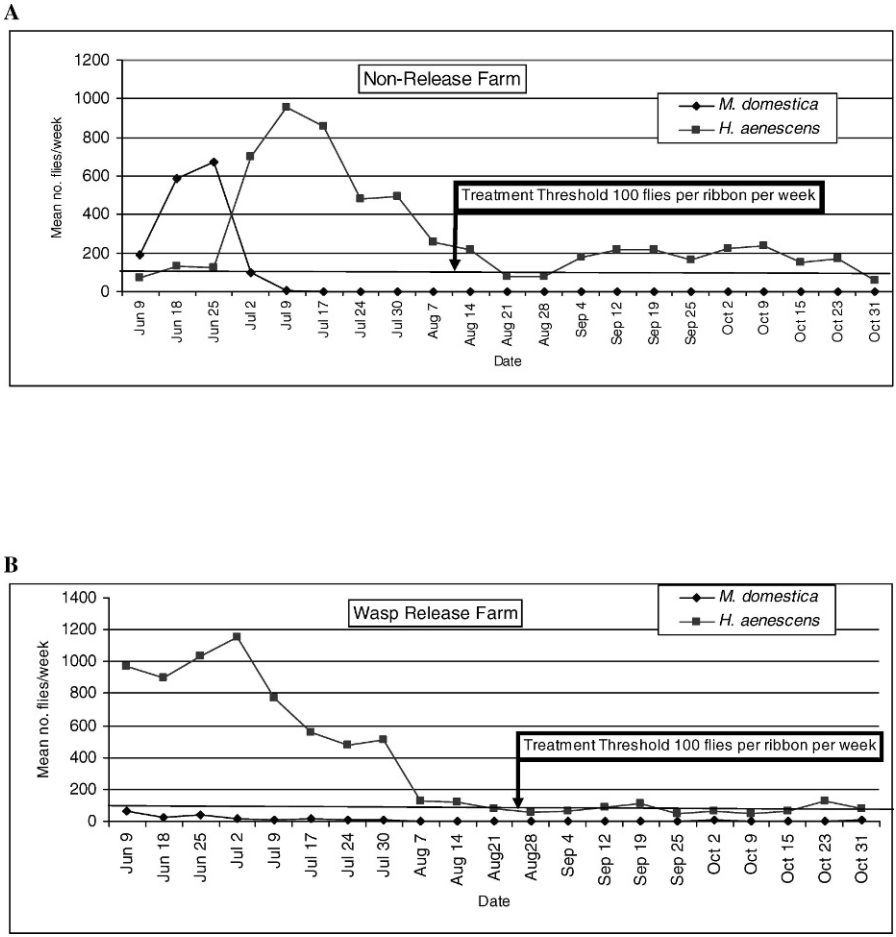


Fig. 2. Mean numbers of *M. domestica* and *H. aenescens* captured on sticky ribbons (exposed for 1 wk at the (A) non-release broiler-breeder egg production farm and those captured at the (B) wasp-release farm from 9 June to 31 October 2003. Treatment threshold was set at fly counts having a mean of 100 flies per ribbon per week (Axtell 1970).

time for manure accumulation and filth fly breeding is shown in Figure 2 for both the non-release farm (Fig. 2A) and the parasite release farm (Fig. 2B).

At the parasite release farm, the numbers of *M. domestica* were below the treatment threshold and throughout the study period of 2003, *M. domestica* numbers were consistently below the treatment threshold, never exceeding the number of *H. aenescens*. During the initial week of 9 June, there was a mean of ≈1,000 *H. aenescens* per sticky ribbon per week found at this farm. By comparison, when filth fly monitoring was initiated at the non- parasite-release farm on 9 June, adult *M. domestica* numbers exceeded both the number of adult *H. aenescens* and the treatment threshold by ≈100 flies per sticky ribbon per week. Approximately 4 wk later *H. aenescens* reached population peak numbers

Table 4. Percentage control of *M. domestica* and *H. aenescens* using sustained pupal parasite releases, calculated from the mean number of flies per ribbon per week collected from the non-release and release farms from 9 June to 31 October 2003.

		M. domestica			*H. aenescens*		
		Mean no. flies/ribbon/week			Mean no. flies/ribbon/week		
Date	Date of wasp release	Non-release farm	Release farm	Percentage control	Non-release farm	Release farm	Percentage control
09 Jun	*	189.5	63.2	67	73.8	973.0	0
18 Jun		589.0	21.5	96	130.4	897.5	0
25 Jun	*	671.0	43.3	94	122.0	1,032.0	0
02 Jul		100.3	14.0	86	696.4	1,153.3	0
09 Jul	*	5.3	5.8	0	957.0	768.5	20
17 Jul		1.0	12.5	0	854.3	556.3	35
24 Jul	*	1.3	7.5	0	479.0	476.3	0
30 Jul		1.0	4.5	0	493.3	512.8	0
07 Aug	*	1.0	2.0	0	256.3	124.0	52
14 Aug		1.0	1.0	0	216.5	117.5	54
21 Aug	*	0.0	0.5	0	82.0	79.0	4
28 Aug		19.5	1.0	100	76.3	58.3	24
04 Sep	*	0.0	1.3	0	178.5	62.3	65
12 Sep		1.0	1.8	0	219.3	90.5	59
19 Sep	*	1.0	0.5	0	215.8	111.0	49
25 Sep		1.0	3.3	0	165.3	51.3	69
02 Oct	*	0.0	8.3	0	224.5	65.5	71
09 Oct		0.0	3.0	0	237.8	50.8	79
15 Oct	*	0.0	2.0	0	153.3	64.8	58
23 Oct		0.0	0.3	0	169.0	126.3	25
31 Oct	*	0.0	4.0	0	61.3	78.5	0

of 957 and 1,153 per sticky ribbon at the non-release farm and parasite release farm respectively. By 9 July at both farms *M. domestica* numbers had fallen to almost zero and few were found on the sticky ribbons thereafter until the end of the study period on 31 October. From the data obtained in this study it appeared that the sustained release of parasitoids had a much smaller impact on the house fly population than did the predation effects of *H. aenescens* larvae.

Comparison of adult *H. aenescens* populations at the two farms showed that at the release farm the parasitoids were causing a 20% reduction in adult *H. aenescens* by 9 July and percent reduction in their numbers increased to the greatest level (79%) during the week of 9 October 2003 (Table 4).

When filth fly monitoring was resumed for year two of the study in May 2004, house fly numbers at the parasitoid release farm were >80% below the number of house flies collected on sticky ribbons at the non-parasite release farm (Table 5). *Hydrotaea aenescens* numbers were 46% lower at the release farm than at the non-release farm (means of 389 and 211 adults per sticky ribbon at the

Table 5. Percentage control of *M. domestica* and *H. aenescens* using sustained pupal parasite releases, calculated from the mean number of flies per ribbon per week collected from the non-release and release farms from 13 May to 12 October 2004.

		M. domestica			*H. aenescens*		
		Mean no. flies/ribbon/week			Mean no. flies/ribbon/week		
Date	Date of wasp release	Non-release farm	Release farm	Percentage control	Non-release farm	Release farm	Percentage control
13 May	*	6.75	1.25	81.5	389	211	46
29 May		4.25	5.25	0	426	374	12
27 May	*	2.75	5.75	0	409	528	0
03 Jun		0.5	1.0	0	608	716	0
10 Jun	*	0.5	0.5	0	546	520	5
17 Jun		1.0	1.75	0	494	693	0
24 Jun	*	0.5	0.75	0	481	582	0
01 Jul		0.25	0.5	0	378	452	0
09 Jul	*	0.0	0.0	0	472	357	24
15 Jul		0.0	0.0	0	355	211	41
22 Jul	*	0.0	0.5	0	321	249	23
29 Jul		0.0	0.0	0	326	186	43
05 Aug		0.25	0.5	0	327	90	72
12 Aug		0.0	0.25	0	322	150	53
19 Aug		0.25	0.25	0	316	64	80
24 Aug		0.0	0.0	0	243	49	80
31 Aug		0.0	0.5	0	276	59	79
07 Sep		0.0	0.0	0	256	95	63
15 Sep		0.0	0.0	0	282	61	78
21 Sep		0.0	0.0	0	247	52	79
28 Sep		0.0	0.25	0	255	77	70
05 Oct		0.0	0.0	0	251	95	62
12 Oct		0.0	0.25	0	206	113	45

non-release and parasite release farms, respectively). No adult house flies were collected on sticky ribbons at the release farm after 1 July while the mean *H. aenescens* numbers were higher at the parasitoid release farm than at the non-release farm until 9 July when percentage control reached 24% at the release farm. After 29 Aug, *H. aenescens* numbers were consistently below 100 flies per sticky ribbon per week except on 12 Aug and 12 Oct. Highest percentage control of *H. aenescens* (80%) occurred on 19 and 24 Aug at the parasite release farm when mean *H. aenescens* numbers were 316 and 243 flies per ribbon at the non-release farm and 49 and 59 flies per ribbon at the parasite release farm.

Discussion

Using percentage of *M. domestica* sentinel pupae parasitized and percent mortality as indicators of parasitoid activity, sustained releases of parasitoids at

the release farm provided a significant increase in parasitism when compared to the data obtained at the non-release farm. Differences in parasitism were most obvious in the second year of the release program (Table 3, Fig. 1).

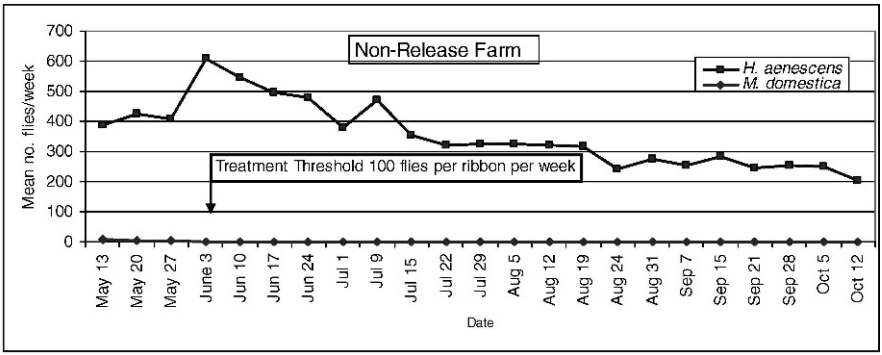
In 2003, the chickens were placed in the houses at the non-release farm 3 wk prior to placing the first sticky fly ribbons in the houses. Filth fly immigration to the houses and fly breeding in the manure had caused *M. domestica* and *H. aenescens* numbers to be at or above the treatment threshold prior to the initiation of the study. Chickens were placed in the houses at the farm selected to receive sustained wasp releases 9 wk prior to placement of the first sticky fly ribbons. Comparison of filth fly species obtained on the 1st week fly ribbons were placed showed house fly numbers were above the treatment threshold of 100 flies per ribbon per week while *H. aenescens* numbers were below the treatment threshold (189.5 and 73.8, respectively) at the non-release farm. Four weeks later house fly numbers had decreased to the treatment threshold (mean of 100.3) while *H. aenescens* numbers had increased to a mean of 897.5 flies per ribbon (Table 4, Fig. 2A). The number of flies on the sticky ribbons was quite different at the farm selected for sustained wasp release.

Mean house fly and *H. aenescens* numbers at the wasp release farm were 63.2 and 972 flies per ribbon, respectively, after the first week's sampling period ended on 9 June 2003 (Table 4, Fig. 2B). There was an approximate 9× difference in the numbers of the *H. aenescens* and *M. domestica*, at this farm when sustained release of parasitic wasps was initiated. Comparing the weekly mean filth fly data at the two farms suggested that the predacious *H. aenescens* larvae reduced the number of house flies to a level at or near zero at both farms. However, mean numbers of *H. aenescens* were approximately 200 flies per ribbon per week at most weekly intervals at the non-wasp release farm for the last 11 wk of the study. Comparison of the data from the two farms in 2003, indicated that the sustained wasp release reduced *H. aenescens* numbers to a level at or just below the treatment threshold during the last 13 wk of the study with percentage reductions as high as 79% on 9 October.

The chickens were placed in the houses at the wasp release farm on 13 and 14 March 2004 and filth fly monitoring ribbons were placed in the houses 9 wk later with the first fly data obtained on 13 May. On 1 and 2 April 2004 the chickens were placed in the houses at the non-release farm and sticky ribbons were placed in the houses 6 wk later on 6 May. Therefore, as in 2003, manure accumulation and filth fly immigration occurred over several weeks prior to initiation of sustained parasite releases and fly monitoring. Adult house flies were almost nonexistent in the houses at both the wasp release and non-release farms when the first ribbons were evaluated on 13 May having means of 6.75 and 1.25 flies per ribbon, respectively. This low number of house flies continued throughout the study period (Table 5, Fig. 3) and was quite different from the numbers of *H. aenescens* found on the sticky ribbons at each weekly interval.

The number of *H. aenescens* collected on the weekly sticky ribbons was higher in the houses at the non-release farm (mean of 389 flies per ribbon), increased to a mean of 608 and then decreased to near 300 flies per ribbon per week on 22 July and remained at or above 300 flies per ribbon per week throughout the remainder of the study period. There was a mean of 211 *H. aenescens* on the sticky ribbons on 13 May and the numbers increased to a mean of 700 flies per ribbon on 3 and 17 June then steadily decreased to on or about the treatment threshold of 100

A



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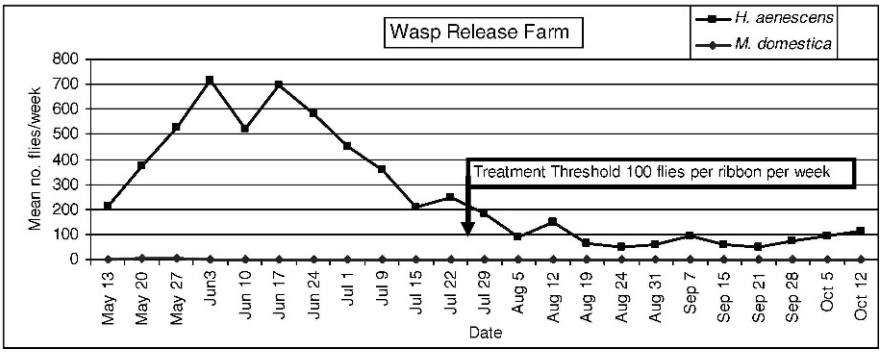

Fig. 3. Mean numbers of *M. domestica* and *H. aenescens* captured on sticky ribbons (exposed for 1 wk at the (A) non-release broiler-breeder egg production farm and those captured at the (B) wasp release farm from 13 May to 12 October 2004. Treatment threshold was set at fly counts having a mean of 100 flies per ribbon per week (Axtell 1970).

flies per ribbon until the study ended on 12 October. It appeared that the parasitoids were selecting *H. aenescens* pupae as hosts because the mean numbers of adult *H. aenescens* collected at the wasp release farm were much lower than the mean numbers collected at the non-release farm. Our *H. aenescens* sentinel bag studies at the wasp release farm confirmed that *M. zaraptor* and other species of wasps readily parasitized *H. aenescens* pupae. Comparison of the data from the two farms in 2004 indicated that sustained wasp release caused an 80% reduction of *H. aenescens* during August. The reduction in adult *H. aenescens* numbers is important due to their importance as vectors of bacteria that cause disease in poultry and humans.

In previous studies we have found DNA of *Campylobacter*, *E. coli* H7, *E. coli* 0157 and E. coli 0157:H7 in 25.5, 3.6, 1.8, and 0.4% of 552 adult *H. aenescens*, respectively, and 19.4, 3.8, 1.3, and 1.3%, in 315 flies, respectively, at two

different turkey farms (Szalanski et al. 2004). In addition, we found similar percentages of positive *Campylobacter* DNA in *H. aenescens* collected from five broiler-breeder egg production facilities (15.9% of 1008 individual flies), (T. M., unpublished data).

No literature was found that showed that any one species of parasitoid had been reported to be more effective than other species in reducing the numbers of *H. aenescens*. All parasitoid host preference studies that have been conducted with *H. aenescens* have been associated with house flies and stable flies or house flies and *Fannia* spp. (Mandeville & Mullens 1990). In 2003, of the three parasitoids released, *M. zaraptor* was the most abundant parasitoid collected in both sentinel house fly and *H. aenescens* pupae. Although *M. zaraptor* was found to occur naturally in broiler-breeder houses in Arkansas, its high abundance at the release farm during both years of this study was likely contributed to the mass release program. The release of *S. cameroni* during the first month did not seem to have a significant impact on overall parasitism since the relative abundance of *S. cameroni* in house fly sentinels for both years was much higher at the non-release site than at the release farm during both years (Table 3).

Due to the gregarious nature of *M. raptorellus*, this parasitoid has been considered an excellent biological control agent of filth flies (Petersen & Currey 1996), substantially lowering production costs compared to solitary species of parasitoids (Floate et al. 2000). Although the majority of females released in 2003 were *M. raptorellus*, this parasitoid did not result in substantial parasitism in sentinel house fly and *H. aenescens* pupae. Kaufman et al. (2001) found similar results for sentinel house fly pupae in New York poultry houses when *M. raptorellus* and *Nasonia vitripennis* Walker were released weekly. The authors in that study suggested that *M. raptorellus* was either unable to compete with *N. vitripennis* or this parasitoid was unable to establish within the poultry house. Our release rates of *M. raptorellus* were similar to the release rates used by Kaufman et al. (2001). However, in our study we did not collect *N. vitripennis* and we did not examine competition among species. We suspect *Muscidifurax raptorellus* released in our study did not establish until the second year of release. Although fewer parasitoids were released in 2004 in comparison to 2003 (Table 2), parasitism by *M. raptorellus* resulted in significant percent parasitism and pupal mortality.

Behavioral characteristics, such as the ability to disperse into the environment and to locate hosts, differ among parasitoid species and may have contributed to the differences in efficacy among the parasitoids used in this mass release study. Pawson & Petersen (1988) conducted a study to determine the dispersal of *M. zaraptor* in dairies and found parasitism of sentinel house fly pupae to occur as far as 8 m from the release point. Tobin & Pitts (1999) examined the dispersal of *M. raptorellus* in a high-rise poultry facility and concluded that this parasitoid had a limited dispersal range that was within 2 m of its release point. *Muscidifurax zaraptor* released in 2003 may have been able to disperse better into the environment in comparison to released *M. raptorellus*. The placement of sentinel bags might have been at the outer dispersal range that *M. raptorellus* successfully locates and parasitizes sentinel pupae. There was also insufficient manure removal in 2003 which may have caused the parasitoids to remain in the houses and thus increasing their population in 2004. Legner (1977) reported that *M. zaraptor* was easily collected near the substrate surface, while *S. cameroni*

and *S. endius* penetrated deeper into the substrate. Although he studied foraging in wheat flakes, similar results have been reported in poultry manure (Rueda & Axtell 1985b, Geden 2002). The sentinel bags used in this study were placed on the surface of the manure, therefore, *M. zaraptor* might have been able to forage more efficiently than *S. cameroni* which might have been foraging for pupae deeper down in the substrate.

The parasitism of *H. aenescens* sentinel pupae was likely underestimated because pupae were only placed in the field in the last 2 mo of 2003. Parasitoid species composition and abundance might have varied throughout the season and this needs to be further addressed.

In 2003, *S. cameroni* was the most abundant parasitoid collected at the non-release farm, with *M. zaraptor* and *S. endius* ranked second and third. In 2004, *Spalangia* spp. were the most dominant. Similar parasitoid species have been collected in other boiler-breeder houses throughout the United States. Dry et al. (2007) collected *S. endius*, *S. cameroni*, *Muscidifurax* spp. and *N. vitripennis* in northwest Arkansas, but reported that *P. vindemiae* to be the predominant parasitoid. Rutz & Axtell (1981) collected seven parasitoid species in North Carolina, of which *S. cameroni*, *Muscidifurax raptor* Girault and Sanders, and *S. endius* were the most abundant. *Nasonia vitripennis* and *M. raptor* were the most predominant parasitoid species observed in New York caged-layer poultry (Rutz & Scoles 1989). No *N. vitripennis* or *P. vindemiae* were collected in our study. Although *N. vitripennis* is considered to play a minor role in fly regulation in warmer regions (Rutz & Scoles 1989), differences in species predominance in our study are probably due to differences in manure conditions between caged-layer (wet manure) and broiler-breeder (generally dry manure) houses (Rutz & Axtell 1981).

The manure conditions could vary between broiler-breeder facility design, which could explain the differences in naturally occurring parasitoid species between our study and that observed by Dry et al. (2007). All broiler-breeder facilities utilized in the studies conducted by Dry et al. (2007) were open-sided houses, wherein air movement was through free air flow by lowering plastic curtains and using 0.9 m diameter or larger ventilating fans to regulate air flow and temperature (North & Bell 1990). In the present study all broiler-breeder facilities were controlled-environment houses using tunnel air ventilation in which the house is completely closed-sided and a negative pressure is created within the facility with air movement and volume regulated by exhaust fans (North & Bell 1990). Thus, parasitoid species apparently vary regarding their ability to gain access to the fly pupation sites beneath the slatted flooring and due to the closed system of the controlled-air facilities probably explains the differences in parasitoid diversity that was found in our study as compared to that of Dry et al. (2007).

The release of parasitoids early in the season before *H. aenescens* and house flies become established may help to augment the population of naturally-occurring parasitoids, and may provide a more effective control of filth flies in these houses. The degree of success in reducing the *H. aenescens* population using parasite releases on poultry farms is especially important when their pathogen carrying importance is considered. In previous studies we have shown that *Campylobacter jejuni*, and *E. coli* O157:H7 were detected in equal or greater numbers of adult *H. aenescens* than in house flies at turkey production facilities (Szalanski et al. 2004). More research is needed to determine the appropriate

release rates of *M. raptorellus* so it can be used efficiently to control filth flies in broiler-breeder houses in Arkansas.

Acknowledgment

We thank M. McClung, L. Gabriel, B. Thomason, and T. Palmer for lab and field assistance. We also thank J. Hogsette, USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL for his generous shipments of *H. aenescens* pupae and D. Taylor, USDA-ARS, Midwest Livestock Insect Research Unit, Lincoln, NE for confirming *Spalangia* samples using PCR-RFLP. Thanks are also extended to Mark Sullivan, Production Manager, OK Foods, Fort Smith, AR for the use of their facilities during this project. This work was supported by the University of Arkansas, Arkansas Agricultural Experiment Station, and by USDA CREES, Special Research Grants, Pest Management Alternatives Grant #2002-34381-12156.

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Sustained Mass Release of Pupal Parasitoids (Hymenoptera: Pteromalidae) for Control of *Hydrotaea aenescens* and *Musca domestica* (Diptera: Muscidae) in Broiler-Breeder Poultry Houses in Arkansas[1]

Tanja McKay,[2] C. Dayton Steelman,[3] Sheri M. Brazil,[3] and Allen L. Szalanski[3]

J. Agric. Urban Entomol. 24(2): 67–85 (April 2007)

ABSRACT *Muscidifurax zaraptor* Kogan and Legner, *M. raptorellus* Kogan and Legner, and *Spalangia cameroni* Perkins were released bi-weekly in two facilities at a broiler-breeder egg production farm in Arkansas during 2003 and 2004. Of the recovered house fly, *Musca domestica* L., sentinel pupae, 18.8% were parasitized in 2003, with *M. zaraptor* being the dominant species (66.8%) and *M. raptorellus* contributing 6.9% of the parasitism. The release of *M. raptorellus* did not result in substantial parasitism in sentinel house fly pupae until the second year of study when *M. raptorellus* was the most dominant species, contributing approximately 61.9% parasitism. At the non-release farm, 13.9% of the sentinel house fly pupae were parasitized, with *S. cameroni* and *M. zaraptor* dominating in 2003. Parasitism at the control farm decreased to 3.4% in 2004. It appeared that sustained releases of parasitoids at the release farm over two years provided a significant increase in house fly pupal parasitism when compared to the percentage of pupae parasitized at the non-release farm. Of the sentinel *Hydrotaea aenescens* (Wiedemann) pupae recovered from the release farm, 9.3% were parasitized in 2003. Filth fly data indicated that the combined predator activity of *H. aenescens* and sustained parasitoid releases reduced *M. domestica* to a level well below the treatment threshold. In addition, the sustained release of parasitoids reduced *H. aenescens* numbers to below the treatment threshold of 100 filth flies per sticky ribbon per week by 9 wk during the latter part of the filth fly season during 2003 and 2004.

KEY WORDS Parasitoids, *Musca domestica*, house fly, *Hydrotaea aenescens*, biological control, poultry

The house fly, *Musca domestica* (L.) and *Hydrotaea aenescens* (Wiedemann) (formerly *Ophyra aenescens*) (Diptera: Muscidae) are common filth flies found in broiler-breeder houses (Davis 1997, Axtell 1999). The house fly is the primary target of most filth fly management programs (Wilhoit et al. 1991). When present in large numbers, house flies not only annoy employees working in these facilities (Thomas & Skoda 1993), but can also reduce aesthetics by leaving fecal and regurgitation spots on structures, light fixtures, and eggs (Axtell 1999). Best

[1]Received 18 January 2008; Accepted 8 May 2008.
Dr. C. Dayton Steelman, Dept. of Entomology, Rm 320, Agric. Bldg., University of Arkansas, Fayetteville, AR, 72701, Phone: (479) 575-2510, Fax: (479) 575-2452, E-mail: dsteelm@uark.edu
[2]Department of Biological Sciences, Arkansas State University – Jonesboro, Arkansas USA.
[3]Department of Entomology, University of Arkansas, Fayetteville, Arkansas USA.

Survey for Microbial Pathogens of the Red Oak Borer (Coleoptera: Cerambycidae) on Northern Red Oak in Northwest Arkansas[1]

Jason M. Meyers,[2,3] Donald C. Steinkraus,[4] Frederick M. Stephen,[4] and Roger E. Gold[3]

J. Agric. Urban Entomol. 24(2): 87–94 (April 2007)

ABSTRACT An outbreak of red oak borer, *Enaphalodes rufulus* (Haldeman), in Arkansas has resulted in millions of dollars in damage to oak trees. A survey for microbial pathogens of late stages of red oak borer in northwest Arkansas forests demonstrated that red oak borer infected with *Beauveria bassiana* were present in 12 of 21 *Quercus* spp. trees. Oak trees dissected in the survey were located in an area of heavy infestation in the Ozark National Forest, Franklin County, Arkansas. Individual trees were selected for the survey based on a likelihood of *E. rufulus* infestation. Overall prevalence of *B. bassiana*-infection was 2.7%. Infected late stage larvae, pupae and adults were found between May and July 2003. Mean number of late stage larval, pupal and adult red oak borers per 0.5 m bole sample was 1.64 (SE ± 0.1). Mean number of *B. bassiana*-infected red oak borer per 0.5 m bole sample was 0.04 (SE ± 0.01). These data demonstrate that *B. bassiana*-infected red oak borers in *Quercus* spp. were found throughout the height of the tree.

KEY WORDS *Enaphalodes rufulus*, *Beauveria bassiana*, *Quercus*, biological control, microbial pathogen, entomopathogenic fungi

Introduction

Many North American trees are damaged by serious insect pests. These tree-infesting insects include historic pests such as *Dendroctonus frontalis* Zimmerman (Scolytidae) (Price & Doggett 1978), recent exotic pests such as Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky (Cerambycidae) (Cavey et al. 1998) and emerald ash borer, *Agrilus planipennis* Fairmaire (Buprestidae) (Childs 2002). Recently, a normally endemic species, the red oak borer, *Enaphalodes rufulus* (Haldeman) (Cerambycidae), increased to epidemic proportions (Stephen et al. 2001).

Oak-hickory forests in Missouri and northwest Arkansas have undergone a significant population increase in the red oak borer. The USDA Forest Service first discovered this population outbreak in 1999, when widespread mortality was

[1]Received 7 July 2008; Accepted 14 August 2008.
[2]Corresponding author (jmmeyers@tamu.edu).
[3]Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, 2143 TAMU, College Station, Texas 77843.
[4]Department of Entomology, 319 Agriculture Bldg., University of Arkansas, Fayetteville, Arkansas 72701.

observed in red oaks (northern red oak, *Quercus rubra*, southern red oak, *Q. falcata*, and black oak, *Q. velutina*) (Stephen et al. 2001). This economically destructive pest also decreases the quality of the wood harvested for commercial use, and may ultimately cause $1 billion in damage in Arkansas (J. Guldin, Southern Research Station, USDA For. Ser., pers. comm.). In addition to economic damage, red oak borer creates a risk to campers, hikers and loggers from weakened tree boles and limbs.

Red oak borer has a two-year life cycle with adults emerging in mid-June to mid-August in odd numbered years (Hay 1969, Galford 1983). The larvae of red oak borer create large galleries within the phloem and xylem (15–25 cm long and 1 cm wide) of *Quercus* spp. trees (Solomon 1995). This insect also has high fecundity with an average of 200 eggs oviposited per female (Donley & Acciavatti 1980) possibly elevating the effects of the outbreak.

Despite the importance of red oak borer, little research has been done on its natural enemies. The primary predators of red oak borer are woodpeckers (Petit et al. 1987), carpenter worms, *Prionoxystus robiniae* Peck (Lepidoptera: Cossidae) (Hay 1974, Ware & Stephen 2006), elaterids (Ware & Stephen 2006) and ants (Donley & Acciavatti 1980, Muilenberg et al. 2008). Cannibalistic behaviors have also been observed in *E. rufulus* (Ware & Stephen 2006). The overall impact of these predators is unclear. Once red oak borer larvae are deep within the heartwood of oak they may be less vulnerable to predation. Determining the impact of microbial pathogens on red oak borer populations is important for understanding red oak borer biology and for evaluating potential biological control agents.

Should the current red oak borer outbreak continue in forest areas or develop in urban areas, control methods will become essential. The objective of this study was to determine what microbial pathogens were naturally infecting late stage larvae, pupae and adults of red oak borer.

Materials and Methods

Twenty-one mature oak trees (19 northern red oak, *Quercus rubra*, 1 white oak, *Q. alba*, and 1 black oak, *Q. velutina*), were harvested between May and July 2003 from Fly Gap and White Rock areas of the Pleasant Hill Ranger District of Ozark National Forest, Franklin County, Arkansas. The trees were selected based on feasibility and safety with respect to the felling and removal of the trees, along with likelihood of red oak borer infestation. Likelihood of red oak borer infestation was based on previously demonstrated methods (Fierke et al. 2005) that found a significant correlation of red oak borer infestation numbers to crown condition and emergence hole density found below 1.5 m. Infested trees were felled, boles cut into 0.5 m long samples, and galleries were revealed by carefully splitting boles with a gas-powered log splitter. The diameter of the harvested oaks at 1.5 m ranged from 24.5 to 36.0 cm (mean, 29.1, SE $\pm$ 0.8), total tree height ranged from 15.2 to 22.5 m (mean, 18.9, SE $\pm$ 0.5), and red oak borer infested bole height ranged from 8.5 to 16.5 m (mean, 6.56, SE $\pm$ 0.12). For analytical purposes, the infested bole was split into tenths of overall infested bole height to normalize the varied tree heights. This stratification offers a more accurate estimate when comparing trees of significantly differing height (Pulley et al. 1977, Hain et al. 1978). For each specimen location, the middle of the

Table 1. Numbers and percentages of mortality, by cause, in 882 late-stage red oak borers collected in northwest Arkansas oaks.

Mortality cause	# infected (%)	% of total late-stage red oak borers
[a]Unknown	30 (53.6)	3.4
B. bassiana	24 (42.9)	2.7
M. anisopliae	2 (3.6)	0.2
Total	56	6.3

[a]Red oak borers found dead in their galleries but not containing nematodes, protozoa, spore-forming bacteria, or entomopathogenic fungi were placed classified as "unknown".

sample height from the ground was used as the height of location. The proportioned location of red oak borer infested bole height ranged from 0.05 to 1.0 (mean, 0.48, SE ± 0.008).

Collecting mature red oak borer larval, pupal and adult stages from oak tree heartwood was labor-intensive. It took ca. 40 hours to split an average of 25 (range 16 to 32) 0.5 m bolt samples per tree in order to collect red oak borers. The entire survey took three people ca. two months of intensive labor to complete. The large amount of labor required to determine the absolute occurrence of healthy and infected red oak borer late instars, pupae and adults limited this study to 21 trees. Larvae were only identified as late stage, because, unfortunately, no studies exist which permit precise identification of red oak borer instars.

All moribund and dead red oak borers were examined for obvious pathogens (nematodes, fungi, spore-forming bacteria, and protozoa) or parasitoids. All dead or moribund red oak borer specimens exhibiting overt signs of fungal mycosis (Lacey & Brooks 1997) were transferred to 100 mm Petri dishes containing moist 90 mm Whatman® filter paper to encourage fungal growth. If fungal pathogens sporulated, the spores were transferred with an inoculating loop from the surface of the cadavers to Sabouraud's dextrose agar containing 1% Bacto yeast extract (Difco, Detroit MI) solid media (SDAY) and 0.1% gentamycin sulfate solution (Sigma, St. Louis MO). The plates were then maintained in the dark at 26°C ± 2. Red oak borer specimens exhibiting bacterial signs were placed in Petri dishes with unmoistened filter paper to prevent saprophytic growth. Samples were taken from specimens infected with a pathogen and identified, if possible, using a phase microscope.

One-way analysis of variance (ANOVA) was used to determine significant differences between bole samples with JMP software (SAS Institute 2000) and means were separated using the Tukey-Kramer HSD test. Statistical analysis was done using JMP 5.0.1 (SAS Institute, 2000, Cary, North Carolina).

Results and Discussion

Out of the 882 late stage larvae, pupae and adults collected and examined, 56 dead or moribund individuals were observed (Table 1). The numbers of red oak borers found within a 0.5 m sample ranged from 0 to 17 with a mean of 1.64 (SE ± 0.1). *Beauveria bassiana*-infected red oak borers were collected from 12 of 21 (57.1%) trees. The number of *B. bassiana*-infected red oak borers found within a

Table 2. Numbers and percentages of healthy, dead, and *B. bassiana*-infected red oak borers by life stage.

Life stage	[a]# healthy (%)	[a]# dead (%)	[b]# of dead infected with *B. bassiana* (%)
Late stage larvae	21 (2.4)	26 (2.9)	18 (32.1)
Pupae	151 (17.1)	16 (1.8)	3 (5.4)
Adults	652 (73.9)	11 (1.2)	1 (1.8)
Unidentifiable stage	3 (0.3)	3 (0.3)	2 (3.6)
Total	827	56	24 (42.9)

[a]Numbers and percentages are based on the total number of red oak borers collected (n=882).
[b]Numbers and percentages are based only on the total number of dead red oak borers (n=56).

0.5 m sample ranged from 0 to 2 with a mean of 0.043 (SE ± 0.01). Late stage red oak borer larvae were the most common stage infected with *B. bassiana* comprising 18 of the 24 dead or moribund red oak borers found in the survey (Table 2). *B. bassiana*-infected red oak borer, while not highly prevalent, were readily discovered in the oaks. Thirty red oak borers died of unknown, unidentified causes (Table 1). No nematodes, protozoa, spore-forming bacteria or parasitoids were found in these dead red oak borers. Based on this study, parasitoids and pathogens, other than fungi, were unimportant mortality factors for late stages of red oak borer. Two red oak borer larvae were infected with *Metarhizium anisopliae*, comprising a very small proportion of red oak borer mortality (Table 1). No parasitoids were recovered from any of the 882 late stage red oak borers collected in this study, suggesting that parasitoids have little impact on late stage red oak borers.

Red oak borer final stage larvae killed by *B. bassiana* were typically darker in color than the creamy yellowish-white hue of healthy larvae, and their bodies were extended and very firm. Generally, the *B. bassiana*-infected larvae and pupae had not sporulated within the galleries (Figs. 1 and 2). *B. bassiana*-infected late stage red oak borer did not, however, exhibit the reddish coloration often associated with certain strains of *B. bassiana* (Benham & Miranda 1953). White mycelia emerged from intersegmental membranes of the abdomen of late stage larvae, pupae and adults and from the membranous joints of the antennae and legs of pupae and adults.

Because it is commonly assumed that *B. bassiana* is usually a soil borne pathogen, it was hypothesized that *B. bassiana*-infected red oak borers would be more likely to occur close to the ground than near the top of the red oak borer-infested oak boles. This assumption was determined to be untrue as *B. bassiana*-infected red oak borers were collected throughout the height of sampled trees (Table 3). There was no statistical difference between means of *B. bassiana*-infected red oak borer in 0.5 m samples contained within their respective proportions (Table 3). There was no statistical difference between means of red oak borer collected from 0.5 m samples contained within their respective proportions of the infested bole (F = 206; df = 9, 527 ; P = 0.032) (Table 4). It was difficult to understand how *B. bassiana* infected late stage larva, pupa and adult stages since they are located deep within the oak trees. Slugs, ants or other

Fig. 1. Healthy late-stage larva of red oak borer (length, 31 mm) (top). *Beauveria bassiana*-infected late-stage red oak borer prior to external sporulation (middle). Larvae infected with *B. bassiana* were firm, hard and slightly longer than healthy larvae. Lower larva was dead of unknown causes. Non-sporeforming bacteria were present. Such larvae were extended in length, soft, and had a dark coloration and bad smell (bottom).

small insects may be able to enter the very small attack hole made by neonate red oak borer and may disseminate these *B. bassiana* spores into the red oak borer gallery. Sap beetles (Nitidulidae) are associated with red oak borer galleries (Hay 1974) and have also been demonstrated as autodisseminators of *B. bassiana* (Dowd & Vega 2003). Previous studies have demonstrated mites are carriers of fungal spores (review, Klepzig et al. 2001) which may also explain *B. bassiana*-infected late stage red oak borers located deep within the heartwood of oaks. Precipitation events have been known to assist in droplet dispersal of fungal spores (Miquel 1883, Fitt et al. 1989) and may aid in dissemination near the trunk of trees. Further conidial dispersal may include aerial dispersal (Harrington 1980, Green et al. 2006). Aerial dissemination of conidia (Roy et al. 2006) may also assist these infections throughout the height of the trees. Some data suggests that *B. bassiana* colonizes corn, *Zea mays*, as an endophyte (Bing & Lewis 1991). It is possible that *B. bassiana* may be an endophyte within oak which would explain *B. bassiana* infections in late stage red oak borers; however, there is no evidence for this at present.

Beauveria bassiana either occurs in or has biological control potential against other wood boring tree pests, including *Scolytus scolytus* (Doberski 1981) and *A. glabripennis* (Dubois et al. 2004). We believe that this survey represents the first observance of natural infections of red oak borer with *B. bassiana*. Previous

Fig. 2. Healthy larva of red oak borer (length, 30 mm) (top). The lower larva was killed by *B. bassiana* which shows typical sporulation (length, 33 mm) (bottom).

studies on red oak borer apparently did not include surveys for microbial pathogens. This may have been because before 1999 red oak borer was not considered to be a major threat to the health of an oak dominated forest (Stephen et al. 2001).

Table 3. Mean (±SE) number of *B. bassiana*-infected late stage red oak borers (ROB) per 0.5 m sample over the normalized infested bole height.

# samples	Sample Ht./Infested bole Ht.	Mean # *B. bassiana*-infected ROB per sample (±SE)
34	0.1	0.029 (0.029)a
57	0.2	0.018 (0.018)a
55	0.3	0.055 (0.031)a
55	0.4	0.073 (0.044)a
51	0.5	0.059 (0.043)a
63	0.6	0.063 (0.038)a
53	0.7	0.057 (0.032)a
57	0.8	0.053 (0.030)a
55	0.9	0 (0)a
57	1	0.018 (0.018)a

*Means with the same letter in a column are not significantly different ($P < 0.05$; Tukey-Kramer HSD). $F = 0.617$; df = 9, 527; $P = 0.783$.

Table 4. Mean (±SE) number of late stage red oak borer (ROB) per 0.5 m sample over the normalized infested bole height.

# samples	Sample Ht./Infested bole Ht.	Mean # ROB per sample (±SE)
34	0.1	0.94 (0.28)ab
57	0.2	2.02 (0.46)ab
55	0.3	2.18 (0.36)a
55	0.4	1.98 (0.27)ab
51	0.5	1.76 (0.26)ab
63	0.6	1.81 (0.30)ab
53	0.7	1.79 (0.37)ab
57	0.8	1.60 (0.28)ab
55	0.9	1.33 (0.25)ab
57	1	0.75 (0.24)b

*Means with the same letter in a column are not significantly different ($P < 0.05$; Tukey-Kramer HSD). $F = 2.06$; df = 9, 527; $P = 0.032$

Acknowledgments

The authors thank the Insect Pathology group, Gabriele Boys, Jon Zawislak, Larry Galligan and Geoff Gardner of the University of Arkansas for assistance with data collection; Forest Entomology group Drs. Damon Crook and Melissa Fierke, and Dana Kinney, Vaughn Salisbury, Brent Kelley, Leah Lucio and Steven Wingard of the University of Arkansas for their advice and assistance. We also thank the late Dr. Patrick Fenn for his advice and critique of early manuscripts. This study was supported in part by a grant from Arkansas Agricultural Experiment Research Enhancement Program.

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Survey for Microbial Pathogens of the Red Oak Borer (Coleoptera: Cerambycidae) on Northern Red Oak in Northwest Arkansas[1]

Jason M. Meyers,[2,3] Donald C. Steinkraus,[4] Frederick M. Stephen,[4] and Roger E. Gold[3]

J. Agric. Urban Entomol. 24(2): 87–94 (April 2007)

ABSTRACT An outbreak of red oak borer, *Enaphalodes rufulus* (Haldeman), in Arkansas has resulted in millions of dollars in damage to oak trees. A survey for microbial pathogens of late stages of red oak borer in northwest Arkansas forests demonstrated that red oak borer infected with *Beauveria bassiana* were present in 12 of 21 *Quercus* spp. trees. Oak trees dissected in the survey were located in an area of heavy infestation in the Ozark National Forest, Franklin County, Arkansas. Individual trees were selected for the survey based on a likelihood of *E. rufulus* infestation. Overall prevalence of *B. bassiana*-infection was 2.7%. Infected late stage larvae, pupae and adults were found between May and July 2003. Mean number of late stage larval, pupal and adult red oak borers per 0.5 m bole sample was 1.64 (SE ± 0.1). Mean number of *B. bassiana*-infected red oak borer per 0.5 m bole sample was 0.04 (SE ± 0.01). These data demonstrate that *B. bassiana*-infected red oak borers in *Quercus* spp. were found throughout the height of the tree.

KEY WORDS *Enaphalodes rufulus*, *Beauveria bassiana*, *Quercus*, biological control, microbial pathogen, entomopathogenic fungi

Introduction

Many North American trees are damaged by serious insect pests. These tree-infesting insects include historic pests such as *Dendroctonus frontalis* Zimmerman (Scolytidae) (Price & Doggett 1978), recent exotic pests such as Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky (Cerambycidae) (Cavey et al. 1998) and emerald ash borer, *Agrilus planipennis* Fairmaire (Buprestidae) (Childs 2002). Recently, a normally endemic species, the red oak borer, *Enaphalodes rufulus* (Haldeman) (Cerambycidae), increased to epidemic proportions (Stephen et al. 2001).

Oak-hickory forests in Missouri and northwest Arkansas have undergone a significant population increase in the red oak borer. The USDA Forest Service first discovered this population outbreak in 1999, when widespread mortality was

[1]Received 7 July 2008; Accepted 14 August 2008.
[2]Corresponding author (jmmeyers@tamu.edu).
[3]Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, 2143 TAMU, College Station, Texas 77843.
[4]Department of Entomology, 319 Agriculture Bldg., University of Arkansas, Fayetteville, Arkansas 72701.

Treatment of Pastures with Diflubenzuron Suppresses Horn Fly, *Haematobia irritans* (Diptera: Muscidae) Development[1]

J. K. Tomberlin,[2,3] K. H. Lohmeyer,[4] and D. Kattes[5]

J. Agric. Urban Entomol. 24(2): 95–101 (April 2007)

ABSTRACT Diflubenzuron is an insect growth regulator labeled for application to pastures and rangeland to suppress grasshopper (Orthoptera: Acrididae) populations. Livestock are permitted access to land immediately after treatment. We hypothesized the development and survivorship of horn fly *Haematobia irritans* (L.) larvae feeding on manure resulting from these animals would not be impacted due to the presence of diflubenzuron residue. Survivorship to the adult stage and percent pupae deformed were recorded for horn flies developing on manure samples from three pastures treated with 59 ml Dimilin 2L® /0.4 hectares. Pastures not treated served as the controls. Manure samples ranged in age from three to 31 d post treatment. This study was replicated in 2004 and 2005. Results were highly variable between site and year. Horn fly survivorship to the adult stage in 11 of 15 sample dates taken was lower in treated than the control manure samples. Accordingly, a significantly greater percentage of deformed pupae was recorded for samples from the treated than the control sites ≤17 d post treatment. Based on this study, our null hypothesis was rejected. Using Dimilin 2L to suppress pasture and range land pests can also impact horn fly populations associated with cattle feeding in these pastures. However, care should be taken to apply adequate pasture coverage ensuring appropriate Dimilin 2L® levels are consumed to achieve the suppression of associated horn flies.

KEY WORDS horn fly, *Haematobia irritans*, diflubenzeron

THE HORN FLY, *Haematobia irritans* (L.), (Diptera: Muscidae) is an important blood-feeding pest of cattle (Da Silva and Mendes 2002). Adults reside on the animal's surface, while their larvae develop in fresh cattle manure deposited in pastures (Lysyk and Steelman 2004). Insecticide applications to the animal's hide are the most common method implemented to suppress associated horn fly populations.

Insect growth regulators (IGR) are a class of insecticides that interfere with insect development (Da Silva et al. 2004). Diflubenzuron is an example of an IGR

[1]Received 23 April 2008; Accepted 8 July 2008.
J. K. Tomberlin, Department of Entomology Texas A&M University, College Station, Texas, Phone: (254) 845-9718, Fax: (254) 845-6035, E-mail: jktomberlin@ag.tamu.edu.
[2]Corresponding author (jktomberlin@ag.tamu.edu).
[3]Department of Entomology, Texas A&M University, College Station, Texas.
[4]USDA-ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.
[5]Department of Agronomy, Agribusiness, Horticulture, and Range Management, Tarleton State University, Stephenville, Texas.

that interferes with chitin synthesis during molting (Garnett and Weseloh 1975), and it can suppress fly pest populations associated with cattle (Da Silva and Mendes 2002). Diflubenzuron applied under laboratory conditions at 1 mg/ 929 cm^2 to a breeding-surface area prevented the emergence of house flies, *Musca domestica* (L.) (Diptera: Muscidae), and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) (Wright 1974). Applications to the breeding area under field conditions at 50 mg/929 cm^2 resulted in 90% suppression of house flies (Wright 1974). House flies were suppressed by 97 and 91% when developing in manure produced by cattle given access to a mineral block containing either 0.1 or 0.05% respectively under laboratory conditions (Wright 1975). Horn flies were suppressed 75 and 83% in manure from cows provided mineral blocks with 0.05 and 0.1% respectively (Barker and Jones 1976). Further laboratory trials recorded 99% mortality of face fly larvae *Musca autumnalis* De Geer (Diptera: Muscidae) provided with manure from cattle fed a ration containing <0.5 mg/kg diflubenzuron (Miller 1974).

Dimilin 2L® (22% diflubenzeron, Chemtura Corporation, Middlebury, CT), is a diflubenzuron formulation used in pastures and rangelands in the United States to suppress arthropod pests, such as the differential grasshopper, *Melanoplus differentialis* (Thomas), (Orthoptera: Acrididae) (Amarasekare and Edelson 2004) and the beet armyworm, *Spodoptera exigua* ((Hübner) (Lepidoptera: Noctuidae) (Decombel et al. 2004), while in other countries, such as Denmark, it is labeled for suppressing house fly populations associated with confined animal facilities (Kristensen and Jespersen 2003).

For most insecticide treatments for grasshoppers or armyworms in pastures, cattle are not allowed to return for a set time period after treatment to prevent the animals from being poisoned. However, this is not the case when using Dimilin 2L with cattle returned to pastures immediately after treatment. Currently, it is not known if treating pastures with Dimilin 2L® and allowing cattle to graze on treated forages will suppress horn flies development in resulting cattle manure. The objectives of this study were to: 1) determine if manure collected from cattle feeding on pastures treated with Dimilin 2L resulted in larval horn fly mortality, and 2) if suppression occurs, the duration of horn fly reduction.

Materials and Methods

Sites in Stephenville, Dublin, and Belton, TX were used in this study. At treated sites in Stephenville and Dublin, Dimilin 2L® was applied aerially at 59 ml/hectare. The treatment site in Belton received the same amount but by tractor with a 9.1 m boom sprayer containing nine nozzles spraying at a rate of 215 L solution/hectare. Aerial sprays were applied at a rate of 22.7 L/0.4 hectare. The control sites received no treatments. The treated sites were as follow: 1) Stephenville, 20.2 hectare and 26 cows with calves; 2) Dublin, 24.3 hectares and 20 cows; 3) Belton, 18.2 hectares and 25 cows. The control sites were as follow: 1) Stephenville, 11.3 hectares and 27 cows and 15 calves; 2) Dublin, 16.1 hectares and 20 cows; 3) Belton, 101.1 hectares and 88 cows. Treatments during year one were applied in Belton on 24 May 2004 and in Stephenville and Dublin on 1 June 2004. A second replication of the study was conducted in 2005 during the same time of year with similar cow numbers.

Three manure samples were taken from each treated and control site at a set interval. Initial manure samples were taken 3 d post treatment. Samples were then taken weekly for four weeks for a total of five sample periods. In order to collect samples, adult cows were monitored for deposition of fecal matter with each sample taken from a manure pat deposited from a different adult cow. Once deposited, approximately 500 ml of each fresh manure pat was placed individually in a 500 ml plastic Solo container, covered with a lid, and stored temporarily in a freezer set at 0°C at the Texas Agriculture Experiment Station (TAES) in Stephenville, TX. Samples were eventually transferred and stored in a freezer set at 0°C at the USDA-ARS Kerrville Research Facility, Kerrville, TX.

In order to determine the effects of Dimilin 2L on horn fly development, the following procedures were implemented. The oldest manure samples were removed from the freezer and allowed to thaw approximately 24 h prior to inoculation with horn fly eggs. Samples typically remained in the freezer for six to eight weeks prior to being examined. For each manure sample, 100 g was removed and placed in a 500 ml drinking cup. One hundred horn fly eggs were taken from a colony maintained at the Kerrville facility and placed on a 2.54 cm diameter filter paper. Filter papers with eggs were then placed on the surface of the manure in each cup and covered with a paper towel held in place with a rubber band. Cups were maintained in a growth chamber set at 27°C and 16L:8D photoperiod. A filter paper with 100 horn fly eggs was placed in a petri dish and stored in the rearing chamber to determine percent hatch. Numbers recorded for each sample were adjusted based on percent mortality recorded for the control horn fly egg sample.

Manure samples were sorted and horn fly pupae removed seven days after inoculation. Number of pupae and deformed pupae per sample was recorded for each sample. Deformed pupae were defined as dumbbell shaped, collapsed, or cigar shaped, while healthy pupae were cylindrical. For each sample, pupae were placed in a petri dish lined with a filter paper labeled with sample collection information. Petri dishes with pupae were covered and returned to the growth chamber. Percent adult emergence for each sample and percent normal and deformed pupae were recorded approximately 10–14 d after being placed in petri dishes. Data from each location were combined and analyzed using Proc ANOVA (SAS Institute 1998). Least significant difference (LSD) test was used following a significant F test ($P < 0.05$) to separate means (SAS Institute 1998). Data presented as percent of response were arcsine square root transformed prior to analysis (SAS Institute 1998).

Results

Data for percent survivorship of horn flies reared on manure from treated and control pastures (Figure 1) were examined. No overall significant difference ($F = 2.86$; df = 1, 39.3; $P = 0.0990$) between treated and control pastures was determined for percent survival of horn flies to the adult stage. However, a significant difference ($F = 24.66$; df = 4, 40.5; $P < 0.0001$) for date was determined. Furthermore, an interaction effect ($F = 5.54$; df = 4, 40.6; $P = 0.0012$) was determined between treatment and sample date. Site was also determined to be significant ($F = 3.91$; df = 2, 35.5; $P = 0.0293$) for survivorship to the adult stage. In contrast, year was not determined to be significantly

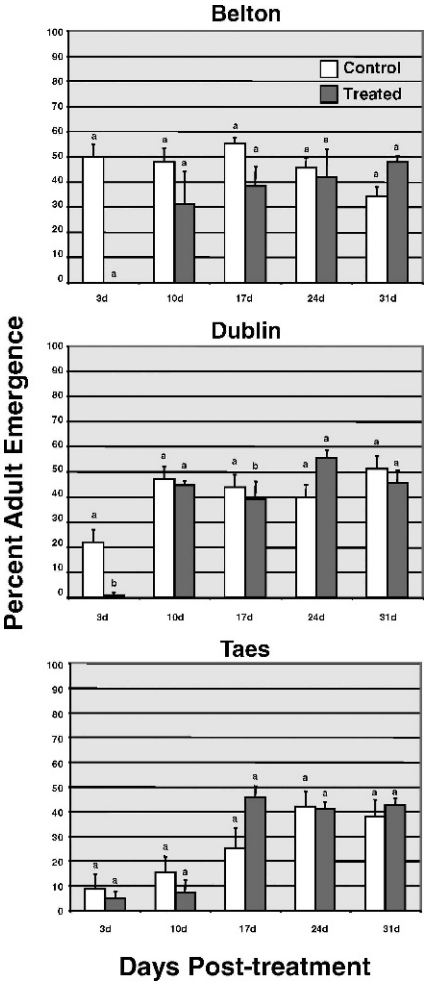

Fig. 1. Percent horn fly adult emergence ±SE when reared on manure sampled three sites treated and untreated with Dimilin 2L®. Means in a column followed by different letters are significantly different ($P \leq 0.05$; LSD, SAS Institute 1998). Values in columns not followed by capital letters were not significantly different across treatments. Percentage data were arcsine transformed prior to analysis.

different ($F = 2.31$; df = 1, 161; $P < 0.1308$). Therefore, data for each year were combined and results are presented in Figure 1. Significantly lower adult emergence was observed in the treated pastures in Dublin at 3 and 17 d post treatment when compared to the untreated control pastures (3d: $F = 80.6$; df = 1, 2; $P < 0.0001$ and 17d: $F = 11.79$; df = 1, 2; $P = 0.0089$).

Data for percent deformed pupae of horn flies reared on manure from treated and control pastures (Figure 2) were also examined. No overall significant difference for percent deformed pupae resulting from horn flies reared on manure

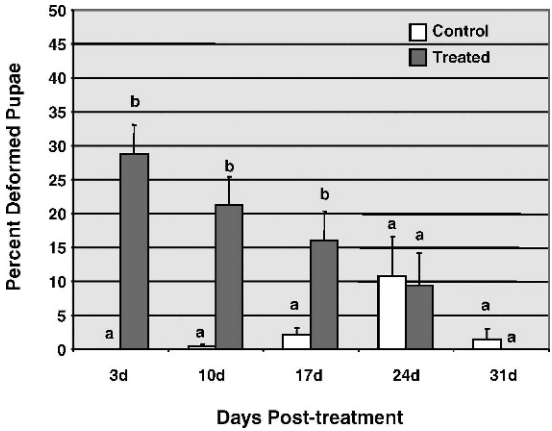

Fig. 2. Percent horn fly pupae deformed ±SE when reared on manure sampled from Dimilin 2L® treated and untreated pastures over time. Means in a column followed by different letters are significantly different ($P \leq 0.05$; LSD, SAS Institute 1998). Values in columns not followed by capital letters were not significantly different across treatments. Percentage data were arcsine transformed prior to analysis.

from treated and control pastures was determined. However, percent deformed pupae by sample date was significantly different. An interaction effect between treatment and date was also recorded. No significant difference in percent deformed pupae by site or year was recorded. Therefore, year and date data were combined and results are presented in Figure 2. Significantly more deformed pupae were observed in the treated pastures at 3, 10, and 17d post treatment when compared to the untreated control pastures (3d: $F = 41.79$; df = 1, 2; $P < 0.0001$; 10d: $F = 27.85$; df = 1, 2; $P < 0.0001$; 17d: $F = 9.48$; df = 1, 2; $P = .0042$).

Discussion

Dimilin 2L is an effective compound for the suppression of pasture and rangeland pests. Additionally, livestock can be returned to sites immediately after treatment. Animals can feed on treated sites without concerns of negative effects due to consumed diflubenzuron passing through the animals and being expelled in their solid wastes. However, an added benefit is that horn flies colonizing resulting wastes are affected.

Results for percent emergence were highly variable across year and site. This effect can be explained in part due to differences between sites. Each site varied in size and the number of cattle per hectare. Such a difference can result in the forage being consumed at much greater rates at one site versus the others. Additionally, consistency in application methodology (i.e. aerial versus tractor) could have resulted in insufficient coverage for some of the sites.

The effect of Dimilin 2L might be better demonstrated in the F_1 generation produced by those individuals to successfully emerge. House flies and stable flies treated topically with a 25% WP difulbenzuron formulation resulted in a 71 and 100% reduction in egg hatch respectively (Wright and Spates 1976). Adult horn

flies housed in cages treated with 1 to 10% diflubenzuron dust resulted in 92 to 89% reduction in hatch of resulting eggs respectively (Kunz and Bay 1977).

The effects of Dimilin 2L on horn fly develop were more apparent when examining percent pupal deformation. Significant levels of percent deformation were recorded for samples taken up to 17 d post treatment. Therefore, although no difference in emergence was recorded between horn flies reared on manure from treated and control sites, a significant impact on the target horn fly population might be present due to reduced mobility and egg hatch from resulting adults.

Although studies examining the use of diflubenzuron for suppressing the horn fly date to the 1970s, we are not aware of it being labeled for such use until Chemtura Corporation added this information to its Dimilin 2L label in 2005. The reason why such a delay occurred between the publishing of Kunz and Bay (1977) and the many other studies on the use of this compound as a feed through and being marketed for horn fly control is not known. Regardless, its use for grasshopper control today can result in indirect benefits due to its impact on horn fly populations associated with livestock placed on these treated sites.

Horn flies represent only one of many species involved in the food web with cattle manure as its foundation. Other insects, such as dung beetles (Coleoptera: Scarabaeidae) depend on the manure as resource for themselves as well as their offspring. Additionally, the parasitic wasp *Muscidifurax raptor*, Girault and Saunders (Hymenoptera: Pteromalidae), as well as many others will target and colonize specific dipteran species commonly associated with the manure. Few data are available on the effects of diflubenzuron on these other arthropods. Dimilin appears to have no effect on the emergence of *M. raptor* or its ability to parasitize other hosts (Ables et al. 1975), but the effects on dung beetles are not known. Future studies need to examine more closely the effects of diflubenzuron on the fecundity of horn flies that successfully pupate in treated manure, as well as, its effects on other biological control agents including dung beetles.

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Treatment of Pastures with Diflubenzuron Suppresses Horn Fly, *Haematobia irritans* (Diptera: Muscidae) Development[1]

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KEY WORDS horn fly, *Haematobia irritans*, diflubenzeron

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[1]Received 23 April 2008; Accepted 8 July 2008.
J. K. Tomberlin, Department of Entomology Texas A&M University, College Station, Texas, Phone: (254) 845-9718, Fax: (254) 845-6035, E-mail: jktomberlin@ag.tamu.edu.
[2]Corresponding author (jktomberlin@ag.tamu.edu).
[3]Department of Entomology, Texas A&M University, College Station, Texas.
[4]USDA-ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, Texas.
[5]Department of Agronomy, Agribusiness, Horticulture, and Range Management, Tarleton State University, Stephenville, Texas.

ISSN 1523-5475

Journal of Agricultural and Urban Entomology

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Volume 24, No. 3 July 2007

THE JOURNAL OF AGRICULTURAL AND URBAN ENTOMOLOGY

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Volume 24 • Number 3 • 2007

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Committee Chairpersons:

Auditing: Merle Shepard, Clemson University, Coastal REC, 2700 Savannah Highway, Charleston, SC 29414: phone 843.402.5393

Honors and Awards: Pat Zungoli, Clemson University, Urban Entomology Lab, 114 Long Hall, Clemson, SC 29634; phone 864.656.3137; fax 864.656.5065

Local Arrangements: Francis Reay-Jones, Clemson University, Pee Dee REC, 2200 Pocket Rd., Florence, SC 29506: phone 843-662-3526 ext. 208; fax 843-661-5676

Long Range Planning: Gerry Carner, Clemson University, 318 Long Hall, Clemson, SC 29634; phone 864.656.5040; fax 864.656.5065

Membership: Pat Zungoli, Clemson University, Urban Entomology Lab, 114 Long Hall, Clemson, SC 29634; phone 864.656.3137; fax 864.656.5065

Nomination: D. Michael Jackson, USDA/ARS /Veg. Lab., 2700 Savannah Highway, Charleston, SC 29414; phone 843.402.5308; fax 843.571.4654

Program: Jeremy K. Greene, Clemson University Edisto REC, 64 Research Rd., Blackville, SC 29817; phone 864.284-3343; fax

Publicity: Alvin Simmons, USDA/ARS/Veg. Lab., 2700 Savannah Highway, Charleston, SC 29414; phone 843.402.5307; fax 843.573.4715

Resolutions: Paula Mitchell, Winthrop Univ. Biology Dept., Rock Hill, SC, 29733; fax 803-323-3448

Influence of Neonicotinoid Insecticides on Infection by *Neozygites fresenii* (Nowakowski) Batko (Entomophthorales: Neozygitaceae) in the Cotton Aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) in South Carolina[1]

Ruly Anwar,[2] Gerald R. Carner,[2] Joseph D. Culin,[2] Hoke S. Hill,[3] and Thomas M. McInnis[4]

J. Agric. Urban Entomol. 24(3): 103–116 (July 2007)

ABSTRACT Incidence of infection by *Neozygites fresenii* (Nowakowski) Batko in the cotton aphid, *Aphis gossypii* Glover was monitored biweekly during the summer of 2002 in a cotton field in Bamberg County, SC. (33°22′02″N, 81°12′14″W). Five treatments were evaluated to determine the effects of insecticides on incidence of *N. fresenii*: acetamiprid, dicrotophos, thiamethoxam, imidacloprid, and control. Aphids were sampled by taking 24 leaves from 12 cotton plants from each plot and placed into 30-ml screw cap vials filled with 70% ethanol. Fungus infection in aphids, numbers of aphids, percentage of winged aphids, and fungus infection in winged aphids were determined from aphids for each plot.

The highest fungus infection occurred on July 12 and 16. Acetamiprid treated plots had infection levels of *N. fresenii* lower than other treatments. Cotton aphid numbers in acetamiprid and thiamethoxam plots were significantly lower than in the dicrotophos treatment and untreated plots. None of the treatments caused reductions in percentage of winged aphids or in infection levels by *N. fresenii* in winged aphids.

KEY WORDS cotton aphid, *Aphis gossypii*, Homoptera, Aphididae, *Neozygites fresenii*, Entomophthorales, neonicotinoid insecticides, dicrotophos

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is an economic pest of cotton in the southeastern and southwestern United States (Steinkraus et al. 1991). High aphid populations can have negative impacts on cotton yield and result in economic losses. Williams (2003) reported that in 2002, the cotton aphid was regarded as the sixth most damaging pest of U.S. cotton. The aphid infested 70.3% of U.S. cotton, causing a 0.119% reduction in yield in 9,307,757 infested acres, resulting in a loss of 31,450 bales. This pest continues to be a concern because of its potential for rapid reproduction and ability to develop resistance. Outbreaks of cotton aphids have been associated with reductions in natural enemy populations and aphid resistance to pesticides (Grafton-Cardwell 1991).

[1]Accepted 12 September 2008.
[2]Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, South Carolina 29634.
[3]Department of Applied Economics and Statistics, Clemson University, Clemson, South Carolina 29634.
[4]Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634.

Three neonicitinoid insecticides (acetamiprid, thiamethoxam, and imidacloprid) and one organophosphate (dicrotophos) have been evaluated for control of the cotton aphid (Gibson et al. 2003, Earnest et al. 2000). Acetamiprid and thiamethoxam have provided adequate control of aphids. Dicrotophos, which previously was the insecticide of choice against this pest, has been reported to be ineffective against the cotton aphid (Earnest et al. 2000). Imidacloprid was less effective in controlling aphids than acetamiprid or thiamethoxam (Gibson et al. 2003).

The entomopathogenic fungus, *Neozygites fresenii* (Entomophthorales: *Neozygitaceae*) has been reported as the causal agent of epizootics in cotton aphid populations. This fungus has been active in reducing aphid populations in the Midsouth and Southeast since 1989 and was determined to be the major natural regulating agent of this pest in this region (Steinkraus et al. 2003). Epizootics of this fungus in cotton aphids generally occur in Arkansas from mid-July to mid August (Steinkraus et al. 1991).

Because of the importance of this fungus in suppressing the cotton aphid, it is essential that any new management tactic be evaluated for its effect on this fungus. Currently, there is no information on the effect of neonicotinoid insecticides on incidence of *N. fresenii*. The objective of this study was to determine effects of the neonicotinoid insecticides, acetamiprid, thiamethoxam, and imidacloprid, and the organophosphate, dicrotophos, on the incidence *N. fresenii* in the field.

Materials and Methods

Four insecticides were evaluated to determine their effects on incidence of *N. fresenii*: acetamiprid (Intruder 70WP, Dupont, Wilmington, DE), dicrotophos (Bidrin SE, Amvac Chemical Corp., Los Angeles, CA), thiamethoxam (Centric 40WG, Syngenta Crop Protection, Greensboro, NC), imidacloprid (Trimax, 4F, Bayer CropScience, Research Triangle Park, NC), and an untreated control. Experimental cotton plots (5415RR variety) were established at the Sandifer Farm, Bamberg County, SC. Cotton was planted on 26 April 2002 in 15 m $\times$ 12 rows plots. The experiment was designed as a split plot randomized block design consisting of four blocks. Dates of sampling were the main factors and insecticide treatments were the subfactors. All insecticide treatments were applied on 19 June and 3 July 2002. In addition, on 26 June, only dicrotophos and imidacloprid were applied. Acetamiprid, thiamethoxam, and imidacloprid were applied at the rate of 0.05 kg (a.i.)/ha and dicrotophos was applied at a rate of 0.56 kg (a.i.)/ha. Karate® was applied for bollworm on 12 and 17 July, 2002 in all plots.

Cotton aphids were sampled twice weekly between 2 July and 23 July 2002. Twelve cotton plants were selected systematically for each plot and two leaves were removed from each of these plants. Leaves were preserved in 30 mL screw cup vials filled with 70% alcohol, and the aphids were later processed in the laboratory to confirm presence of *N. fresenii*. Percent of aphid infection was determined from numbers of all aphids including winged aphids per plot by dividing the numbers of aphids with fungus by the total numbers of aphids sampled, then multiplying by 100. Microscope slide squash mounts in lactophenol fuchsin were made for aphids collected on 2, 9, 16, 19, and 23 July and each aphid was examined with a microscope for secondary conidia, hyphal bodies, conidiophores, primary conidia, and resting spores (Steinkraus et al. 1991).

Aphids were classified into one of the following six categories based on Steinkraus et al. (1995): (1) uninfected, (2) with secondary conidia attached to aphid's leg, antennae or body, (3) with hyphal bodies, (4) with conidiophores and primary conidia, (5) with resting spores, and (6) with saprophytic fungi.

Aphid numbers for each plot were determined by counting aphids in samples from each plot. Percentage of winged aphids was obtained from the proportion of winged aphids in the total of sampled aphids in each plot. Fungus infection levels in winged aphids were obtained from numbers of infected winged aphids in numbers of all winged aphids from each plot.

All data were analyzed for treatment effects using ANOVA (PROC GLM SAS Institute Version 9.1 2004). Numbers of aphids were transformed using $\log(\sqrt{x} + 0.05)$ and percentage values such as % fungus infection in aphids (total of winged and non-winged aphids), % winged aphids in aphid populations, and % fungus infection in winged aphids were arcsine transformed to homogenize variance among treatments. For all significance factors, Tukey's test was used to determine differences between means at $P < 0.05$.

Results and Discussion

Infection levels by *N. fresenii*. Effects of neonicotinoid insecticides on fungus infection by *N. fresenii* are shown in Table 1 and Figure 1. Infection levels of *N. fresenii* varied significantly between insecticide treatments ($F = 2.86$, df = 21, $P = 0.0004$). Infection by this fungus occurred in the field for the first time on July 2 in all treatment plots except the acetamiprid plots and the levels were below 20% in all plots. On July 5 and 9, fungus infection levels in untreated plots were higher than in insecticide treated plots, but these levels did not differ significantly and infection levels still remained low, except for untreated plots (20.13%). After July 9, percent infection rose gradually and the highest infection levels were reached in mid-July (16 July). On 19 July, percent infection decreased and resting spores occurred in dicrotophos and thiamethoxam plots. Significant differences in infections levels among treatments occurred only on July 12 and 16. On July 12, infection increased dramatically in all plots with rates as high as 95% in the thiamethoxam treatments and 85% in the untreated check. Infection levels by *N. fresenii* were much lower in acetamiprid and dicrotophos plots than in thiamethoxam and untreated plots. The imidacloprid treatment had fungus infection levels lower than in untreated plots, but these were not significantly different. On July 16, lower infection levels occurred only in acetamiprid plots and these were only significantly different from the dicrotophos plots. Infection levels remained high in most of the treatments until aphids disappeared from the plots. By July 23, only a few aphids were found in dicrotophos and untreated control treatments and there were no aphids in the other treatments (Table 1).

Aphid populations. Numbers of cotton aphids obtained from samples per plot varied significantly between insecticide treatments ($F = 24.05$, df = 4, $P < 0.0001$) and between sampling dates ($F = 8.80$, df = 5, $P < 0.0001$). However, numbers did not differ significantly on individual dates ($F = 1.63$, df = 20, $P = 0.0679$). The highest numbers of cotton aphids obtained per plot occurred in the dicotrophos treatment and these were significantly different from neonicotinoid insecticide treatments. Untreated plots had aphid numbers lower than dicrotophos, but these were not significantly different. Numbers of aphids in

Table 1. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* infection levels in cotton aphid, *Aphis gossypii* at Bamberg County, SC. in July 2002.

	% infection by *N. fresenii* in each insecticide treatment				
Date	Acetamiprid	Dicrotophos	Thiamethoxam	Imidacloprid	Untreated
6/28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7/2	0.00 ± 0.00	8.50 ± 9.15	2.75 ± 5.50	10.20 ± 5.81	19.75 ± 11.15
7/5	7.92 ± 9.17	12.00 ± 4.32	5.00 ± 10.00	6.58 ± 13.16	14.83 ± 13.79
7/9	5.63 ± 6.57	8.00 ± 9.09	8.75 ± 10.31	6.00 ± 5.89	20.13 ± 13.74
7/12	38.75 ± 35.06 c	39.52 ± 15.02 c	95.39 ± 6.74 a	42.78 ± 24.72 bc	83.38 ± 12.72 ab
7/16	38.47 ± 21.31 b	81.15 ± 10.47 a	78.42 ± 4.15 ab	58.14 ± 18.26 ab	56.61 ± 18.90 ab
7/19	18.33 ± 17.06	66.59 ± 29.88	52.23 ± 8.36	46.68 ± 30.16	72.57 ± 13.94
7/23	-	100.00 ± 0.00	-	-	92.62 ± 7.34

Means within a date followed by the same letter are not significantly different $P > 0.05$.
Means without letters in the same row are not significantly different $P > 0.05$.

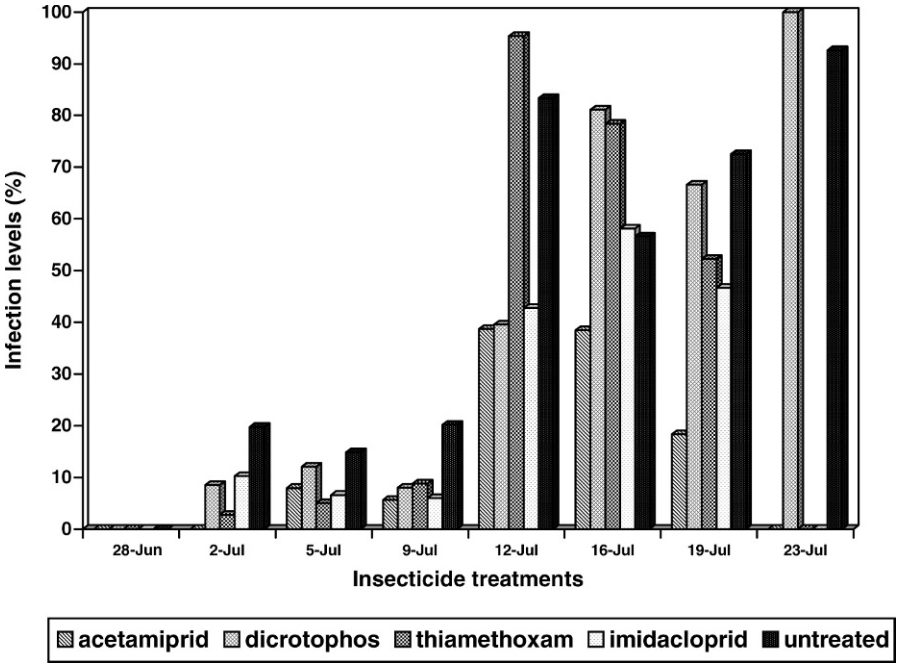

Fig. 1. Effect of three neonicotinoid and organophosphate insecticides on *N. fresenii* infection levels in cotton aphid, *Aphis gossypii* in Bamberg County, SC in July 2002.

acetamiprid and thiamethoxam plots were significantly lower than in untreated plots. The highest numbers of aphids were collected in midseason (July 9, 12 and 16), with peak aphid numbers occurring on July 12. This number was significantly higher than those on July 2, 5, and 19 (Table 2).

Winged aphid numbers. Percentage of winged aphids in aphid samples did not differ significantly between insecticide treatments (F = 1.37, df = 21, P = 0.1602). However, winged aphid populations changed over time (F = 5.26, df = 6, P = 0.0002). Winged aphids occurred for the first time in the field on July 2, except for untreated plots. The highest levels of winged aphids occurred on July 5 in all treatment plots with an average of 18.56%. The one exception to this was the dicrotophos treatment which peaked on July 9. Table 3 shows that thiamethoxam, acetamiprid, imidacloprid, and untreated plots had the highest levels of winged aphids (45.00%, 24.17%, 12.28%, and 11.33%, respectively). The highest level of winged aphids in the dicrotophos plots occurred on July 9 and was only 2.50%. Average of percentage winged aphids differed between sampling dates with levels on July 5 and 9 higher than on other dates (Table 3).

Fungus infection in winged aphids. Infection levels in winged aphids were not affected by either insecticides or date of sampling (F = 1.17, df = 46, P = 0.2617). However, infection occurred on all sample dates (July 2–July 23). Fungus Infection did not occur in untreated plots on the first three sampling dates, but levels were high in the last three sampling dates. In acetamiprid plots, the first infection occurred on July 9 (6.25%) and levels reached a peak on July 19

Table 2. Effect of three neonicotinoid and one organophosphate insecticides on numbers of the cotton aphids, *Aphis gossypii* collected from insecticide treated plots at Bamberg County, SC in July 2002.

	Numbers of cotton aphids in each insecticide treatment (aphids/plot)					
Date	Acetamiprid	Dicrotophos	Thiamethoxam	Imidocloprid	Untreated	Average
7/2	18.50 ± 21.25	50.00 ± 0.00	8.75 ± 4.50	27.25 ± 17.45	42.50 ± 15.00	29.40 ± 19.98 cd
7/5	16.00 ± 16.25	50.00 ± 0.00	3.50 ± 1.29	12.00 ± 7.39	28.25 ± 25.28	21.95 ± 20.63 d
7/9	14.00 ± 7.53	38.25 ± 23.50	25.25 ± 17.04	41.75 ± 16.50	47.505.00	33.35 ± 18.54 abc
7/12	36.25 ± 2.36	49.50 ± 1.00	29.50 ± 13.18	48.0 ± 02.94	51.00 ± 1.83	42.85 ± 13.31 a
7/16	21.25 ± 10.87	51.50 ± 1.73	32.50 ± 11.68	48.50 ± 3.79	49.25 ± 0.96	40.60 ± 13.77 ab
7/19	9.75 ± 6.55	47.50 ± 3.11	11.7 ± 53.59	31.5 ± 018.63	49.75 ± 2.99	30.05 ± 19.24 bcd
average	19.29 ± 15.96 c	47.79 ± 9.71 a	18.54 ± 14.47 c	34.83 ± 17.44 b	44.71 ± 13.49 ab	

Means within a row or column followed by the same letter are not significantly different $P > 0.05$.
Means without letters in the same row are not significantly different $P > 0.05$.

Table 3. Effect of three neonicotinoid and one organophosphate insecticides on percentage of winged aphid in cotton aphid populations, *Aphis gossypii*, collected from insecticide treated plots at Bamberg County, SC in July 2002.

	% winged aphids in each of the insecticide treatments					
Date	Acetamiprid	Dicrotophos	Thiamethoxam	Imidacloprid	Untreated	Average
7/2	9.38 ± 11.97	2.00 ± 1.63	10.84 ± 13.16	3.61 ± 5.24	0.00 ± 0.00	3.29 ± 7.00 b
7/5	24.17 ± 19.08	0.00 ± 0.00	45.00 ± 52.60	12.28 ± 15.89	11.33 ± 15.29	18.56 ± 28.58 a
7/9	16.47 ± 22.73	2.50 ± 5.00	8.52 ± 6.25	4.50 ± 5.26	0.00 ± 0.00	6.40 ± 11.44 a
7/12	3.75 ± 3.50	0.50 ± 1.00	1.55 ± 1.99	1.00 ± 2.00	2.93 ± 1.95	1.95 ± 2.35 b
7/16	0.00 ± 0.00	2.42 ± 1.81	0.00 ± 0.00	1.09 ± 1.27	2.53 ± 2.51	1.21 ± 1.75 b
7/19	5.28 ± 6.11	2.21 ± 2.55	0.00 ± 0.00	0.00 ± 0.00	0.49 ± 0.98	1.59 ± 3.37 b
Average	8.28 ± 9.86 a	5.87 ± 9.86 a	3.33 ± 9.86 a	1.66 ± 9.86 a	1.60 ± 1.07 a	

Means within a row or column followed by the same letter are not significantly different $P > 0.05$.
Means without letters in the same row are not significantly different $P > 0.05$.

(50%). Thiamethoxam plots had infected winged aphids only on July 5, 9, and 12 with a peak level of 45% on July 12. Imidacloprid plots had infected winged aphids on all sampling dates except July 19 with a peak on July 16 (50%). The highest infection level of winged aphids occurred in dicrotophos plots on July 16 (62.50%) (Table 4).

Fungus development stages. On July 2, *N. fresenii* was found in all plots, except for acetamiprid plots (Fig. 2a). Fungus stages at that time were dominated by hyphal bodies, with some secondary and primary conidia. On July 5 (Fig. 2b) and 9 (Fig. 3a), all plots had *N. fresenii* with hyphal bodies and primary and secondary conidia. Fungus infection at that time remained low, except in untreated plots which reached 20%. Peak fungus infection occurred on July 12 (Fig. 3b) and 16 (Fig. 4a). Hyphal bodies of the fungus still remained high in all plots. Fungus infection increased dramatically, especially in thiamethoxam and untreated plots. On July 16, fungus infection levels in thiamethoxam and dicrotophos plots were high. However, levels in dicrotophos plots were only significantly different from the acetamiprid treatment. Fungal stages in dicrotophos plots on July 16 were dominated by hyphal bodies, primary conidia, and saprophytic fungi. This was different from thiamethoxam plots where hyphal bodies were the dominant stage. On July 19, all categories of fungal stages occurred in the field including resting spores and saprophytic fungi (Fig. 4b). At the end of season (Fig. 5), the fungus only occurred in dicrotophos and untreated plots. In dicrotophos plots, saprophytic fungi dominated while in untreated plots there were both hyphal bodies and saprophytic fungi.

Discussion. On July 12 and 16, infection levels in acetamiprid plots were significantly lower ($P < 0.05$) than in untreated plots. This compound is also the most effective in reducing aphid populations (Gibson et al., 2003). Thus, it appears that acetamiprid had an indirect effect on the fungus by delaying the onset of the epizootic in the lower population of aphids. However, this effect was brief, since there were no significant differences in infection levels for any of the treatments on July 5, 9, and 19.

There is usually a pattern that fungus infection by *N. fresenii* occurs in high aphid populations. Also, winged aphids are usually produced when aphid populations are high. However, winged aphid numbers were higher in insecticide treatments. Lower fungus infection levels in acetamiprid plots occurred in the presence of lower aphid numbers, but these plots were higher in numbers of winged aphids. It may be that winged aphids were moving into these plots from neighboring plots where aphid populations were higher.

Acetamiprid was the only material that appeared to suppress infection levels throughout the test, with significantly lower infection levels recorded on July 12 and 16. On the other hand, infection in thiamethoxam plots, which started out low on July 2, rebounded to levels higher than in the check plots, but not significantly different, on July 12 and 16.

Some insecticides such as acetamiprid and thiamethoxam are effective in reducing aphid populations (Gibson et al. 2003). Table 2 in our results shows that these neonicotinoid insecticides had lower aphid numbers per plot compared to dicrotophos and untreated plots. Aphid populations in these plots tended to have lower infection than in untreated and dicrotophos plots. Thus, it appears that these two insecticides have an indirect effect on *N. fresenii*. Earnest et al. (2000) also reported that dicrotophos was not effective against aphids in 1999 compared

Table 4. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* infection levels in winged cotton aphids, *Aphis gossypii* in Bamberg County, SC in July 2002.

	% infection by *N. fresenii* in insecticide treatments					
Date	Acetamiprid	Dicrotophos	Thiamethoxam	Imidacloprid	Untreated	Average
7/2	0.00 ± 0.00	25.00 ± 50.00	0.00 ± 0.00	25.00 ± 50.00	0.00 ± 0.00	10.00 ± 30.78 a
7/5	0.00 ± 0.00	0.00 ± 0.00	6.25 ± 12.50	25.00 ± 50.00	0.00 ± 0.00	6.25 ± 22.76 a
7/9	6.25 ± 12.50	15.00 ± 30.00	25.00 ± 50.00	33.33 ± 47.14	0.00 ± 0.00	15.92 ± 32.66 a
7/12	25.00 ± 50.00	0.00 ± 0.00	50.00 ± 57.74	12.50 ± 25.00	62.50 ± 47.87	30.00 ± 44.13 a
7/16	0.00 ± 0.00	62.50 ± 47.87	0.00 ± 0.00	50.00 ± 57.74	66.67 ± 47.14	35.83 ± 46.60 a
7/19	50.00 ± 57.74	37.50 ± 47.87	0.00 ± 0.00	0.00 ± 0.00	25.00 ± 50.00	22.50 ± 41.28 a
Average	13.54 ± 20.32 a	16.40 ± 20.32 a	21.25 ± 20.32 a	19.17 ± 20.32 a	23.33 ± 24.07 a	

Means within a row or column followed by the same letter are not significantly different $P > 0.05$.
Means without letters in the same row are not significantly different $P > 0.05$.

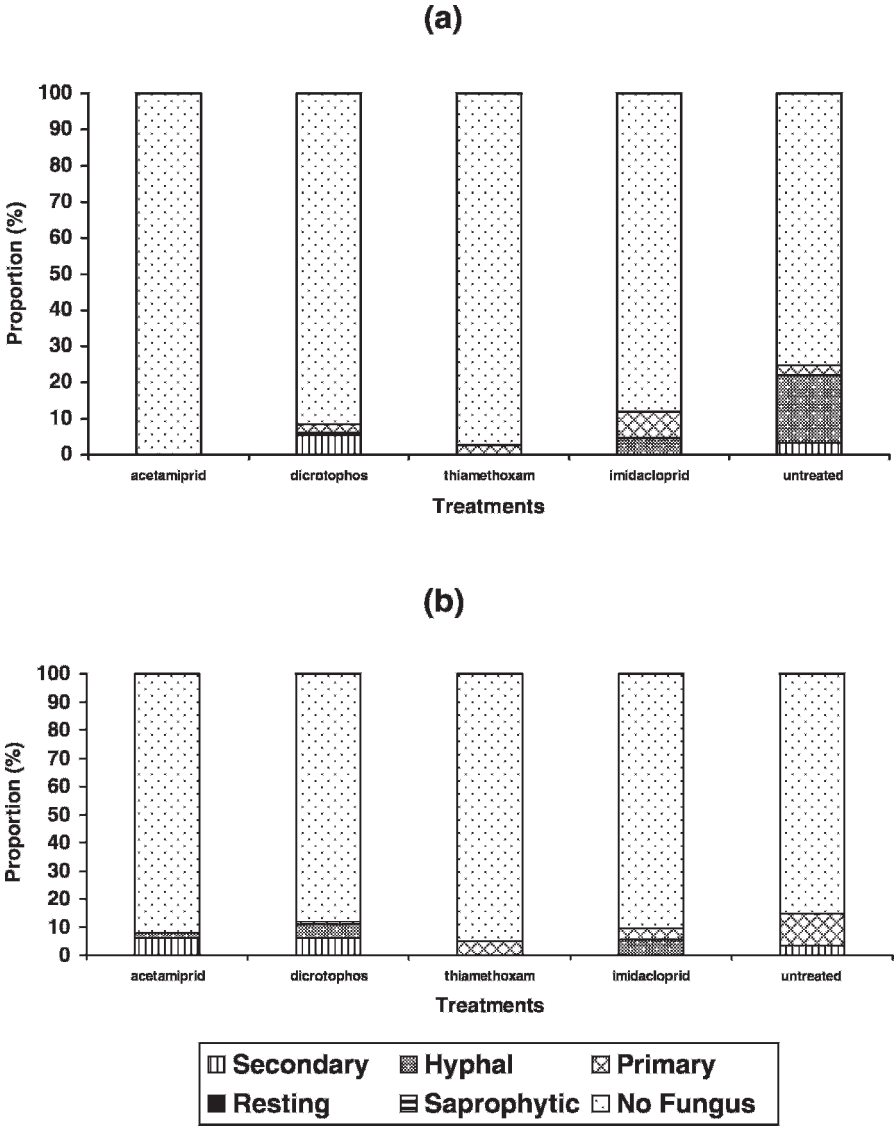

Fig. 2. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* development stage in cotton aphid, *Aphis gossypii* in Bamberg County, SC on 2 July (a) and 5 July (b), 2002.

with early years. However, high aphid infection only occurred in untreated plots. These data indicate that dicrotophos may have a deleterious effect on *N. fresenii* because of the negative correlation between aphid populations and fungus infection on July 12.

N. fresenii is one of the most important natural enemies of the cotton aphid. Our data showed that insecticide treatments did not affect winged aphid

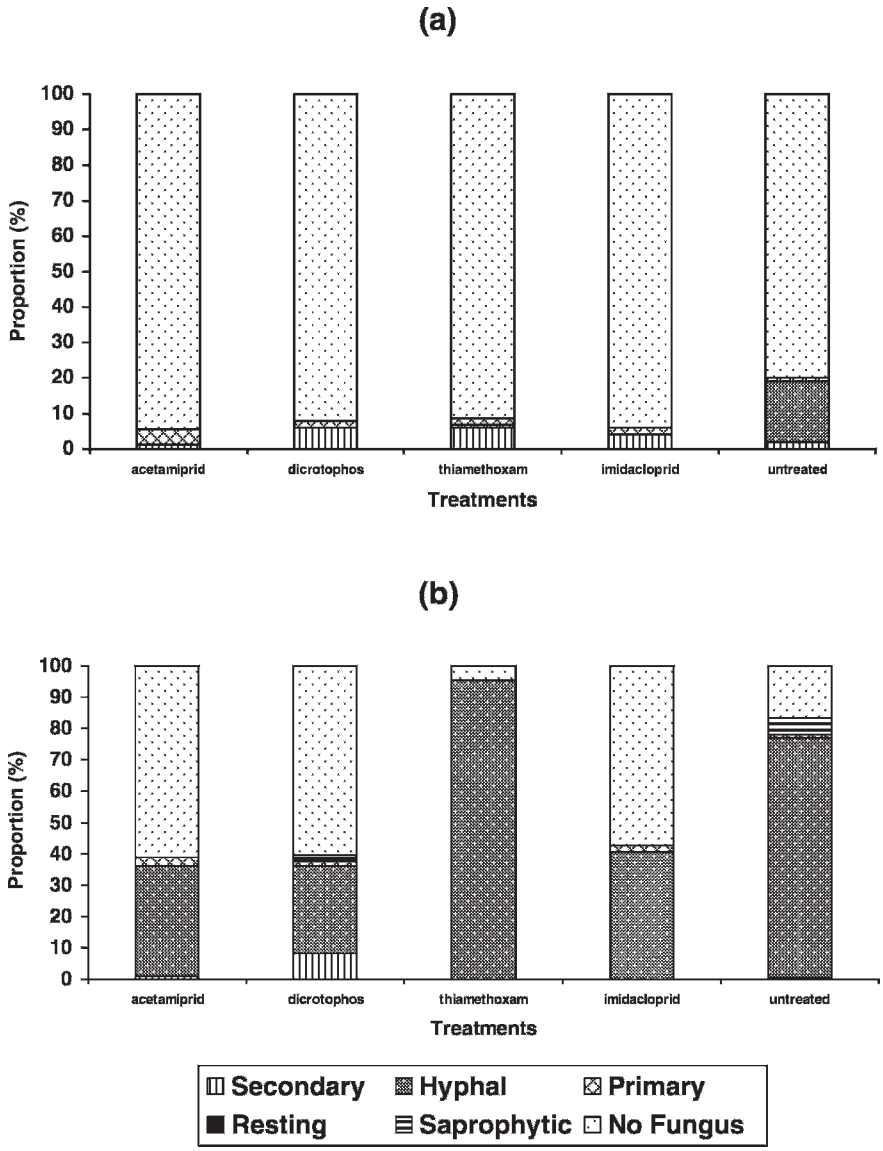

Fig. 3. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* development stage in cotton aphid, *Aphis gossypii* in Bamberg County, SC on 9 July (a) and 12 July (b), 2002.

production and fungus infection by *N. fresenii* in winged aphids. However, winged aphid productions in untreated plots for every sampling date were lower than in insecticide treatments. Winged aphids in neonicotinoid insecticide treated plots, especially in the acetamiprid treatment, were higher than in untreated plots. Conway et al. (2003) reported that imidacloprid-treated cotton

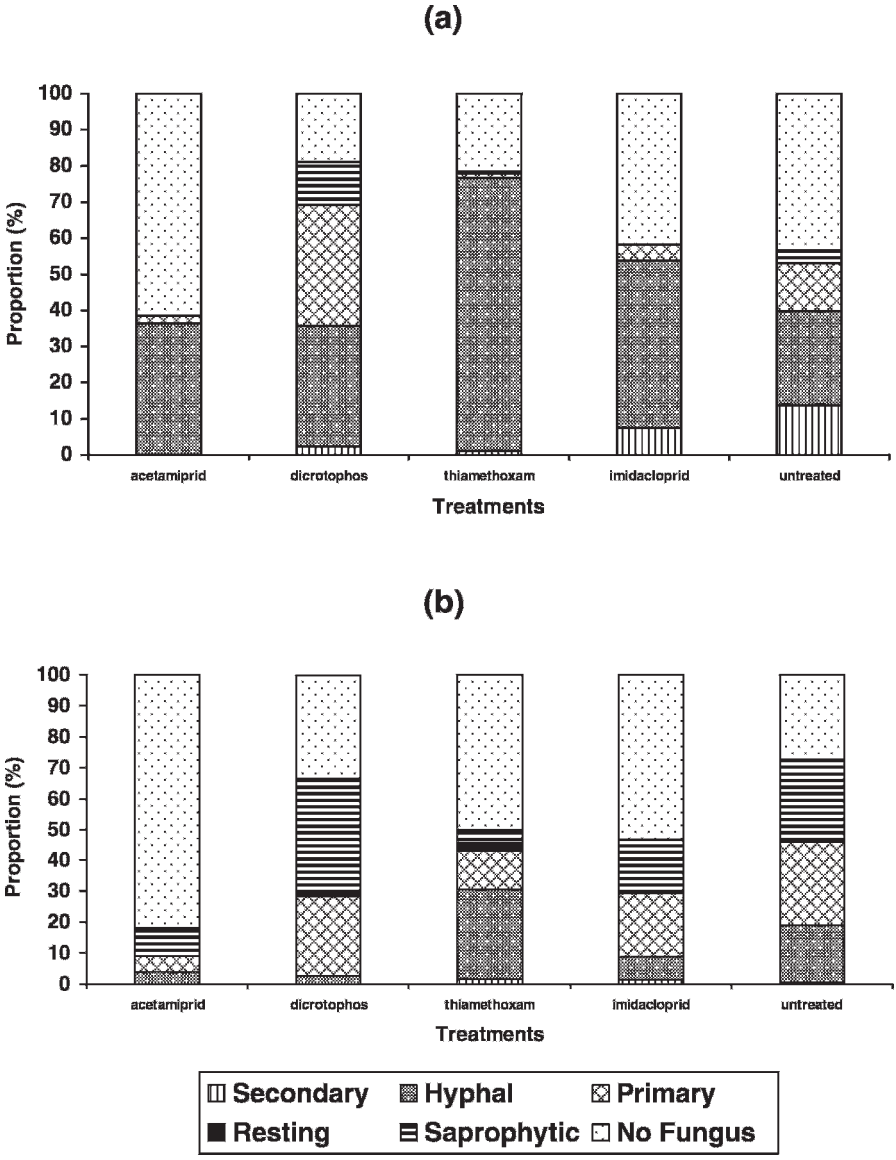

Fig. 4. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* development stage in cotton aphid, *Aphis gossypii* in Bamberg County, SC on 16 July (a) and 19 July (b), 2002.

had 12% alatae offspring compared with only 2% in the control plants. However, fecundity of treated alatae decreased in the treated plots compared to the control (4.9 offspring/adult compared with 9.2 offspring/adult in control plants). Kay and Steinkraus (2004) mentioned that cotton aphids infected by *N. fresenii* produced significantly less offspring and honeydew than uninfected aphids.

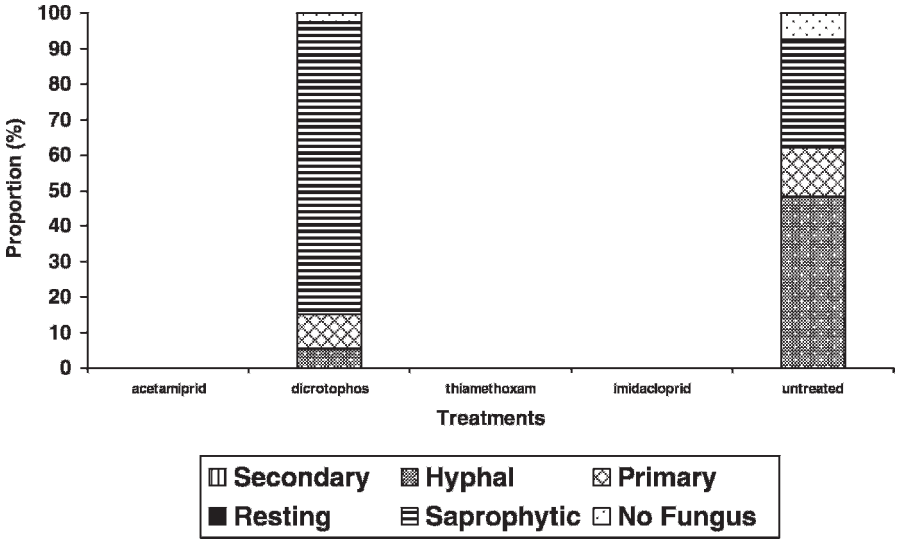

Fig. 5. Effect of three neonicotinoid and one organophosphate insecticides on *N. fresenii* development stage in cotton aphid, *Aphis gossypii* in Bamberg County, SC on 23 July, 2002.

On 19 July, there were resting spores in the field, although we only found these in dicrotophos and thiamethoxam plots. Pell et al. (2001) mentioned that resting spores are the most important way that Entomophthorales survive periods when the host is not present or active. In our study, the end of the epizootics of this fungus occurred when these spores were formed. Several days after that, our sampling did not detect cotton aphids or the fungus in the field.

The fungus life cycle is quite rapid and time from the initial aphid contact with secondary conidia to the death of the host and fungal sporulation can be as short as 3 days (Steinkraus et al. 1993). Therefore, it is likely that there were multiple fungus life cycles that occurred in the field during one season. For almost all dates, every fungus stage could be found in the field. This is further evidence that overlapping generations occurred in the field. Also, all stages of the cotton aphid were susceptible to this fungus.

From these studies, we can conclude that acetomiprid and thiomethoxam are the two most effective materials for suppression of cotton aphid populations. However, fungus infection levels were significantly higher in thiomethoxam than in acetomiprid plots. There could be two explanations for this. Thiomethoxam may cause aphids to be more susceptible to the fungus, or acetomiprid may inhibit development of the fungus. In spite of high aphid numbers in dicrotophos and untreated plots, highest aphid infection only occurred in untreated plots.

Acknowledgments

We thank Dr. Sam Turnipseed and Dr. M. Sullivan for help in setting up the field studies. We are also grateful to Amy Gibson and the Edisto REC crews for helping in the field work. The authors also thank to Eric Benson and Merle Shepard for reviewing earlier

versions of this manuscript. This is technical contribution number 5481 of the South Carolina Agricultural Experiment Station, Clemson University.

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Influence of Neonicotinoid Insecticides on Infection by *Neozygites fresenii* (Nowakowski) Batko (Entomophthorales: Neozygitaceae) in the Cotton Aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) in South Carolina[1]

Ruly Anwar,[2] Gerald R. Carner,[2] Joseph D. Culin,[2] Hoke S. Hill,[3] and Thomas M. McInnis[4]

J. Agric. Urban Entomol. 24(3): 103–116 (July 2007)

ABSTRACT Incidence of infection by *Neozygites fresenii* (Nowakowski) Batko in the cotton aphid, *Aphis gossypii* Glover was monitored biweekly during the summer of 2002 in a cotton field in Bamberg County, SC. (33°22′02″N, 81°12′14″W). Five treatments were evaluated to determine the effects of insecticides on incidence of *N. fresenii*: acetamiprid, dicrotophos, thiamethoxam, imidacloprid, and control. Aphids were sampled by taking 24 leaves from 12 cotton plants from each plot and placed into 30-ml screw cap vials filled with 70% ethanol. Fungus infection in aphids, numbers of aphids, percentage of winged aphids, and fungus infection in winged aphids were determined from aphids for each plot.

The highest fungus infection occurred on July 12 and 16. Acetamiprid treated plots had infection levels of *N. fresenii* lower than other treatments. Cotton aphid numbers in acetamiprid and thiamethoxam plots were significantly lower than in the dicrotophos treatment and untreated plots. None of the treatments caused reductions in percentage of winged aphids or in infection levels by *N. fresenii* in winged aphids.

KEY WORDS cotton aphid, *Aphis gossypii*, Homoptera, Aphididae, *Neozygites fresenii*, Entomophthorales, neonicotinoid insecticides, dicrotophos

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is an economic pest of cotton in the southeastern and southwestern United States (Steinkraus et al. 1991). High aphid populations can have negative impacts on cotton yield and result in economic losses. Williams (2003) reported that in 2002, the cotton aphid was regarded as the sixth most damaging pest of U.S. cotton. The aphid infested 70.3% of U.S. cotton, causing a 0.119% reduction in yield in 9,307,757 infested acres, resulting in a loss of 31,450 bales. This pest continues to be a concern because of its potential for rapid reproduction and ability to develop resistance. Outbreaks of cotton aphids have been associated with reductions in natural enemy populations and aphid resistance to pesticides (Grafton-Cardwell 1991).

[1]Accepted 12 September 2008.
[2]Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, South Carolina 29634.
[3]Department of Applied Economics and Statistics, Clemson University, Clemson, South Carolina 29634.
[4]Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634.

Estimating *Cephus cinctus* Wheat Stem Cutting Damage – Can We Cut Stem Counts?[1]

Héctor Cárcamo,[2] Toby Entz, and Brian Beres

J. Agric. Urban Entomol. 24(3): 117–124 (July 2007)

ABSTRACT In the last decade, the wheat stem sawfly [*Cephus cinctus* Norton (Hymenoptera: Cephidae)] has resurged as an important pest of wheat in the Canadian prairies and continues to be a chronic pest in the Northern Great Plains of the USA. Ecological and management studies to determine egg and larval infestation, damage and parasitoid attack rates, require laborious dissections of stems collected at various spatial scales. We used a statistical simulation study to determine the minimum number of stems required to estimate these response variables at the level of a sub-sample (e.g., within a plot). The number of stems required to estimate sawfly cutting damage and parasitoid attack to larvae was strongly and negatively related with the response variable. At moderate to high levels of sawfly pressure where the stems cut by larvae exceeds 40%, it is possible to reduce stem counts to 50 stems; however, in the 10% cutting range, up to 200 stems are needed for accurate estimates. These values were similar for sample size required to estimate larval parasitism but egg infestation of stems, when levels surpass 70%, can be determined with as few as 30 stems.

KEY WORDS wheat stem sawfly, Monte Carlo simulation, sub-sampling, sawfly damage

Cephus cinctus is historically an important insect pest of wheat in the Canadian prairies and the Great Plains of the USA. Larvae develop and feed inside the lumen of thick-stemmed grasses and most cereal crops, destroying plant tissue and removing nutrients; upon maturation, larvae migrate to the base where they girdle the stem from the inside to plug the stem above their overwintering chamber (Criddle 1922). Most damaged stems break at this point and topple to the ground thereby causing additional harvest losses (Beres et al. 2007). Annual losses in the Canadian Prairies may surpass tens of millions of dollars during outbreak years.

Estimating damage risk for management purposes (e.g., http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm10584) or determining population parameters for ecological studies (Filipy et al. 1985) require sampling stems from experimental plots or at several points within commercial fields. Nansen et al. (2005a) determined within field spatial distribution of adult damage, immature populations (2005b) and number of sampling points needed to determine infestation

[1]Accepted 20 June 2008.
[2]Corresponding author (carcamoh@agr.gc.ca).
Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403-1st Avenue South, Lethbridge, Alberta T1J 4B1, Canada.

at the field scale to make harvesting decisions (2005c). Their work showed that ten samples at field edges are enough to estimate sawfly damage under most conditions. However, there is limited data on how large each sample needs to be to determine egg infestation of stems, stem cutting and parasitism levels by *Bracon cephi* within each sampling point. Traditionally, researchers have collected around 50 stems from each sampling point (e.g., Holmes 1982), although there is no statistical validation for this number. At the other extreme, as few as a single stem per sampling point have been used in large spatial scale studies where numerous points are sampled in a large field (Nansen et al. 2005c). Therefore, the objectives of this simulation study were to determine minimum number of stems per sampling point (e.g., a plot or transect points within a farm) required to estimate (1) proportion of stems infested with eggs, (2) proportion of sawfly larvae parasitized by *B. cephi* and (3) proportion of stems cut by sawfly. We also used data from an ongoing study to investigate if estimates of metric traits such as larval weight, stem diameter and pith expression were influenced by sample size.

Materials and Methods

The first data set was obtained from a study planted in late April or early May from 2003–2005 at the Agriculture and Agri-Food Canada wheat stem sawfly nursery (Beres et al. 2005) 10 km west of Lethbridge, Alberta. Ten wheat genotypes representing a range of susceptible and resistant lines to sawfly damage (Table 1) were planted in plots, 3 × 4 m, separated by 2 m alleys sown to winter wheat. In early September, a sample of wheat stems was collected near the middle of each plot by excavating approximately 30 cm of plant material from 3 adjacent rows. Plants were placed in large paper bags and stored at 10°C until the stems could be dissected to determine sawfly cutting or parasitism. Outside stem diameter for each main stem and tiller in a plant was measured about 1 cm below the second node at 0, 45 and 90°C with a digital caliper.

The second data set was collected from an ongoing study to assess effects of seeding rates, cultivars and their blends on sawfly management (Table 1). Large plots, 30 m × 50 m, were planted in early May 2006 in a farmer's field near the town of Nobleford, approximately 25 km northwest of Lethbridge. From 5 to 7 July 2006, 2 samples of 0.5 m, 20 m apart and 15 m from any edge were excavated and dissected on the same day to estimate sawfly egg and larval densities per stem. Furthermore, each internode was rated for pith expression of the lumen using a scale of 0 (hollow) to 5 (completely solid) as per the protocol of DePauw and Read (1982). These data sets were then used for statistical sub-sampling as described below.

For each genotype and year, SAS® (SAS 2005) was used to perform a Monte Carlo simulation by randomly selecting with replacement from 2 to 50 (within plots) or 2 to 200 (over plots or years) sub-samples 1000 times from all available samples to estimate the sawfly population trait of interest or the stem diameter or pith expression. The means and standard deviation over the 1000 replications were then calculated for each variety, year, and sample size. The estimated percent damage calculated from all the samples that were collected was assumed to be the best estimate and values equal to plus and minus 20% of this estimated value were calculated. For each variety and year, the minimum sample size required was defined as the smallest sample size (i.e., mean plus or minus its

Table 1. Stem lumen, wheat class, and literature reference for wheat genotypes used in simulation study to determine sample sizes needed to estimate sawfly population traits. Treatments at Nobleford included, in addition to the 3 cultivars, a harrow and control and a blend of 1:1 of the solid and hollow stemmed cultivars; a split plot of 4 seeding rates at 150, 250, 350, 450 seeds/m row was superimposed in each replicate block. CWAD = Canada Western Amber Durum, CWRS = Canada Western Red Spring Wheat.

Cultivar	Stem type	Class	Reference
Coalhurst site			
AC Navigator	Hollow	CWAD	Clarke et al. 2000
Kyle	Hollow	CWAD	Townley-Smith et al. 1987
AC Barrie	Hollow	CWRS	McCraig et al. 1998
AC Cadillac	Hollow	CWRS	DePauw et al. 1998
McKenzie	Hollow	CWRS	Graf et al. 2003
AC Abbey	Solid	CWRS	DePauw et al. 2000
AC Eatonia	Solid	CWRS	DePauw et al. 1994
B9973B03&AC4AW	Solid	Experimental line	Clarke et al. 1998
B9973B03&AG2AT	Solid	Experimental line	Clarke et al. 1998
G9608B1-L12J11BF02	Solid	Experimental line	Clarke et al. 1998
Nobleford site			
CDC Go	Hollow	CWRS	NA
Lillian	Solid	CWRS	DePauw et al. 2005
AC Avonlea	Hollow	CWAD	Clarke et al. 1998

standard deviation was fully within the 20% interval calculated from the best estimate). Minimum required sample sizes were also obtained for 10% intervals. These intervals were chosen following a previous study by Nansen et al. (2005c) where such intervals are considered adequate for management purposes.

Results and Discussion

There was a very strong and inverse relationship between levels of sawfly damage (stem cutting) and the number of stems required to estimate it (Fig. 1, Table 2). At the Coalhurst site, damage levels were moderate during the study period, around 20–40%, and decreased towards the end of the study in 2005, as the result of parasitoid attack. Typically, a sub-sample of 50–100 stems has been used in past studies of sawfly damage (e.g., Cárcamo et al. 2005). From our simulation, a 50 stem sub-sample is adequate to estimate damage at the pressures we experienced at Coalhurst and growers could use this sampling intensity to make decisions about how to minimize harvest losses using swathers or pick up reels attached to their combines. For ecological studies where greater precision is needed, up to 125 stems would be required at moderate sawfly pressure (10–30% cutting). Under very low levels of sawfly pressure where damage is under 10% of wheat stems, researchers interested in population

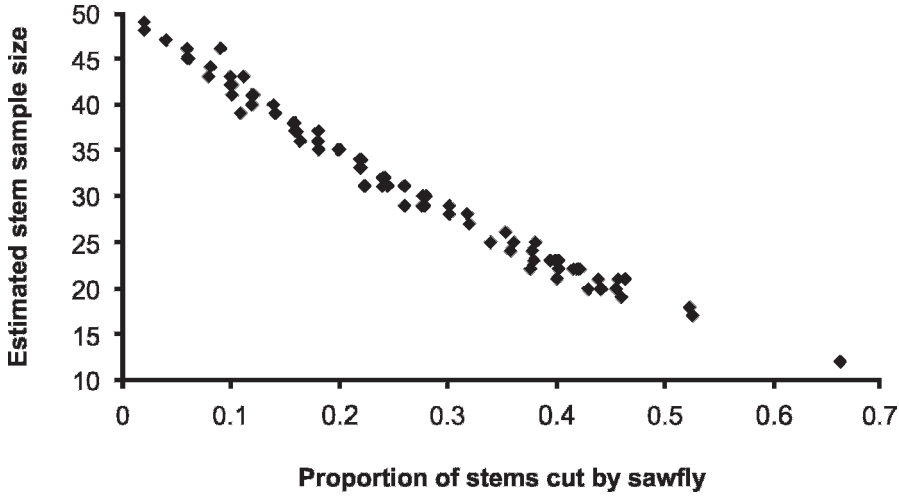

Fig. 1. The scatterplot shows the minimum number of stems that were required in a Monte Carlo simulation so that the estimated proportion of stems cut were within a 20% confidence interval of the observed proportions obtained from a field study near Nobleford, Alberta, planted in 2006.

dynamics would require around 200 stems to estimate larval densities within plots or sampling points along commercial field transects. Fortunately, ascertaining damage levels can be done in the field in a relatively short time and doubling the number of stems counted in a sub-sample post harvest, as done in the Alberta survey (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm10584), only adds a few minutes at each point within the field.

Determining egg infestation, for life table studies, is a very labour intensive task that requires dissection and careful inspection of numerous stems. This proportional variable is also highly negatively correlated with sample size; the more stems with eggs, the fewer stems needed (Table 2, Fig. 2). However, under high levels of sawfly pressure, several eggs may be found in one stem, the first larva to hatch will cannibalize the others so that only one larva survives in each stem (Holmes 1982). In 2006 at Nobleford, where this data was collected, egg infestation was relatively high (mean of 57%) in all plots and according to our simulation results, 30–40 stems were sufficient to estimate the proportion infested with eggs with a confidence interval of 10%. If a producer wished to get a general idea of potential damages based on egg infestation, as few as 20 stems per sample would be sufficient. At low sawfly population levels, where fewer than 40% of stems carry eggs, the sub-sample size would need to increase substantially to over 50 and 100 stems, for management and ecological studies, respectively.

An important aspect of wheat stem sawfly population dynamics is larval mortality caused by the parasitoid *B. cephi* (Holmes et al. 1963). This variable is best estimated post harvest by dissecting all stems including sawfly cut "stubs" where mature larvae may be attacked (albeit at low levels) by second generation parasitoids. In our simulation study from the Coalhurst data set, we pooled all plots within each genotype from all years to obtain a larger sample of stems with sawfly larvae parasitized. In some cases, a solid stemmed genotype supported

Table 2. Summary of simulation results to estimate adequate sub-samples of stems per sampling point required to estimate various sawfly population parameters[a].

	Within a plot (up to 50 stems)						Over plots or over years (200 stems or more)				
Variable	# observations used	Confidence interval (%)	Coefficient intercept	X	X^2	r^2	# observations used	Intercept	X	X^2	r^2
Egg infestations at Nobleford											
	135	10	1.03	−0.01	NA	0.84	40	0.99	−0.0089	0.000028	0.99
	152	20	1.01	−0.03	0.00038	0.98	40	0.99	−0.03	0.00031	0.99
Sawfly-cut stem proportion at Coalhurst site											
	93	10	1.59	−0.03	NA	0.98	30	0.59	−0.0029	NA	0.82
	99	20	0.95	−0.03	NA	0.99	30	0.58	−0.0071	0.000024	0.96
Parasitism by *B. cephi* At Coalhurst At Nobleford											
	107	10	0.78	0.0041	0.00031	0.65	9	0.72	−0.0022	NA	*0.37*
	109	20	0.80	−0.01	NA	0.70	10	0.54	−0.0023	NA	*0.29*
	123	10	0.94	−0.01	NA	0.50	39	0.91	−0.0052	0.000013	0.90
	145	20	0.95	−0.02	0.00029	0.80	40	0.78	−0.0052	NA	0.87
Larval weights at Coalhurst											
	30	10	0.01	−0.00010	NA	*−0.02*	10	0.014	−0.00025	NA	*−0.04*
	30	20	0.01	−0.00085	NA	*0.10*	10	0.016	−0.0013	NA	*0.31*

X = sample size.
X^2 = curvature of the regression line.
[a]italics denotes $P > 0.05$ for r^2 values.
NA = not needed, line essentially straight.

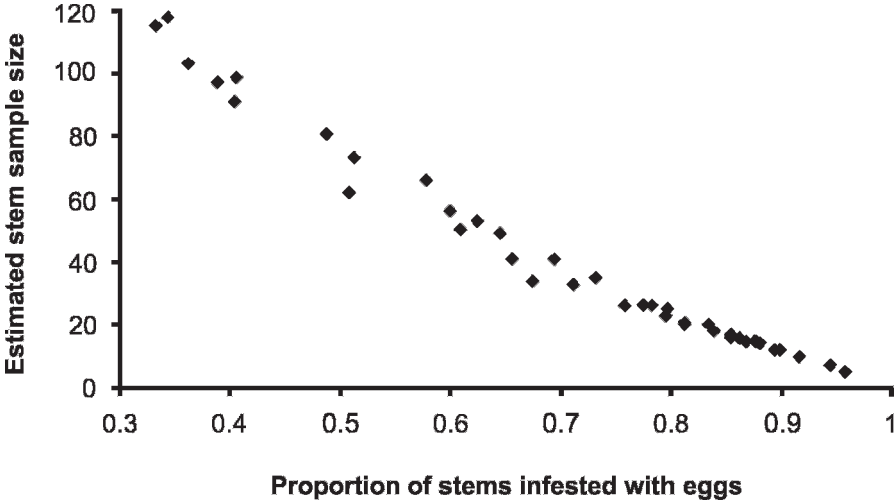

Fig. 2. The scatterplot shows the minimum number of stems that were required in a Monte Carlo simulation so that the estimated proportion of stems infested with sawfly eggs were within a 10% confidence interval of the observed egg infestations obtained from a field study near Nobleford, Alberta, planted in 2006.

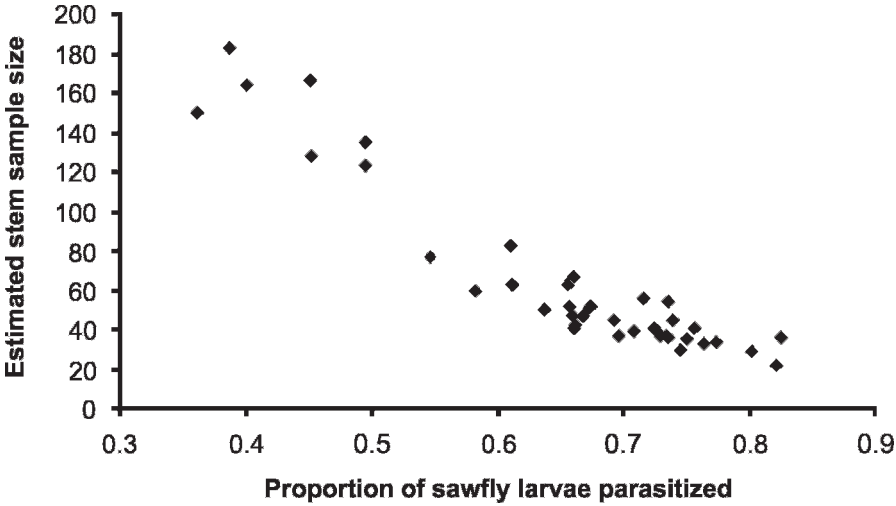

Fig. 3. The scatterplot shows the minimum number of stems that were required in a Monte Carlo simulation so that the estimated proportion of sawfly larvae parasitized by *Bracon cephi* were within a 10% confidence interval of the observed proportions obtained from a field study near Nobleford, Alberta, planted in 2006.

very few larvae with no parasitoids; therefore, it was excluded from the analysis. A sample of 50 stems was adequate to estimate parasitism levels over 50% with a 10% confidence level (Fig. 3). Under conditions when parasitism levels are as high as 80% as observed at the Nobleford site in 2006, ($r^2 > 0.80$ when stems pooled over plots, Table 2), a small sample of only 30 stems would correctly estimate parasitism level at a sample point.

Relationship between sample size and the estimate of pith expression of AC Lillian (solid stem), CDC Go (hollow) and AC Avonlea (hollow) or the outside stem diameters were also investigated as done for insect parameters. None of the plant trait estimates were influenced by sample size. For example, the average pith expression of individual internodes or their average, were highly variable. At the 10% confidence interval a sub-sample of 4 or 14 stems would produce estimates of solidness that ranged from 1–3 in our ranking scale. Given such high variability, it is advisable to process as many stems as logistically possible (>20) to estimate pith expression and stem diameter within a sub-sample.

In conclusion, the number of stems required to estimate sawfly cutting damage as well as parasitoid attack to larvae at the level of a sample (e.g., within plots) is strongly and negatively related to the level of sawfly damage and parasitism, respectively. At moderate to high levels of sawfly pressure where the stems cut by larvae and their parasitism by *B. cephi* exceeds 40 and 50%, respectively, it is possible to reduce stem counts to 50 stems to estimate both variables; however, at low populations of sawfly and parasitoids, up to 200 stems are needed for accurate estimates. Egg infestations can be determined relatively accurately with as few as 30 stems at high sawfly populations when over 60% of stems become infested with eggs.

Acknowledgments

We thank T. Larson, S. Kendrick, R. Dyck, K. Cole, C. Herle, S. Simmill, B. Lounds, C. Klima, S. Leusink, T. Pernitsky, C. Aceytuno and J. Goettel for their assistance in maintaining the plots and collecting the data and B. van Hezewijk for constructive comments on an earlier manuscript draft. This study was financed through the A-base and Matching Investment Initiative of Agriculture and Agri-Food Canada made possible through the funds provided by the Western Grains Research Foundation for cereal improvement at AAFC to BB.

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Estimating *Cephus cinctus* Wheat Stem Cutting Damage – Can We Cut Stem Counts?[1]

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J. Agric. Urban Entomol. 24(3): 117–124 (July 2007)

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Estimating damage risk for management purposes (e.g., http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm10584) or determining population parameters for ecological studies (Filipy et al. 1985) require sampling stems from experimental plots or at several points within commercial fields. Nansen et al. (2005a) determined within field spatial distribution of adult damage, immature populations (2005b) and number of sampling points needed to determine infestation

[1]Accepted 20 June 2008.
[2]Corresponding author (carcamoh@agr.gc.ca).
Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403-1st Avenue South, Lethbridge, Alberta T1J 4B1, Canada.

Laboratory Evaluation of Dinotefuran and Novaluron Amended Baits Against *Paratrechina* sp. nr. *pubens*[1]

Jason M. Meyers[2] and Roger E. Gold[3]

J. Agric. Urban Entomol. 24(3): 125–136 (July 2007)

ABSTRACT With the introduction of an invasive pest ant species, *Paratrechina* sp. nr. *pubens*, it has become imperative to develop novel control technologies. There is currently no published research concerning dinotefuran and novaluron against pest ants. *Paratrechina* sp. nr. *pubens* workers and brood were exposed to baits containing dinotefuran and novaluron at varied concentrations. Liquid bait amended with dinotefuran was applied in the laboratory against *P*. sp. nr. *pubens*. Mean percent mortality of *P*. sp. nr. *pubens* was typically higher as the concentration increased at both three (F = 7.28; df = 28; $P < 0.001$) and seven (F = 7.28; df = 28; $P < 0.001$) d post-treatments. Three day observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven d post treatment showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; $P < 0.01$, df = 1; $\chi^2 = 7.09$; $P < 0.01$, respectively). The use of dinotefuran was highly efficacious against *P*. sp. nr. *pubens*, and is recommended for further laboratory research and initial field research. Corn grit bait amended with novaluron was applied in the laboratory against *P*. sp. nr. *pubens* workers and brood. At four wk results did not reveal significant differences among concentrations with active ingredient and controls (F = 1.504, df = 3, 27, P = 0.239). Results of the study were inconclusive regarding the efficacy of novaluron against *P*. sp. nr. *pubens*. The findings of this study emphasize the difficulties in maintaining incomplete colonies of *P*. sp. nr. *pubens* that contain brood under laboratory settings.

KEY WORDS Dinotefuran, novaluron, *Paratrechina*, bioassay, neonicotinoids, invasive, exotic, bait, IGR

Neonicotinoids comprise a class of insecticide that is very effective against a great variety of insects. Neonicotinoids demonstrate agonistic activity on arthropod postsynaptic nicotinic acetylcholine receptor sites (Tomizawa & Yamamoto 1993, Miyagi et al. 2006). Dinotefuran, *N*-methyl-N′nitro[*N*″-[(tetrahydro-3-furanyl)methyl]guanidine, has insecticidal activity that includes both neuron-excitatory and neuron-blocking mechanisms (Kiriyama & Nishimura 2002). Dinotefuran is a 3rd generation neonicotinoid with broad spectrum activity against insects (Wakita et al. 2003). Typically known as and used in agricultural products (Elbert et al. 1998), neonicotinoids effectiveness has been further expanded to the control of urban insect pests (e.g., Premise®, Maxforce®

[1]Received 9 July 2008; Accepted 15 August 2008.
[2]Corresponding author (jason.meyers@basf.com).
[3]Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, 2143 TAMU, College Station, TX 77843.

Granular Fly Bait, and Advantage® (imidacloprid) for control of termites, flies, and fleas and ticks). Dinotefuran insecticidal activity has previously been demonstrated across a few insect groups including houseflies, *Musca domestica* (Kiriyama et al. 2003), mosquitoes (Corbel et al. 2004) and cockroaches (Mori et al. 2001, Kiriyama & Nishimura 2002, Miyagi et al. 2006). Neonicotinoids have proven to have a low toxicity to mammals (Kiriyama & Nishimura 2002, Corbel et al. 2004).

Novaluron, 1-[3-cloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, is an insect growth regulator (IGR) that has been used against a variety of arthropods (Ishaaya et al. 2003, Su et al. 2003, Cabrera et al. 2005). However, there is no published research of this IGR against formicid species. Although IGRs have adverse affects against other ant species in the laboratory (Banks et al. 1983, Kabashima et al. 2007), field control of ants using (IGR) baits can be difficult due to their temporally dynamic nutritional needs. Sustainable amounts of an IGR must be maintained within the colony and be available to the brood in an effective dose during molts. These difficulties are compounded by the inactivity of IGRs on worker and alate castes. Colony death occurs when lack of worker replacement and natural death of adult castes take place (Banks et al. 1983).

There is currently no published research concerning dinotefuran efficacy against ants. An invasive ant species, *Paratrechina* sp. nr. *pubens*, has created numerous problems in southeast Texas since 2002 (Meyers & Gold unpublished data). This invasive species has caused numerous electrical shortages of a variety of apparatuses, ecological dominance, companion animal avoidance of outdoors, and they are an immense nuisance due to their high density in urban and commercial environments (Meyers & Gold unpublished data). Since its introduction, this tramp ant has spread to 25 geographically distinct locations in five Texas counties. According to field observations from pest control operators, and preliminary laboratory studies, very few commercially available bait matrices are attractive to *P.* sp. nr. *pubens*. It is imperative to discover attractive and successful bait matrices as part of a temporally comprehensive control strategy for the management or eradication of *P.* sp. nr. *pubens* populations. Typical control tactics for urban ant pest population management of *P.* sp. nr. *pubens* have been inadequate due to its remarkable population densities. Novel control measures should be evaluated regarding population management of *P.* sp. nr. *pubens*. Successful control research tactics will likely be integrated into an overall management program for *P.* sp. nr. *pubens* control or eradication.

Invasive social insects can create ecologically devastating results (Moller 1996, Chapman & Bourke 2001, Holway et al. 2002). Social behaviors of ants create a weakness that can be exploited during the control process. Shared resources, trophallaxis, cannibalism, communication, and grooming are all avenues for an increase in treatment efficacy. This is particularly evidenced by the horizontal transmission of active ingredients (AI's), as has been demonstrated in cockroaches (Kopanic & Schal 1999), termites (Ibrahim et al. 2003) and ants (Soeprono & Rust 2004). Invasions by social insects often encompass large geographical regions, are detrimental to agricultural systems and natural communities, and are expensive to control (Vinson 1986, Vander Meer et al. 1990, Williams 1994). The ease of application of aerially applied pesticides is a desirable character for a management program for invasive species. Baits could

be integrated into an overall management program. These programs have been historically evaluated (e.g., Mirex against the red imported fire ant, *Solenopsis invicta* [Banks et al. 1973]), and more recently for termites as "Operation Full Stop" for the Formosan subterranean termite, *Coptotermes formosanus*, in New Orleans, Louisiana (Ring et al. 2001).

The use of baits for eradication of ants has been reviewed (Stanley 2004). The use of baits has proven successful against other invasive species behaviorally similar to *P.* sp. nr. *pubens*. Unicolonial ants, such as the Argentine ant, *Linepithema humile* (Krushelnycky et al. 2004), and the yellow crazy ant, *Anoplolepis gracilipes* (Abbott & Green 2007), have been successfully controlled despite high densities. Containment of an early detected invasive species may afford time to successfully manage or eradicate incipient populations (Krushelnycky et al. 2004).

These studies evaluated the biological activity of dinotefuran and novaluron against *P.* sp. nr. *pubens*. The objective for these studies was to determine mortality ratios of *P.* sp. nr. *pubens* at various concentrations of dinotefuran and novaluron amended into liquid and a corn grit bait matrices, respectively. This study constitutes an initial effort to find control alternatives for *P.* sp. nr. *pubens* in Texas.

Materials and Methods

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Each of thirty plastic boxes, 9 cm high × 15 × 30, coated with fluon, contained 100 *Paratrechina* sp. nr. *pubens* workers collected from Pasadena, TX (29°36.748 N, 95°03.313 W). Workers were collected from laboratory maintained queenright colonies of moderate size containing brood. Glass tubes, 1.6 × 15 cm were placed in each box, containing deionized water with a cotton plug. Tubes were covered with solid color construction paper for darkening purposes. Five replications at each of five concentrations of dinotefuran (0.00006, 0.00012, 0.00025, 0.0005 and 0.001%) were used, along with five replications of the product with no AI (blank). Concentrations were selected based on the suggestions provided by the manufacturer. Insecticide was provided in aqueous solution at 0.001%. All dilutions were made using 20% sucrose in deionized water. Ants were starved for 24 h prior to exposure. Two mL droplets of dinotefuran or blank were placed on the bottom of each box. Observations were made at 1, 2, 3, 4, 5, 24, 48, 72, and 168 h after application, and moribund ants were counted. By the end of the study the numbers of live ants were counted as opposed to the number of dead, as it became apparent that the ants were cannibalistic. Counts of dead or live ants were made after the 24 h starvation period and statistical analysis was conducted accordingly.

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean percent mortality in treatments. Means were separated using Tukey-Kramer HSD test. LD_{50} and LD_{90} values of mortality response to treatments were analyzed using PROC PROBIT (SAS Institute 2000, Cary, North Carolina).

Evaluation of novaluron against *P.* sp. nr. *pubens*. Novaluron was administered at various concentrations to *P.* sp. nr. *pubens* in granular form using ACAB matrix (0.1, 0.25, 0.5, and 0.0% AI). *Paratrechina* sp. nr. *pubens* were starved for 24 h pre-treatment. The colonies were allowed to feed on the bait for 1 week, after which the bait container was removed. Throughout the length of the experiment, *P.* sp. nr. *pubens* were offered 25% honey-water and crickets.

Fig. 1. *P*. sp. nr. *pubens* workers adhered to a crystallized droplet of dinotefuran.

Each replicate consisted of 100 workers and 50 brood (small egg clusters, larvae, and/or pupae). Two colonies were field-collected and laboratory-raised from which the experimental units were derived. All replicates were placed in plastic boxes, 9 cm high × 15 × 30, coated with fluon and provided glass containers fitted with water-wicks (Fig. 1). Colonies were exposed to CO_2 until movement ceased and individual workers and brood could easily be counted and removed using a camel-hair paint brush. Colonies were placed in clear Petri dishes (3.5 × 1.0 cm) containing dental stone substrate for observational purposes and moisture retention. Into the top of each Petri dish, two holes were made to allow for worker movement. Post-treatment observations of worker and brood numbers, including abnormal behaviors, were made at each time interval. Deviation from the original colony numbers were used to determine efficacy of novaluron concentrations. Each concentration of novaluron was repeated seven times. Based on previous experiments with *P*. sp. nr. *pubens* colonies, replications for this study were maintained in a growth chamber at ~29.5°C and ~64.5% humidity. All treatments and replicates were done with a completely randomized block design (CRBD).

Post-treatment counts were conducted by exposing surviving *P*. sp. nr. *pubens* to CO_2 until rapid movements ceased and workers and brood could be counted. To determine efficacy of novaluron, observations of live and dead workers and brood were made at 3, 7, 14, and 28 d post-treatment. Efficacy was determined based on the comparisons of reduction of post-treatment counts from pre-treatment counts. The evaluation of dinotefuran against *P*. sp. nr. *pubens* revealed that workers are cannibalistic. Temperature and humidity data were taken every hour throughout the experiment using a HOBO Data Logger (Onset Computer, Bourn, MA).

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean mortality (workers) and survival (larvae) in treatments (JMP, SAS Institute, Cary, NC). Means were separated using Tukey's HSD test.

Table 1. Mean dinotefuran-treated *P.* sp. nr. *pubens* mortality rates with doses using five replications of 100 ants per arena.

Concentration (%)	Mean % mortality in five replications @ 3dat[ab]	Mean % mortality in five replications @ 7dat[ac]
0.001[d]	78.82 a	89.17 a
0.0005	63.36 a	82.51 ab
0.00025	58.61 ab	88.62 a
0.00012	44.82 abc	87.61 a
0.00006	16.47 bc	57.15 b
Blank	3.31 c	4.18 c

[a]Means in the same column followed by the same letter are not significantly different ($P < 0.05$; Tukey-Kramer HSD).
[b]$F = 7.28$; df = 28; $P < 0.001$.
[c]$F = 26.57$; df = 28; $P < 0.0001$.
[d]For statistical purposes, this dose had only four replications.
dat = days after treatment.

Results

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Mean percent mortality of *P.* sp. nr. *pubens* was typically higher as the concentration increased at both three ($F = 7.28$; df = 28; $P < 0.001$) and seven ($F = 7.28$; df = 28; $P < 0.001$) d post-treatments (Table 1). There were no significant differences between the four highest concentrations for both post-treatment observations. Three d observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven d post treatment (Table 2) showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; $P < 0.01$, df = 1; $\chi^2 = 7.09$; $P < 0.01$, respectively).

Evaluation of novaluron against *P.* sp. nr. *pubens*. One-Way ANOVA was conducted to determine any bias in replication placement within the growth chamber. This analysis found no bias within replications ($F = 0.38$, df = 6, 101, $P = 0.89$).

There were no statistical differences found between treatments throughout time. No statistical differences were found between means of dead workers by treatment throughout time (Table 3). At 14 d post-treatment, the only statistically significant differences ($P = 0.028$) were found between treatments of live larvae (Table 4). However, the results did not differentiate the control means from two of the AI treatments (0.1 and 0.5%). The results for both dead workers and live larvae were inconclusive.

Discussion

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Dinotefuran caused more mortality in *P.* sp. nr. *pubens* than did blank controls. With the relatively low LD_{90} values, these data indicate high efficacy of dinotefuran to control *P.* sp. nr. *pubens*. These data also indicated that dinotefuran caused sufficient mortality to warrant further testing in both the laboratory and field;

Table 2. Probit regression of mortality data to dinotefuran-treated *P.* sp. nr. *pubens* workers at different time intervals with LD values in percent active ingredient.

# replications	Days after treatment	Slope ± SE	LD_{50} (95% FL)	LD_{90} (95% FL)	χ^2
30	3	0.99 (0.37)	0.0003 (0.00008–0.0008)	0.005 (0.001–137.75)	7.20
30	7	0.84 (0.32)	1.67×10^{-5} (3.08×10^{-9}–5.53×10^{-5})	0.00055 (0.00025–0.037)	7.09

Table 3. Mean # of dead *P.* sp. nr. *pubens* workers throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of dead workers throughout time (d)[a]			
Treatment (AI%)	3[b]	7[c]	14[d]	28[e]
0.10	9.14 (3.13) a	21.14 (3.26) a	32.29 (5.68) a	55.14 (4.74) a
0.25	6.86 (3.13) a	14.43 (3.26) a	22.00 (3.35) a	41.14 (5.49) a
0.50	4.57 (0.95) a	20.86 (5.39) a	32.00 (6.39) a	53.43 (6.3) a
0.0 (Control)	9.00 (3.18) a	22.29 (5.81) a	32.57 (5.37) a	59.00 (8.03) a

[a]Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).
[b]$F = 0.818$, df = 3, 27, $P = 0.497$.
[c]$F = 0.644$, df = 3, 27, $P = 0.595$.
[d]$F = 0.937$, df = 3, 27, $P = 0.438$.
[e]$F = 1.504$, df = 3, 27, $P = 0.239$.

however, the delivery system of dinotefuran will need modification for field tests. Applying this bait matrix with corn grit or other food substances may decrease evaporation and crystallization rate, along with increasing the likelihood that workers will be able to allocate the bait to remaining colony members.

High survival ratio within the control replications suggests an unbiased analysis of the experiment. However, extraneous factors such as crystallization (Fig. 1) of dinotefuran, and cannibalism may have affected mortality in this no-choice test. Crystallization of the bait may not have allowed for continued feeding past ca. 48 h (Fig. 1). Some individuals became adhered to the product and therefore died *in situ*, which may have adversely affected spread of the insecticide throughout the remaining workers. Crystallization may have caused a differential availability of dinotefuran within the formulation. The primary dissipation route for dinotefuran may be through aqueous photolysis (~1.3 d). Sorting and

Table 4. Mean # live *P.* sp. nr. *pubens* larvae throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of live larvae throughout time (d)[a]			
Treatment (AI %)	3[b]	7[c]	14[d]	28[e]
0.10	20.71 (1.52) a	6.71 (1.29) a	3.86 (1.18) ab	0.17 (0.17) a
0.25	23.29 (1.69) a	7.57 (1.09) a	1.57 (0.65) b	0.00 (0) a
0.50	20.43 (1.88) a	9.00 (1.42) a	5.86 (1.96) ab	1.00 (0.45) a
0.00 (Control)	27.00 (2.04) a	8.71 (1.51) a	7.29 (1.11) a	0.83 (0.65) a

[a]Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).
[b]$F = 2.894$, df = 3, 27, $P = 0.056$.
[c]$F = 0.628$, df = 3, 27, $P = 0.604$.
[d]$F = 3.589$, df = 3, 27, $P = 0.028$.
[e]$F = 1.620$, df = 3, 27, $P = 0.216$.

separation of the dead individuals from the living group of workers would not have allowed for the opportunity of cannibalism. This cannibalistic behavior towards exposed individuals of social insects increases the transmission of an insecticide throughout the population (Kopanic & Schal 1999, Ibrahim et al. 2003, Soeprono & Rust 2004). Given the relative stability of dinotefuran, this is likely the case regarding its interaction with *P*. sp. nr. *pubens* both physiologically and behaviorally. It is unknown whether *P*. sp. nr. *pubens* workers were cannibalistic toward healthy or moribund workers, or simply consume cadavers as part of a normal behavioral assemblage. Although no counts were taken of major body parts (head, thorax, or abdomen), observations indicate that consumption of the head was considerably less likely than the thorax or abdomen. Further studies on horizontal transfer of insecticide through cadaver maintenance or cannibalism should be investigated in *P*. sp. nr. *pubens*. Metabolic dissipation pathways of dinotefuran should also be investigated. These findings may indicate the reasoning for high horizontal transmission through behaviors (trophollaxis, grooming or other) or cannibalistic insects.

The relative success of this laboratory study warrants further laboratory evaluations and initial field efficacy investigations. These findings may assist pest control operators during their efforts to control the numerically superior pest.

Evaluation of novaluron against *P*. sp. nr. *pubens*. Despite a supposed ideal environment of temperature and humidity (29.45°C ± 0.007, 64.57% ± 0.09, respectively), workers and brood of replications began dying at a surprising rate. Because of this, the original experiment was cancelled and performed again. The initial experiment was run under the same parameters (with exception of 100 brood rather than 50) and was considered inconclusive. Statistical analyses were conducted on the truncated data, and no apparent biases were found within the experiment. We believe that this demonstrated the difficulties in maintaining *P*. sp. nr. *pubens* in colony-form with low worker numbers and without the presence of queens.

Paratrechina sp. nr. *pubens* provisioned bait granules (both control and AI) (Figs. 2 & 3). The workers placed the bait inside the Petri dish, or upon and around the water-wick. This created an ideal environment (high moisture) for fungal growth. These facts may have hampered the ability to perform a more informative test; however, if statistical differences were to be found, they would have likely occurred at greater than 28 d post-treatment.

Formicid species often demonstrate temporal fluctuation of food resource consumption. This is not an ideal situation for IGR efficacy testing. For efficacy of IGRs to be expressed, there needs to be enough titer within a specific time interval (i.e., during larval molt). Because formicids select alternative food resources throughout time, administering an IGR can be a difficult task. Nevertheless, based on these results, this product could not be used for the control of *P*. sp. nr. *pubens*; however, additional laboratory and field research needs to be done. The laboratory studies should include whole colony tests with natural ratios of brood workers and queens. If used in the field, it would likely be most effective to broadcast large quantities of the bait during early spring as large numbers of brood are maturing.

Although not supported from these results, there remains the possibility that novaluron is effective against *P*. sp. nr. *pubens*. Another study conducted during experiments with red imported fire ant, *Solenopsis invicta*, found that methoprene was not effective against another *Paratrechina* sp. (Sanchez 2005).

Fig. 2. This picture demonstrates the provisioning of bait and subsequent fungal growth associated with the high humidity and the clustering behavior of *P*. sp. nr. *pubens*. The discoloring (yellowing) of the wick seen here is typical of all field-collected colonies maintained in the laboratory.

In that study, an increase in *Paratrechina terricola* populations were found in trees located in areas treated with methoprene.

A previous experiment (Meyers et al., unpublished data), field observations, and communication with various pest control operators with clientele affected by *P*. sp. nr. *pubens*, suggested that the current label rate for ACAB (abamectin) is not effective. The currently recommended rate of 1.5 lbs per acre is unlikely to create or sustain control of the numerically dense *P*. sp. nr. *pubens* populations. If an additional AI was integrated into the product, or an increase in the current broadcast rate, the efficacy of ACAB may increase. If additional bait amounts were used, the efficacy of the product would likely increase. The field effectiveness of this product at current label and expanded usage should be assessed against *P*. sp. nr. *pubens* in early spring.

P. sp. nr. *pubens* are considerably attracted to the ACAB matrix in the laboratory and field. It is therefore recommended that ACAB with novaluron be tested against large laboratory colonies (with a full compliment of castes). Field observations suggest an immense increase in numbers of *P*. sp. nr. *pubens* brood and worker members during early spring. During this period foraging for food sources high in protein is needed for brood production. ACAB contains a marine lipid based attractant. Therefore, this product may be a viable option as part of a temporally dynamic control program against *P*. sp. nr. *pubens*.

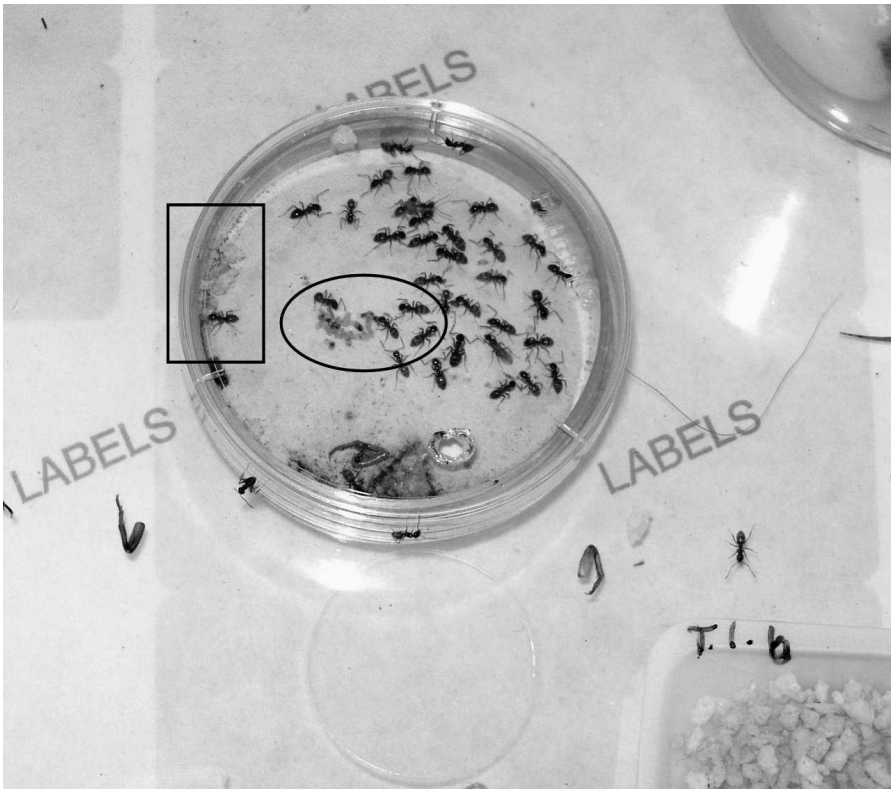

Fig. 3. This picture demonstrates the provisioning of the bait inside the Petri dish. The square shows provisioned bait granules for 0.1% AI treatment. The circle shows workers tending several larvae.

The results of this laboratory study underscores the difficulties of maintaining relatively small, queenless colonies of *P*. sp. nr. *pubens*. Although it is not known whether the lack of queens adversely affected the outcome of the study, it could be one of the contributing factors.

Acknowledgments

The authors would like to thank the late Dr. Harry Howell for assistance with the experimental design. This project was partially supported by a grant from Whitmire Micro-Gen and the Endowed Chair of Urban Entomology at Texas A&M University, Department of Entomology.

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Laboratory Evaluation of Dinotefuran and Novaluron Amended Baits Against *Paratrechina* sp. nr. *pubens*[1]

Jason M. Meyers[2] and Roger E. Gold[3]

J. Agric. Urban Entomol. 24(3): 125–136 (July 2007)

ABSTRACT With the introduction of an invasive pest ant species, *Paratrechina* sp. nr. *pubens*, it has become imperative to develop novel control technologies. There is currently no published research concerning dinotefuran and novaluron against pest ants. *Paratrechina* sp. nr. *pubens* workers and brood were exposed to baits containing dinotefuran and novaluron at varied concentrations. Liquid bait amended with dinotefuran was applied in the laboratory against *P*. sp. nr. *pubens*. Mean percent mortality of *P*. sp. nr. *pubens* was typically higher as the concentration increased at both three (F = 7.28; df = 28; $P < 0.001$) and seven (F = 7.28; df = 28; $P < 0.001$) d post-treatments. Three day observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven d post treatment showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; $P < 0.01$, df = 1; $\chi^2 = 7.09$; $P < 0.01$, respectively). The use of dinotefuran was highly efficacious against *P*. sp. nr. *pubens*, and is recommended for further laboratory research and initial field research. Corn grit bait amended with novaluron was applied in the laboratory against *P*. sp. nr. *pubens* workers and brood. At four wk results did not reveal significant differences among concentrations with active ingredient and controls (F = 1.504, df = 3, 27, P = 0.239). Results of the study were inconclusive regarding the efficacy of novaluron against *P*. sp. nr. *pubens*. The findings of this study emphasize the difficulties in maintaining incomplete colonies of *P*. sp. nr. *pubens* that contain brood under laboratory settings.

KEY WORDS Dinotefuran, novaluron, *Paratrechina*, bioassay, neonicotinoids, invasive, exotic, bait, IGR

Neonicotinoids comprise a class of insecticide that is very effective against a great variety of insects. Neonicotinoids demonstrate agonistic activity on arthropod postsynaptic nicotinic acetylcholine receptor sites (Tomizawa & Yamamoto 1993, Miyagi et al. 2006). Dinotefuran, *N*-methyl-N′nitro[*N*″-[(tetrahydro-3-furanyl)methyl]guanidine, has insecticidal activity that includes both neuron-excitatory and neuron-blocking mechanisms (Kiriyama & Nishimura 2002). Dinotefuran is a 3rd generation neonicotinoid with broad spectrum activity against insects (Wakita et al. 2003). Typically known as and used in agricultural products (Elbert et al. 1998), neonicotinoids effectiveness has been further expanded to the control of urban insect pests (e.g., Premise®, Maxforce®

[1]Received 9 July 2008; Accepted 15 August 2008.
[2]Corresponding author (jason.meyers@basf.com).
[3]Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, 2143 TAMU, College Station, TX 77843.

Survey of Natural Enemies of the Sweetpotato Whitefly (Hemiptera: Aleyrodidae) in Ten Vegetable Crops in Egypt

Alvin M. Simmons[1] and Shaaban Abd-Rabou[2]

J. Agric. Urban Entomol. 24(3): 137–145 (July 2007)

ABSTRACT The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) is a worldwide pest in diverse agroecosystems. There are numerous species of predators and parasitoids that are associated with this pest. Climate and species of vegetation can dramatically affect the distribution and incidence of these natural enemies. A field survey was conducted to determine the incidence of the primary natural enemies of *B. tabaci* in 10 vegetable crops in Egypt. Fifteen species of natural enemies of *B. tabaci* were observed, including 5 species of predators and 10 species of parasitoids. *Coccinella septempunctata* L. was the most commonly found predator, and it was found in four of the crops. The parasitoids consisted of four species of *Encarsia* and six species of *Eretmocerus* which represents 71% of the known aphelinid parasitoid species of *B. tabaci* in Egypt. *Eretmocerus aegypticus* Evans and Abd-Rabou was the most commonly encountered parasitoid species; it was found in five of the crops. To date, this species has only been reported in Egypt. These results help define the diversity of natural enemies of *B. tabaci* among vegetable crops.

KEY WORDS Aleyrodidae, *Bemisia tabaci*, biological control, predator, parasitoid, vegetable, sweetpotato whitefly, *Encarsia*, *Eretmocerus*

The B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (also reported as *B. argentifolii* Bellows & Perring), along with other members of the *Bemisia* complex, is a serious problem of agricultural crops on a global scale. The application of synthetic pesticides is the strategy that is most commonly used by growers to control whiteflies. However, although some compounds are less disruptive than others, the use of insecticides as a pest management option can have adverse effects on beneficial organisms (Haynes 1988, Longley & Jepson 1996, Simmons & Jackson 2000, Simmons & Abd-Rabou 2005a). Numerous species of parasitoids and predators are associated with *B. tabaci* (Lopez-Avila 1986, Abd-Rabou 1998, 1999, Gerling et al. 2001). These natural enemies of *B. tabaci* are found in diverse agroecosystems around the world. A wide diversity of generalists predators feed on *B. tabaci* (Gerling 1990, Cock 1994, Nordlund & Legaspi 1996, Gerling et al. 2001). Several studies have been conducted on the importance of the beneficial fauna attacking *B. tabaci* in agricultural systems (e.g., Onillon 1990, Cohen et al. 1993, Legaspi et al. 1996, Abd-Rabou 1999). Surveys have been conducted from cultivated and wild host plants (Bennett et al.

[1]U.S. Vegetable Laboratory, USDA, Agricultural Research Service, 2700 Savannah Highway, Charleston, South Carolina 29414. E-mail: alvin.simmons@ars.usda.gov
[2]Ministry of Agriculture, ARC, Plant Protection Research Institute, 7 Nadi El-Said, Dokki, Giza, Egypt.

1990, Simmons 1998, Trujillo et al. 2004), and new species attacking *B. tabaci* continue to be found. Aphelinids in the genera *Encarsia* and *Eretmocerus* are the most prevalent parasitoids of *Bemisia* (Polaszek et al. 1992). At least 64 species of *Encarsia* and *Eretmocerus* are known (Gerling et al. 2001, Schmidt et al. 2001, Heraty & Woolley 2002, Manzari et al. 2002, Oliveira et al. 2003, Abd-Rabou 2006, Abd-Rabou & Ghahari 2007, Evans 2007). Some are more cosmopolitan than others. In a recent survey, a new parasitoid species, *Er. aegypticus* Evans and Abd-Rabou, was identified based on specimens collected from *B. tabaci* in Egypt (Abd-Rabou & Evans 2002).

Different climates can dramatically affect the population and distribution of *Bemisia* and its community of natural enemies (Gerling 1984, Watson et al. 1992, Simmons & Elsey 1995, Simmons 1998). Moreover, populations of natural enemies and rates of parasitism by parasitoids of *B. tabaci* can greatly vary based on diverse host plants (Simmons et al. 2002, Simmons & Abd-Rabou 2005b). Surveys can help identify the incidence of whitefly parasitoids and predators in a given region or locality, and their association with plants, and may facilitate their role or potential role as a pest management component. The objective of this study was to determine the incidence of the primary predators and parasitoids of *B. tabaci* in 10 common vegetable crops in Egypt.

Materials and Methods

Ten diverse vegetables crops were established in experimental field plots in 2005. The plant species were: broccoli (*Brassica oleracea* var. *botrytis* L., 'Soltani'), cowpea [*Vigna unguiculata* (L.), 'Fetriat'], cantaloupe [*Cucumis melo* L. var. *aegyptiacus* (Sickenb.) Hassib,'El-Masry'], cabbage (*Brassica oleracea* var. *capitata* L.,'Balady'), cucumber (*Cucumis sativus* L., 'Balady'), eggplant (*Solanum melongena* L., 'Aswad'), green bean (*Phaseolus vulgaris* L., 'Broncho'), sweetpotato [*Ipomea batatas* (L.) Lam., 'Abis'], sugar beet (*Beta vulgaris* L. 'Giza 21'), and tomato (*Lycopersicon esculentum* Miller, 'Castel Rock'). One planting of a given crop species was established in one of five regions (Fayum, Garbiya, Qalyubiya, Minufiya, and Sharqiya Governorate) in Egypt. Pesticides were not used in the study. Each field plot was 0.13 ha. The planting started during the beginning of June 2005. Samples were collected from mid-July until mid-October in 2005. The start and end of the sample dates were not the same for all crops during a 14-week period. Leaf samples were obtained from each field during 12 consecutive weeks per field. The weekly samples consisted of 30 leaves that were randomly selected from separate plants in a zigzag pattern in each field, and the leaves were taken from the center of the selected plants. The number of adult predators of *B. tabaci* was determined based on direct observation of each selected leaf while the leaf was still attached to the plant. The leaf samples were then detached and taken to the laboratory.

With the aid of a microscope, all immature stages of *B. tabaci* were counted on leaf samples except for the eggs stage and the first nymphal instar. The leaves were then held in well-ventilated 0.5 liter cardboard containers and the whiteflies and parasitoids were allowed to emerge (according to Simmons and Abd-Raou 2005a). All adult specimens of *B. tabaci* and its parasitoids found at the bottom of the rearing containers were then collected. The parasitoids were then identified and counted, and percent parasitism was estimated based on the

Table 1. Mean numbers of *B. tabaci* and its predators in 10 vegetable crops.

Crop	Site[a]	Mean no. *B. tabaci* per wk	Mean number of predators per 30 leaves per week				
			C. carnea	*C. Septem-punctata*	*Deraeo-coris* sp.	*Orius* sp.	*Geocoris* sp.
Broccoli	Q	1410.3	0	0	0	0	95.7
Cantaloupe	M	223.3	19.9	0	26.2	0	0
Cabbage	Q	1537.3	0	193.8	74.9	0	0
Cowpea	S	1103.8	0	88.8	0	0	0
Cucumber	Q	756.6	0	0	0	0	15.2
Eggplant	Q	285.0	25.6	0	0	17.7	0
Green bean	Q	416.4	0	23.2	0	0	0
Sweetpotato	G	503.5	0	26.0	0	25.3	0
Sugar beet	Q	277.3	11.3	0	0	0	0
Tomato	F	185.8	0	0	0	0	0

[a]F=Fayum governorate; G=Garbiya governorate; Q=Qalyubiya governorate; M=Minufiya governorate; S=Sharqiya governorate in Egypt.

number of parasitoids relative to the number of whiteflies counted. Identification of natural enemies was confirmed based on the examination of adults mounted in Hoyer's medium (Noyes, 1982) and by comparing them with pinned and voucher specimens. The parasitoids were identified to species and the predators were identified to either species or genus.

Results and Discussion

Five species of predators and 10 species of parasitoids were detected among 10 vegetable crops (Tables 1 and 2). Because sampling time and locations were not standard, and leaves among crops were different sizes, no direct comparisons can be made on quantification of insect species among the different crops. However, information on parasitism is presumed to be independent of leaf size. No predator of *B. tabaci* was observed on tomato (Table 1). Four crops (broccoli, cucumber, eggplant, and sugar beet) each harbored single predator species; these represented a total of three species. The most commonly found predator was *Coccinella septempunctata* L. (the seven-spotted lady beetle) which was found in four crops (cabbage, cowpea, green bean, and sweetpotato). This species is widespread in Egypt (Abdel-Gawaad et al. 1990, Abd-Rabou 1999). A temperature range of 25–35°C is optimal for biological process and feeding behavior of *C. septempunctata* (Xia et al. 1999), and such temperatures are common during the summer growing season in the regions of our study. In a survey conducted in Pakistan, Khan et al. (2001, and other papers cited therein) found *C. septempunctata* feeding on *B. tabaci*, and on numerous other taxa of pests, on a wide diversity of agronomic and row crops. Each of the other species of predators [*Chrysoperla carnea* (Stephens), *Deraeocoris* sp., *Orius* sp. and *Geocoris* sp.] was represented in 2–3 crops. Only one predator species (*C. carnea*) was observed in

Table 2. Mean numbers of *B. tabaci* and its parasitoids in 10 vegetable crops.

Crop	Site[a]	Mean no. *B. tabaci* per wk	Mean number of parasitoids per 30 leaves per week									
			Encarsia				*Eretmocerus*					
			inaron	*lutea*	*mineoi*	*sofia*	*aegypticus*	*corni*	*diversicilatus*	*emiratus*	*eremicus*	*hayati*
Broccoli	Q	1410.3	46.3	0	0	0	125.3	0	0	0	76.8	0
Cantaloupe	Q	223.3	0	0	0	0	8.8	0	0	0	0	0
Cabbage	Q	1537.3	0	0	0	200.4	0	0	0	0	159.5	0
Cowpea	S	1103.8	0	0	0	106.3	94.1	0	0	0	0	0
Cucumber	Q	756.6	0	0	9.6	13.8	0	0	0	0	0	17.1
Eggplant	Q	285.0	0	16.9	0	0	15.6	0	0	0	0	0
Green bean	Q	416.4	0	23.9	19.2	0	23.4	0	0	0	0	0
Sweetpotato	G	503.5	0	0	0	0	0	0	41.9	0	0	0
Sugar beet	Q	277.3	0	22.7	0	14.2	0	0	0	0	0	15.5
Tomato	F	185.8	15.3	0	0	0	0	8.3	0	15.0	0	0

[a]Site F= Fayum governorate; G= Garbiya governorate; Q = Qalyubiya governorate; M = Minufiya; S = Sharqiya governorate in Egypt.

the two leguminous crops (cowpea and green bean) which were located in separate regions. This generalist predator is native to the holarctic region and feeds on aphids, mites, whiteflies and other species (Frank & McCoy 1994). Between the two *Cucumis* plant species (located in different regions) and between the two *Brassica* species (located in the same region), the crops did not harbor the same species complex of whitefly predators (Table 1).

Four species of *Encarsia* and six species of *Eretmocerus* were collected in the survey (Table 2). Cantaloupe and sweetpotato each harbored a single species of different *Eretmocerus* parasitoids (Table 2). Three crops (cabbage, cowpea, and eggplant) each harbored two species of parasitoids which represented a total of four species. The remaining five crops each harbored three species of parasitoids which represented nine species. *Er. aegypticus* was encountered in five of the crops in Qalyubiya and Sheryia goveronerates. Currently, this species has only been reported to occur in Egypt, and the previously documented distribution were Cairo, Giza, and New Valley governorates (Abd-Rabou 2006). The two *Brassica* plant species shared two of five species of parasitoids located on those crops. Among the four species of parasitoids found on the two *Cucumis* plant species, none was in common on the two crops.

Er. eremicus and *Er. hayati* were both found in two separate crops, while *Er. corni*, *Er. diversicilatus* and *Er. emiratus* were each found in single crops (Table 2). *Encarsia sofia* was found in four crops. This is a species which is found throughout the Old World (Heraty & Polaszek 2000). *En. lutea* was found in three crops, while *En. inaron* and *En. mineoi* were both found in two crops (Table 2). Overall parasitism among the crops was relatively low (18% in broccoli, 4% in cantaloupe, 23% in cabbage, 18% in cowpea, 5% in cucumber, 11% in eggplant, 16% in sweetpotato, 13% in sugar beet, and 13% in tomato), although we suspect that parasitism may have been underestimated because it was based on the ability of the insects to complete development on the detach leaf samples.

The number of each species as well as the combined number of parasitoids and number of predators tended to increase in each crop during the sampling period (Fig. 1). Notable exceptions were counts of whitefly and parasitoids in cantaloupe, and parasitoids in tomato. During the season, the counts of parasitoids were generally greater than the counts of predators in most crops (except for cantaloupe, eggplant, and sweetpotato).

Biological control of field populations of *B. tabaci* spp. had been attempted by the introduction, conservation and augmentation of natural enemies (Goolsby et al. 2005). A laboratory study with five species of common generalist predators indicated that those predators vary in their preference of whitefly host stage and vary in the amount of handling time in feeding (Hagler et al. 2004). Our survey was not set up to quantify the impact of the natural enemies on infestation by *B. tabaci*. It is not known if there were any interactions within and between guilds of the beneficial species. However, predation of parasitized *B. tabaci* has been shown to be less by some species of coccinellids as compared with when they feed on unparasitized whitefly nymphs (Al-Zyound & Sengonca 2004, Zang & Liu 2007). Moreover, interspecific competition was demonstrated among parasitoids of *B. tabaci* (Bográn et al. 2002).

In Egypt, 14 species of *Encarsia* and *Eretmocerus* are known to parasitize *B. tabaci* (Abd-Rabou 2006). No new species were detected in our survey. Four species of *Encarsia* and *Eretmocerus* (*En. davidi*, *En. formosa*, *En. mundus*, and

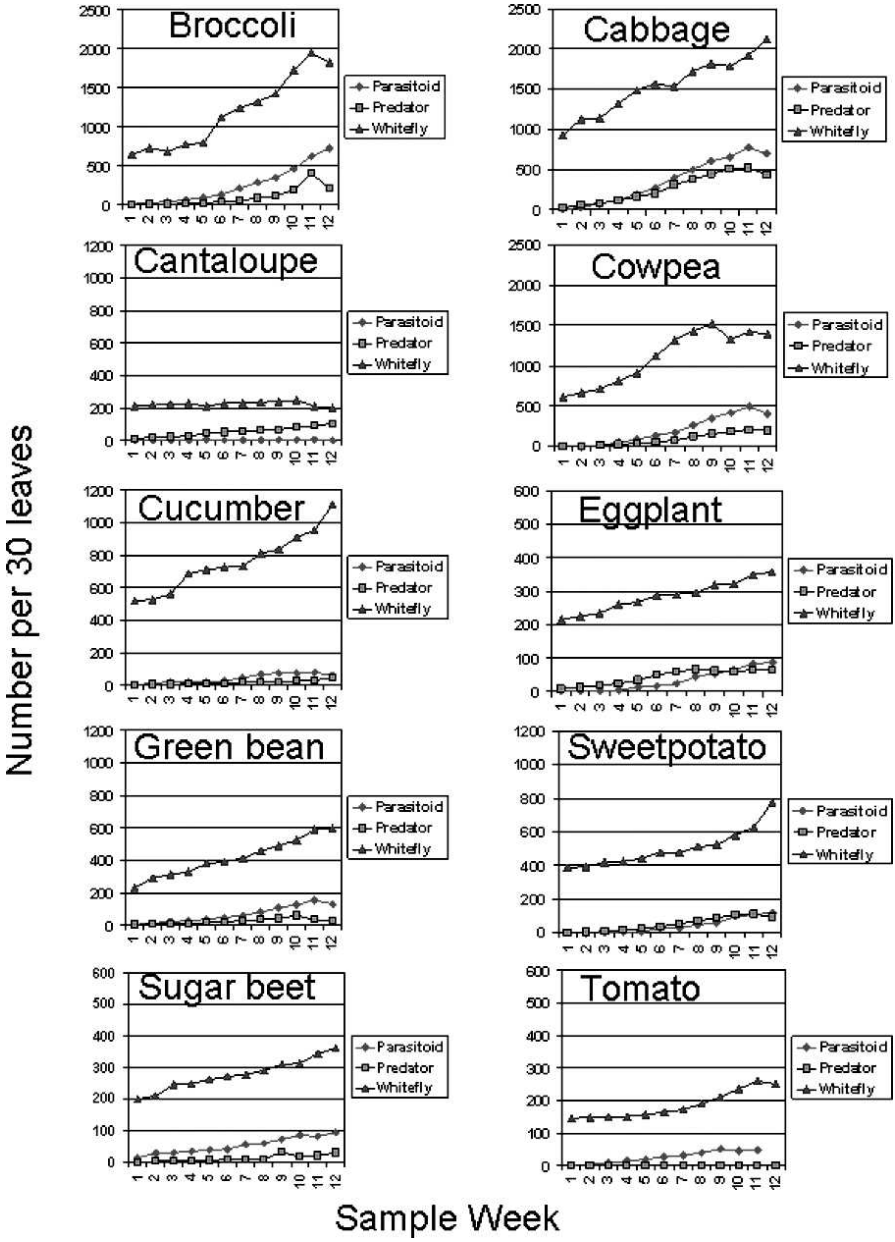

Fig. 1. Numbers of immature parasitoids and adult predators of *Bemisia tabaci*, and number of *B. tabaci* on 30 leaves over 12 weeks in 10 different vegetable crops.

Er. elegans) that are known to occur in Egypt (Abd-Rabou 2006) were not detected in our survey. Information from this survey is helpful for the understanding of the association of predators and parasitoids with *B. tabaci* in vegetable crops.

Acknowledgments

Appreciation is given to Jesusa C. Legaspi and Tong-Xian Liu for their suggestions on an earlier version of the manuscript. This publication is sponsored by the U.S.–Egyptian Science and Technology Joint Fund in cooperation with the USDA and Egyptian Ministry of Agriculture under Project Number OTH8-022-001. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Survey of Natural Enemies of the Sweetpotato Whitefly (Hemiptera: Aleyrodidae) in Ten Vegetable Crops in Egypt

Alvin M. Simmons[1] and Shaaban Abd-Rabou[2]

J. Agric. Urban Entomol. 24(3): 137–145 (July 2007)

ABSTRACT The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) is a worldwide pest in diverse agroecosystems. There are numerous species of predators and parasitoids that are associated with this pest. Climate and species of vegetation can dramatically affect the distribution and incidence of these natural enemies. A field survey was conducted to determine the incidence of the primary natural enemies of *B. tabaci* in 10 vegetable crops in Egypt. Fifteen species of natural enemies of *B. tabaci* were observed, including 5 species of predators and 10 species of parasitoids. *Coccinella septempunctata* L. was the most commonly found predator, and it was found in four of the crops. The parasitoids consisted of four species of *Encarsia* and six species of *Eretmocerus* which represents 71% of the known aphelinid parasitoid species of *B. tabaci* in Egypt. *Eretmocerus aegypticus* Evans and Abd-Rabou was the most commonly encountered parasitoid species; it was found in five of the crops. To date, this species has only been reported in Egypt. These results help define the diversity of natural enemies of *B. tabaci* among vegetable crops.

KEY WORDS Aleyrodidae, *Bemisia tabaci*, biological control, predator, parasitoid, vegetable, sweetpotato whitefly, *Encarsia*, *Eretmocerus*

The B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (also reported as *B. argentifolii* Bellows & Perring), along with other members of the *Bemisia* complex, is a serious problem of agricultural crops on a global scale. The application of synthetic pesticides is the strategy that is most commonly used by growers to control whiteflies. However, although some compounds are less disruptive than others, the use of insecticides as a pest management option can have adverse effects on beneficial organisms (Haynes 1988, Longley & Jepson 1996, Simmons & Jackson 2000, Simmons & Abd-Rabou 2005a). Numerous species of parasitoids and predators are associated with *B. tabaci* (Lopez-Avila 1986, Abd-Rabou 1998, 1999, Gerling et al. 2001). These natural enemies of *B. tabaci* are found in diverse agroecosystems around the world. A wide diversity of generalists predators feed on *B. tabaci* (Gerling 1990, Cock 1994, Nordlund & Legaspi 1996, Gerling et al. 2001). Several studies have been conducted on the importance of the beneficial fauna attacking *B. tabaci* in agricultural systems (e.g., Onillon 1990, Cohen et al. 1993, Legaspi et al. 1996, Abd-Rabou 1999). Surveys have been conducted from cultivated and wild host plants (Bennett et al.

[1]U.S. Vegetable Laboratory, USDA, Agricultural Research Service, 2700 Savannah Highway, Charleston, South Carolina 29414. E-mail: alvin.simmons@ars.usda.gov
[2]Ministry of Agriculture, ARC, Plant Protection Research Institute, 7 Nadi El-Said, Dokki, Giza, Egypt.

Formica perpilosa, an Emerging Pest in Vineyards[1]

Kris Tollerup,[2] Michael K. Rust,[3] and John H. Klotz[3]

J. Agric. Urban Entomol. 24(3): 147–158 (July 2007)

ABSTRACT *Formica perpilosa* Wheeler is a serious economic ant pest on table grapes grown in the Coachella Valley, California, and Hermosillo, Sonora, Mexico. This ant aggressively tends hemipteran pests, such as the vine mealybug, *Planococcus ficus* Signoret, and disrupts natural control by predators and parasitoids. Efforts are underway to develop control measures against *F. perpilosa* using granular bait, yet little is known about the colony life cycle or foraging characteristics of this ant. We studied the seasonal activity, mating behavior, and density and spatial characteristics of *F. perpilosa* nests in vineyards as well as its foraging and recruitment behavior. Nests were active from early February to mid-October. Mating flights occurred in early August and again in the first two weeks of September and new colonies were founded by a single queen. *F. perpilosa* rapidly colonized a new, non-infested vineyard with ca. 9% of the vines infested after 1.5 y. In September the proportions of infested vines at 5, 20, and 30 y old vineyards were 18.6, 21.8, and 16.2%, respectively. This ant is seasonally polydomous and nest density increased ca. two-fold at the 5 and 20-year old vineyards between February and September. Foraging and recruitment primarily occurred up to 6.39 m from a home nest. The implications of these studies for controlling *F. perpilosa* using low-toxic bait delivery systems are discussed.

KEY WORDS *Formica perpilosa*, ant pest, vineyards, bait, control

The ant, *Formica perpilosa* Wheeler, is native to Arizona, Lower California, Nevada, New Mexico and Northern Mexico (Wheeler 1913, Ward 2005). Wheeler (1902) first described this ant as *Formica fusca* subsp. *subpolita* var. *perpilosa* but later revised the species as *Formica perpilosa* (Wheeler 1913). Most species within the genus *Formica* are found in boreal and temperate forests (Fisher and Cover 2007); however, *F. perpilosa* is unusual in that it is found in riparian habitats and irrigated lands in hot-desert regions (Wheeler 1913).

In its native habitat, *F. perpilosa*. nests at the base of desert trees and shrubs such as white-thorned acacia, *Acacia contricta* Benth, and mesquite, *Prosopis juliflora* (Mol.) (Wheeler and Wheeler 1986, Wagner 1997). Workers forage primarily on the plant that their nest is associated with collecting nectar from extrafloral nectaries, honeydew from various hemipterans, and exudate from lycaenid butterfly larvae (Schumacher and Whitford 1974, Wagner and Kurina 1997). It also acts as a generalist predator within plants and as a predator and scavenger of small arthropods on the soil surface (Schumacher and Whitford

[1]Submitted 25 April 2008, Accepted 12 August 2008.

[2]Rutgers Agricultural Research and Extension Center, Bridgeton, New Jersey 08302. E-mail: tollerup@aesop.

[3]Department of Entomology, University of Califomia, Riverside, California 92521.

1974). Colonies are seasonally polydomous (Wagner 1997) and the number of foraging workers ranges from 2000 to 3500 (Schumacher and Whitford 1974). *Formica perpilosa* is an economic pest on table grape grown in the Coachella Valley, Riverside County, California, and Hermosillo, Sonora, Mexico because it tends hemipterans such as the vine mealybug, *Planococcus ficus* (Signoret), and citrus mealybug, *Planococcus citri* (Risso), reducing the effectiveness of the predators and parasitoids of these pests. In Coachella Valley vineyards, *F. perpilosa* may also play a critical role in sustaining populations of *P. ficus* by providing underground shelter during the winter and extreme summer temperatures (Tollerup 2007). It has also has been collected on grapefruit ca. 100 km northwest of the Coachella Valley in Hemet.

In California and Mexico, growers currently rely on the contact insecticide, chlorpyrifos (Lorsban) to manage *F. perpilosa*, as well as other ant pests. In vineyards, the efficacy of chlorpyrifos is limited to ca. six to eight weeks (Klotz et al. 2003). Colonies rebound after this period because the insecticide kills only the aboveground workers that contact it leaving the majority of workers and queens within nests unaffected. California growers may soon lose insecticides with chlorpyrifos as the active ingredient. The compound has been detected in several major bodies of water in California including the San Joaquin and Merced Rivers and the Sacramento/San Joaquin River Delta (Helliker 2004). This class of agricultural insecticide is currently under reevaluation by the California Department of Pesticide Regulation.

Toxic baits provide an alternative to contact insecticides (Williams et al. 1997, Klotz et al. 2000, Klotz et al. 2004). However, to develop a bait and effectively employ its use, it is important to understand the biology of the target species. For example, extensive studies on the biology and foraging behavior of *Linepithema humile* (Mayr) and *Solenopsis invicta* (Buren) have been instrumental in the development of effective control measures for these pests (Silverman and Brightwell 2008, Williams 2001).

With a similar goal in mind for *F. perpilosa*, the purpose of this study was to determine the: 1) colony life cycle including its seasonal activity and mating behavior, 2) density and spatial characteristics of its nests within vineyards and dispersal mechanisms, and 3) characteristics of foraging including distance and its effect on the rate of recruitment.

Material and Methods

Study Sites. Infestations of *Formica perpilosa* occur throughout the Coachella Valley in table grape vineyards. We conducted preliminary visual surveys of 25 vineyards between 2002 and 2005 and found it nesting at all sites. Four vineyards were selected for this study to provide a representative cross-section of vineyard-age and location within the Coachella Valley. The vineyards ranged from 1.5 to approximately 30 y of age. The sites were drip irrigated with either two emitters placed approximately 0.5 m to either side of the trunk or a single emitter placed next to the trunk. The vines were cane-pruned and planted with a spacing of ca. 2.13 m between vines and 3.67 m between rows. A single 0.40 ha (500 vines) block was selected at each site.

Site 1 was a Midnight Beauty Seedless Grape vineyard and was ca. 18 mo old at the time it was surveyed. The vineyard was located on the west side of the

Coachella Valley in the Oasis area and planted on a newly cleared site with excessively-drained, gravelly-sand soil. The site was under construction when the experiment began and was selected to determine the rate and spatial characteristics of *F. perpilosa* infestations as they spread to new vineyards. Its southeast corner was adjacent to the northwest corner of a five-year old vineyard heavily infested with *F. perpilosa*. The corners of the two vineyards were separated by a dirt road ca. 22 m wide. Vines at site 1 were surveyed for the presence/absence of an *F. perpilosa* nest in October 2005.

Site 2 was located ca. 0.5 km northwest of site 1. The vineyard was also planted with Midnight Beauty Seedless Grape and approximately five years old at the time of the survey. The soil type was excessively-drained, gravelly-sand. The block at site 2 was surveyed in 2004 during February (early season) when *F. perpilosa* nests begin activity and during September (late season).

Site 3 was a Thompson Seedless Grape vineyard located on the east side of the valley in the Mecca area, and site 4 was a Superior Seedless Grape vineyard located ca 2 km north of site 2. Sites 3 and 4 were ca. 20 and 30 y of age, respectively, and planted in well-drained fine sandy-loam soil. Vines at site 3 were surveyed for the presence/absence of an *F. perpilosa* nest in both February and September 2004 while site 4 was surveyed in September 2004.

Colony Life Cycle. An ant colony consists of a group of workers and their associated reproductives housed in a single nest (i.e., monodomy) or multiple interconnected nests (DeBout et al. 2007). In this study, we could not always distinguish between single-nest and multiple-nest colonies. Thus, we use the term nest to indicate a group of workers housed in a single structure with or without the presence of reproductives.

One or two times per month, 10 to 20 individual nests were randomly selected at sites 2, 3, or 4 and inspected for the presence of brood and adult reproductives. Individual nests were inspected by removing the top 5–10 cm of soil and debris around the entrance to expose the shallow brood chambers.

Nest Density and Spatial Distribution. Vines infested with *F. perpilosa* have a distinct debris pile at their base consisting of small pieces of plant material and soil (Fig. 1). The presence of a debris pile was used as a criterion to indicate the existence of an *F. perpilosa* nest. Surveys were conducted by walking between vine-rows and visually inspecting the base of each vine for a debris pile. Infested vines were flagged and their location within the block was noted.

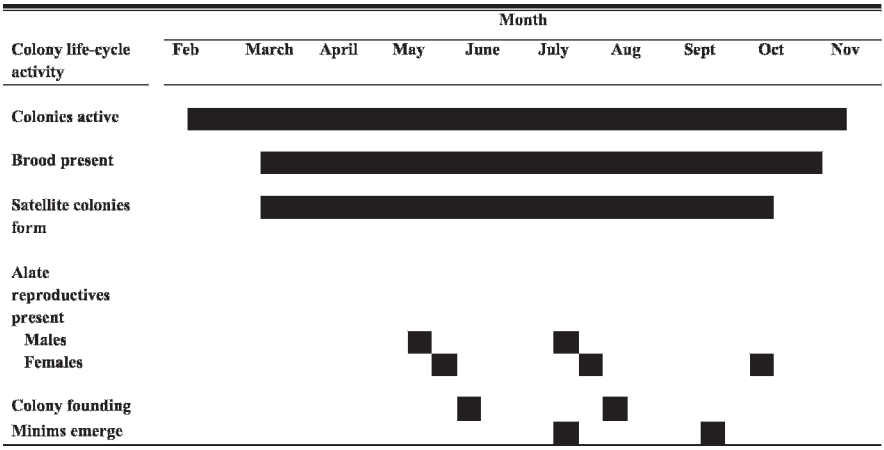
Foraging Characteristics. To determine the average maximum daytime foraging distance of *F. perpilosa*, nests were selected that did not have another within-row nest up to 32 m away. Foragers within the proximity of the nest were followed to determine the one furthest from the nest. This was accomplished by conducting a visual search of the soil surface within the vine-row and beneath the canopy. Searches began at the nest entrance and covered successive areas measuring 2.13×0.7 m, the length between vines and the approximate width of the vine canopy. Upon finding at least one forager within the area, the search ceased and moved to the next similar sized area away from the nest. This procedure continued until no foragers were found for 60 s, at which point the search moved back to the area where the last forager was observed. When that ant was found, it was offered a small amount of non-toxic granular anchovy bait. In each case, the ant immediately seized a bait granule in its mandibles and began its homeward journey. The forager was observed until it entered a nest.

Fig. 1. A *Formica perpilosa* nest at the base of an ca. 5-years old grapevine in the Coachella Valley. The debris pile consists primarily of soil and small pieces of plant material. The presence of a debris pile was used as a criterion for an *F. perpilosa* infestation.

In July 2005, experiments were conducted at sites 2 and 3 to determine the rate at which *F. perpilosa* foragers recruit nest mates to a food source. Each recruitment trial was conducted by placing a pile of ca. 10g non-toxic anchovy bait on the ground within a vine-row at distances of 2.13, 4.56, or 6.39 m from a nest. These distances were based on preliminary results of the above mentioned experiment on maximum daytime foraging distance. Recruitment was measured by counting the number of foragers that removed bait during a 30-minute period. Ant counts were taken for 60 s every two minutes beginning when the first forager removed bait.

Statistical Analysis. To determine the spatial characteristics of *F. perpilosa* nests within each plot, maps were constructed using x, y, z coordinates. Vine spacing (2.13 m between vines by 3.67 m between vine rows) was used as the (x, y) coordinate. For example, the first vine within row (l) was designated as position (0, 0); the second and third vines were designated as positions (0, 2.13) and (0, 4.26); and, the first, second, and third vines within row (2) were designated as positions (3.67, 0), (3.67, 2.13), and (3.67, 4.26). The z coordinate was a binomial variable (0 = no nest, 1 = nest). Maps were constructed using SigmaPlot 8.0 (Systat 2005) and the total number of nests per plot was calculated. A 5×2 contingency table was used to determine if the number of nests in the blocks differed among the vineyards. To determine differences between vineyards and survey dates, a series of pair-wise comparisons were performed. The alpha value was adjusted to 0.01 using the Sidak-Dunn procedure to indicate significance (Sokal and Rohlf 1995).

Table 1. Colony life-cycle of *F. perpilosa* in Coachella Valley vineyards.



Nest spatial characteristics were analyzed by first calculating a coefficient of dispersion (CD) value for each site (Sokal and Rohlf 1995). The CDs were calculated using two different sampling grids of 100, 5-vine subplots (five contiguous vines within the same row), and 100, 5-vine subplots (five contiguous vines across rows). Coefficients of dispersion provide a measurement of the spatial relationship among objects, in this case ant nests. Values greater than one indicate that nests have a clumped distribution and the presence of one nest increases the likelihood of encountering an additional nest. Conversely, values less than one indicate that nests tend to repel each other and as the value decreases there is a lower likelihood of encountering closely neighboring nests. Values close to one indicate that no spatial relationship exists and nests are evenly distributed (Sokal and Rohlf 1995).

The distribution data of infested vines within subplots was then analyzed using a binomial distribution goodness-of-fit test. Then, the nearest distance between nests was determined using the nearest neighbor procedure in SYSAT 12 (Systat 2007).

The average maximum foraging range was calculated using weighted means ± SD. Recruitment data were regressed at each distance tested using ant counts as the dependent variable and time as the independent variable using SYSAT 12 (Systat 2007).

Results and Discussion

Colony life-cycle. In the Coachella Valley colonies became active in early February and remained so until early November (Table 1). As the colonies began their seasonal activity, the debris pile around their nest entrances increased in size. This occurred most noticeably from the first part of June through July and indicates that nest size rapidly increased during this period. Larvae and pupae began to appear in the shallow nest chambers in March and were found through October.

Fig. 2. Immature *F perpilosa* queens collected from a shallow nest chamber at the base of a grapevine in the Coachella Valley during October 2005.

In its native habitat, *F. perpilosa* establishes temporary satellite nests (seasonally polydomy) (Wagner and Martinez del Rio 1997). In polydomous species, satellite nests are typically abandoned at the end of the season when the nests coalesce, although in some species, they can remain occupied over several seasons (DeBout et al. 2007). In Coachella Valley vineyards, colonies began establishing satellite nests in early April approximately 3–4 weeks after brood appeared. Workers were observed removing pupae from their parent nest and transporting them to satellite nests at the base of neighboring vines. The debris piles at the entrances of the satellite nests quickly developed and were not visually distinguishable from a parent nest. Satellite nests were established only within the same vine-row as a parent nest and at a distance between 2.13 to 6.39 m (one to three vines away). While polydomy is often associated with polygyny (Wilson 1971), we did not find more than one queen per colony.

Female alate reproductives appeared during the early part of June, August, and October (Table 1). Male reproductives were observed twice during the season, in May and July, approximately two weeks prior to female reproductives. Not all colonies produced reproductives and the two sexes were not observed together in the same nest. The female reproductives do not form a pupal case (Fig. 2) as do the workers and males.

The majority of alates left their colony in early June and August. At these times we observed several individual dealated queens within vine-rows excavating their nests at the base of vines. The first workers, or minims, emerged from nascent colonies (Fig. 3) in July and again in September approximately 3–4 weeks after colony founding. In claustral colony founding

Fig. 3. A nascent *F perpilosa* nest at the base of a grapevine ca. 18 mo old in the Coachella Valley. Nascent colonies appear during July and September approximately three to four weeks after colony founding.

species such as *F. perpilosa*, newly mated queens search the soil surface for a suitable site and excavate a small single-chamber nest (Wilson 1971). The queen then seals herself in the nest and rears the first brood using her metabolized wing muscles and fat body as a food source (Wilson 1971).

Mating of *F. perpilosa* probably occurs in flight, although we did not determine what cues initiated flights or where mating took place. The males of species which gather in aerial swarms are generally much smaller in comparison to queens and have reduced or vestigial mandibles (Holldobler and Wilson 1990). In the case of *F. perpilosa*, the males are approximately half the size of the queens and the mandibles are much reduced.

Nest density and spatial distribution. The youngest vineyard in this study became infested after 1.5 y with approximately 9% of the vines (111 nests/ha) infested (Table 2). In September, the proportion of infested vines at the mature vineyards (5, 20, and 30 year-old sites) were 18.6 (230 nests/ha), 21.8 (269 nests/ha), and 16.2% (200 nests/ha), respectively. No significant difference in the number of infested vines occurred among the mature vineyards in September; however, they had significantly more infested vines than the 1.5-year-old vineyard. During February, at the early period of the season, the proportion of infested vines equaled 6.9 (119 nests/ha) and 11.2% (138 nests/ha) at the five (Fig. 4) and 20-year old vineyards, respectively. This was significantly lower than in September and statistically similar to the 1.5-year-old vineyard (Table 2).

In random samples of 100 vines in each of the mature vineyards, *P. ficus* was closely associated with *F perpilosa*. Mealybugs were found on 41.3 ± 3 .51% (mean ± SD) of *F perpilosa* infested vines in July and 67.4 ± 4.16% of infested vines in October (data not shown). Mealybug were not found in the absence of ants.

Table 2. Density and spatial characteristics of *F. perpilosa* nests.

Vineyard age (yrs)	Site (mo. surveyed)	[a]No. of nests per 500-vine block	Mean nests per plot (5 vines)	[b]CD (within-row plots)	CD (between-row plots)	Avg. distance ± SD (m) between nests
1.5	1 (Oct.)	45 a	0.45	1	1.22	5.0 ± 3.11
5	2 (Feb.)	48 a	0.48	0.69*	0.78	5.85 ± 3.11
5	2 (Sept.)	93 b	0.93	0.85	0.77	3.6 ± 1.81
20	3 (Feb.)	56 a	0.56	0.81	1.13	4.99 ± 2.18
20	3 (Sept.)	109 b	1.09	0.57**	0.93	3.67 ± 1.25
30	4 (Sept.)	81 b	0.81	0.64*	0.82	4.49 ± 2.03

[a]Numbers with the same letter are not significantly different. Statistical tests were conducted using a 5×2 contingency and multiple pair-wise comparisons to determine differences between vineyards and survey dates ($P = 0.01$).
[b]CD is the coefficient of dispersion. An asterisk indicates that the value differs from a binomial distribution ($P = 0.05$).

The distribution of nests at all sites and survey dates tended to be distributed evenly (CD = 1) or repelled (CD < 1) in both the within and between-row subplots (Table 2). We expected that the CDs of the within-row subplots would be greater than one (clumped distribution) given the relatedness among satellite nests and that they are established only within the same vine-row as their parent nest. The average distance between nearest neighbor nests among all the sites ranged between (mean ± SD) 3.6 ± 1.81 and 5.85 ±3.11 m (Table 2). These distances are

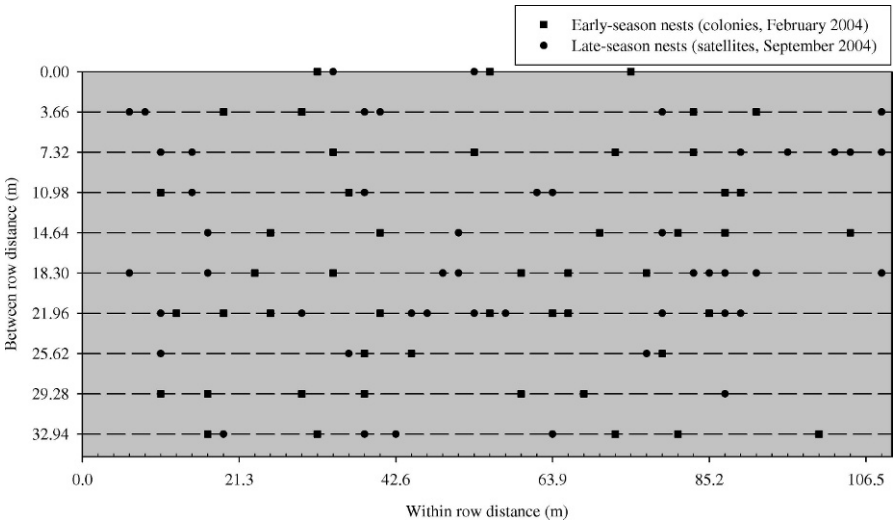


Fig. 4. Infestation map of site 2 showing vines infested with *F. perpilosa* nests in February 2004 (early-season) and satellite nests that were established by September 2004. A block equals ca. 0.4 ha (500 vines) planted at a spacing of 2.13 m between vines and 3.67 m between vine-row.

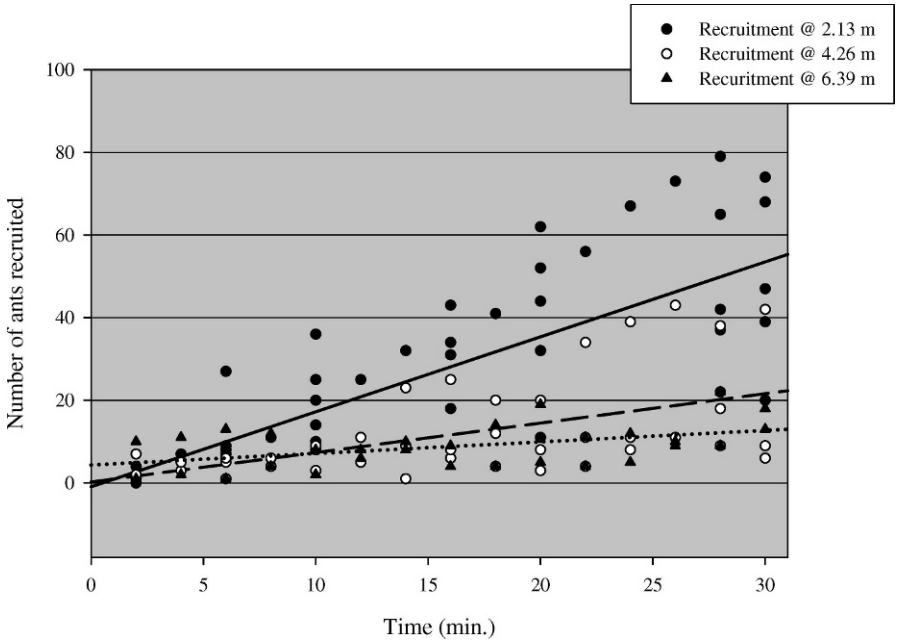


Fig. 5. Relationship between the number of ants recruited to a food source and time at three different distances. Regression equations are: 2.13 m, $y = -0.94 + 1.82x$, ($r^2 = 0.61$, $P < 0.0001$, n = 58); 4.26 m, $y = 0.23 + 0.71x$, ($r^2 = 0.28$, $P < 0.0001$, n = 45); 6.39 m, $y = 4.34 + 0.28x$, ($\sim = 0.19$, $P = 0.01$, n = 30).

similar to those observed in its native environment. In their natural habitat, distances ranged between 3 to 5 m depending on the location of suitable nesting sites. The colony founding behavior of *F. perpilosa* plays a role in both the rapid infestation of newly planted vineyards and in limiting the number of new colonies in mature vineyards. Suitable nesting sites are to a great extent occupied in mature vineyards and the rarity of nascent colonies suggests that newly mated queens may be killed by other ants while attempting claustral colony founding. The mating flights allow *F. perpilosa* to disperse over a large area and rapidly colonize newly planted vineyards.

Foraging characteristics. The daytime maximum within-row foraging distance of *F. perpilosa* ranged between 2.13 and 14.68 m with approximately 69% of the foraging occurring between 2.13 and 6.39 m of a home nest. The weighted average maximum foraging distance was 6.12 ± 9.81 m (mean ± SD). A significant rate of recruitment occurred at 2.13, 4.26, and 6.39 m away from a nest; however, the rate decreased considerably beyond 2.13 m (Fig. 5).

F. perpilosa is both a diurnal and nocturnal forager with peak activity occurring between 0400 and 0600 h (Schumacher and Whitford 1974). At a soil surface temperature greater than 45.2 ± 0.6°C (mean ± SD) foraging does not occur (Schumacher and Whitford 1974). Our experiments were conducted between June and September when soil surface temperature exceeded this and foraging did not occur between or across vine-rows. Workers that collected bait all began their

journey home in the same direction and entered the first nest they encountered. These data suggest that more than one nest did not forage from the same food source i.e., foraging territories do not overlap. Foraging territories appeared to be well defined even though neighboring nests were related in many instances.

Control. Given the widespread distribution of *F. perpilosa* within the Coachella Valley, the high density of nests in vineyards, and its association with *P. ficus*, this ant has a potential to cause substantial economic damage. In a previous study (Tollerup et al. 2004), we showed the efficacy of granular baits in controlling *F. perpilosa* in vineyards. The results of this study provide critical information for determining the application rates and timing of these granular baits. In developing a baiting program for *F. perpilosa* in vineyards a primary consideration is the delivery system. Bait stations are ideal because they prevent exposure of the crop to insecticide and do not interfere with biological control programs. Additionally, bait stations can be designed to reduce risk to non-target ant species.

Controlling *F. perpilosa* with bait delivered in stations, however, requires that the entire vineyard be monitored in order to locate all nests. The debris pile at the base of infested vines provides a reliable criterion for determining if an infestation is present. However, since debris piles tend to be small and more difficult to see during the early season, it is best that monitoring be done in the last week of May or first week of June.

Although there can be ca. half the number of nests during the early season an application of bait would not be effective at this time. The larval stages, especially the later instars, are the only members of a colony that can feed on solid foods (Abbot 1978). Thus it is important that a sufficient number of larvae be present prior to treating with a granular bait, and this occurred by late May to early June.

It is unlikely that a single station will affect control of multiple nests since daytime foraging and recruitment activity occurred primarily within 6.39 m of a home nest and multiple nests did not forage from a common station. Consequently, each nest in the vineyard should be treated with a bait station. However, due to the mating flights and claustral colony founding of *F. perpilosa*, bait stations will not effectively prevent reinfestion or infestations into new vineyards.

Between 2000 and 2006 the granular baits Clinch (0.011% abamectin) (Norvartis Crop Protection Inc., Peters, South Australia), and Esteem (pyriproxyfen) (Valent USA Corporation, Walnut Creek, California) were registered for use as broadcast baits in avocado, almonds, citrus, and walnuts. Although these baits are not effective against *F. perpilosa*, they do control other agricultural pest species such as imported fire ants, *Solenopsis invicta* Buren and pavement ants, *Tetramorium caespitum* (L.).

However, we advocate the application of granular ant baits in stations rather than broadcasted in order to reduce environmental contamination and improve efficacy. Pyriproxyfen for instance is adsorbed by suspended organic material and remains biologically active in soil for up to two months (Sullivan 2000). Pyriproxyfen is stable in light with a half-life of between 6.8 to 8.5 d (Sullivan 2000), while the half-life of abamectin is only 5 to 10 h (Wislocki et al. 1989).

Acknowledgments

We thank Edna Diaz and Marina Hannosh for their assistance with collecting data. We would also like to thank Dr. Carmen Gispert for her help in finding experimental sites and

Dr. Subir Ghosh for his advice on the statistical analysis. Funding for this project was in part provided by the University of California, Statewide Integrated Pest Management Program and the California Desert Grape Administrative Committee. We would like to especially thank the growers that participated in this project and provided us with access to experimental sites.

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Formica perpilosa, an Emerging Pest in Vineyards[1]

Kris Tollerup,[2] Michael K. Rust,[3] and John H. Klotz[3]

J. Agric. Urban Entomol. 24(3): 147–158 (July 2007)

ABSTRACT *Formica perpilosa* Wheeler is a serious economic ant pest on table grapes grown in the Coachella Valley, California, and Hermosillo, Sonora, Mexico. This ant aggressively tends hemipteran pests, such as the vine mealybug, *Planococcus ficus* Signoret, and disrupts natural control by predators and parasitoids. Efforts are underway to develop control measures against *F. perpilosa* using granular bait, yet little is known about the colony life cycle or foraging characteristics of this ant. We studied the seasonal activity, mating behavior, and density and spatial characteristics of *F. perpilosa* nests in vineyards as well as its foraging and recruitment behavior. Nests were active from early February to mid-October. Mating flights occurred in early August and again in the first two weeks of September and new colonies were founded by a single queen. *F. perpilosa* rapidly colonized a new, non-infested vineyard with ca. 9% of the vines infested after 1.5 y. In September the proportions of infested vines at 5, 20, and 30 y old vineyards were 18.6, 21.8, and 16.2%, respectively. This ant is seasonally polydomous and nest density increased ca. two-fold at the 5 and 20-year old vineyards between February and September. Foraging and recruitment primarily occurred up to 6.39 m from a home nest. The implications of these studies for controlling *F. perpilosa* using low-toxic bait delivery systems are discussed.

KEY WORDS *Formica perpilosa*, ant pest, vineyards, bait, control

The ant, *Formica perpilosa* Wheeler, is native to Arizona, Lower California, Nevada, New Mexico and Northern Mexico (Wheeler 1913, Ward 2005). Wheeler (1902) first described this ant as *Formica fusca* subsp. *subpolita* var. *perpilosa* but later revised the species as *Formica perpilosa* (Wheeler 1913). Most species within the genus *Formica* are found in boreal and temperate forests (Fisher and Cover 2007); however, *F. perpilosa* is unusual in that it is found in riparian habitats and irrigated lands in hot-desert regions (Wheeler 1913).

In its native habitat, *F. perpilosa*. nests at the base of desert trees and shrubs such as white-thorned acacia, *Acacia contricta* Benth, and mesquite, *Prosopis juliflora* (Mol.) (Wheeler and Wheeler 1986, Wagner 1997). Workers forage primarily on the plant that their nest is associated with collecting nectar from extrafloral nectaries, honeydew from various hemipterans, and exudate from lycaenid butterfly larvae (Schumacher and Whitford 1974, Wagner and Kurina 1997). It also acts as a generalist predator within plants and as a predator and scavenger of small arthropods on the soil surface (Schumacher and Whitford

[1]Submitted 25 April 2008, Accepted 12 August 2008.

[2]Rutgers Agricultural Research and Extension Center, Bridgeton, New Jersey 08302. E-mail: tollerup@aesop.

[3]Department of Entomology, University of Califomia, Riverside, California 92521.

Instar, Diet and Time of Day at Infestation in Relation to Establishment of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith), On Whorl Stage Sorghum[1]

Johnson J. Zeledon and Henry N. Pitre[2]

J. Agric. Urban Entomol. 24(3): 159–163 (July 2007)

ABSTRACT Field experiments were conducted to determine establishment of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), infestations on whorl stage sorghum when larvae in different stages were reared on either sorghum foliage or wheat germ based diet and artificially inoculated onto the plants. No differences were observed in numbers of larvae per plant on days 1, 2, 6 or 12 after infestation when plants were inoculated with third instar larvae reared on sorghum foliage or wheat germ diet. Inoculations using first or third instars at day 1, 6 or 12 after infestation were not compared because of non-significant interactions. However, at day 2 after infestation and when first instar larvae were used, the mean numbers of larvae per plant were greater when inoculations were made at 7:00 am and 1:00 pm than at 6:00 pm, whereas when third instar larvae were used a greater number of larvae was on the infested plants when inoculated at 7:00 am than at 1:00 pm or 6:00 pm. For experimentation, it is better to inoculate whorl stage sorghum with first or third instar fall armyworm larvae reared on either sorghum foliage or wheat germ diet during early morning than later during the day to obtain suitable survival of larvae to successfully establish infestations of this pest.

KEY WORDS fall armyworm, artificial diet, sorghum infestations

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), is commonly cited as a major crop pest in the Americas (Wiseman et al. 1966). It is considered one of the most important constraints to corn production in Latin America (Andrews 1988). Larvae feed on sorghum foliage, as well as on the panicles (Henderson et. al. 1966). In the southern United States of America the FAW is considered a serious pest on both corn and sorghum (Wiseman and Davis 1979, Isenhour et al. 1985).

Entomologist and plant breeders use a hand held device commonly identified as a bazooka/inoculator (Wiseman et al. 1980) to transfer first instar FAW larvae (Wiseman and Duncan 1989, Starks and Burton 1979) onto corn lines to be screened for insect resistance. On sorghum, a less suitable host than corn for the development of this insect, information regarding successful inoculations of FAW larvae on sorghum is limited in the literature to studies conducted in the 1960s and 1970s (Henderson et al. 1966, Starks and Burton 1979).

[1]Received 25 April 2008; Accepted for publication 10 May 2008.
[2]Corresponding author (hnp1@msstate.edu).
Department of Entomology and Plant Pathology, Mississippi State, Mississippi 39762 US.

Studies related to FAW development and susceptibility to insecticides when reared on different artificial diets and host plants have been conducted. Wood et al. (1981) reported that FAW collected from rice and reared on artificial diet and later placed on a sorghum diet were more susceptible to insecticide than FAW larvae that were moved from artificial diet to Bermuda grass, corn, cotton or soybean. This might suggest that sorghum has some adverse effects on larval development and mortality when larvae are fed for some time on one diet and then moved to feed on sorghum. However, Bailey and Chada (1968) concluded that the change in FAW diet from wheat germ based diet to sorghum leaves or from sorghum leaves to wheat germ diet did not affect larval development and had negligible effect on pupal weight.

Insects are routinely mass reared in the laboratory to be used to artificially inoculate and evaluate crop plants for insect resistance. From such studies new sorghum varieties and hybrids have been developed (Wiseman and Duncan 1989, Diawara et al. 1990, Diawara et al. 1991). Experiments were conducted to determine the success of artificial infestations of sorghum when first and third instar larvae reared on two diets and placed onto whorl stage sorghum using a bazooka/inoculator device at different times of day. Knowledge of the success of FAW artificial infestations of sorghum is important for establishing studies to evaluate host plant resistance to this insect pest, methods of insect pest control, degrees of insect damage to plants, and accurate economic threshold levels.

Material and Methods

Two simultaneous studies were conducted during the 2002 growing season at the Mississippi Agricultural and Forestry Experiment Station Plant Science Research Farm, Oktibbeha County, MS to obtain basic information on artificial infestation procedures with FAW. Fall armyworm egg masses were obtained from the USDA/ARS Laboratory at Starkville, Mississippi, allowed to hatch in the laboratory and larvae were then reared to first or third instars on a wheat germ diet or sorghum foliage (Pioneer '83G66') at 25°C and 95% RH prior to use in field studies.

The grain sorghum hybrid (Pioneer '83G66') was planted in the field with 0.97 m between rows at a rate of 173,000 p1ants/ha on 10 May 2002. Dual II Magnum® herbicide (Syngenta International AG, P.O. Box CH 4002-2, Basel, Switzerland) was applied at the rate of 1.1 kg AI/ha. Larvae of designated instar were placed onto whorl stage plants using a hand-held bazooka/inoculator and infestation success was recorded. Plant phenology, as described by Vanderlip (1993), was used to determine the plant growth stage at treatment and observation times.

Effect of time of day and instar. Sorghum plants were infested at three times during the day with larvae that fed on artificial diet. Treatment plots were three rows, each 3 m long arranged in a randomized complete block design with four replications. Infestations, either 10 first or third instar larvae per plant, were made at 7:00 a.m., 1:00 p.m. or 6:00 p.m. when plants were 25 d old (early growth stage 3, 8 leaves). Larvae were mixed with 20/40 corn cob grit, calibrated to deposit the desired number of larvae directly into the whorl using the bazooka/inoculator. All plants (ca. 50) in the middle row of each plot were infested. Infestations were made when rainfall was not expected for several days. At each sampling interval, number of larvae per plant was recorded from three plants per plot using a destructive sampling procedure. Plants were clipped at ground level

Table 1. Survival of fall armyworm larvae on sorghum after artificial infestation of first or third instar larvae at different times during the day. 2002.

		Mean number of larvae per plant after infestation[1]			
Infestation time	Instar	Day 1[2]	Day 2[3]	Day 6[2]	Day 12[2]
7:00 a.m.	1	4.0	4.1 ab	4.0	2.6
1:00 p.m.	1	3.9	4.2 a	3.9	1.7
6:00 p.m.	1	4.4	3.2 bc	4.1	1.8
7:00 a.m.	3	3.5	4.0 ab	3.5	1.6
1:00 p.m.	3	4.3	2.4 c	2.2	1.1
6:00 p.m.	3	2.8	3.0 c	3.1	1.4

[1]Mean of 10 larvae per plant.
[2]Mean separations were not conducted because of a non-significant interaction.
[3]Mean numbers in the column followed by the same letter are not significantly different ($P > 0.05$). LSMEANS, SAS (1999).

and the whole plant was carefully examined for the presence of larvae on days 1, 2, 6 and 12 after infestation. Data were analyzed using analysis of variance and when appropriate, means were separated using Fisher's protected LSD (SAS 1999).

Effect of food source. Fall armyworm larvae were reared to third instar in the laboratory on artificial diet or sorghum foliage and then used to infest sorghum plants at early growth stage 3 at the rate of 10 larvae per plant. Infestations were made at sundown (6:00 p.m.). Treatment plots were as described in the previous study. All plants (ca. 50) in the middle row of each plot were infested when rainfall was not expected for several days. Plants in each plot were destructively sampled to determine number of larvae per plant at days 1, 2, 6 and 12 after infestation and data were analyzed as in the previous study.

Results and Discussion

Effect of time of day and instar. There was no significant interaction between instar and time of day at infestation for number of larvae per plant on day 1 after infestation ($F = 0.73$; df = 2, 15; $P = 0.4997$) (Table 1). The independent effects of instar and time of day at infestation for number of larvae per plant were not significant ($F = 0.80$; df = 1, 15; $P = 0.3840$ and $F = 0.118$; df = 2, 15; $P = 0.8397$, respectively). By day 2 after infestation, a significant interaction was observed between instar and time of day at infestation on number of larval per plant ($F = 4.15$; df = 2, 15; $P = 0.0360$) (Table 1). A significantly greater number of first instar larvae were on plants inoculated at 1:00 p.m. compared with third instar larvae placed on sorghum at the same time. A generally greater number of larvae were on plants inoculated at 7:00 a.m. or 1:00 p.m. for first instar and 7:00 a.m. for third instar than at later times during the day for respective instars (Table 1). No significant interactions between instar and time of day at infestation for number of larvae per plant were observed on

Table 2. Effect of food source on survival of third instar fall armyworm on sorghum after artificial infestation. 2002.

	Mean number larvae per plant diet after infestation[1]			
Diet	Day 1	Day 2	Day 6	Day 12
Wheat germ	6.3 a[2]	4.7 a	2.0 a	0.8 a
Sorghum foliage	6.0 a	4.3 a	2.3 a	1.0 a

[1]Mean of 10 larvae per plant.
[2]Mean numbers in a column followed by the same letter are not significantly different ($P > 0.05$). LSMEANS, SAS (1999).

days 6 and 12 after infestation ($F = 0.50$; df = 2, 15; $P = 0.6142$ and $F = 1.56$; df = 2, 15; $P = 0.2426$, respectively) (Table 1). There was no significant effect of time of day at infestation on number of larvae per plant ($F = 0.70$; df = 2, 15; $P = 0.5110$), but the effect of instar at infestation was significant ($F = 4.48$; df = 2, 15; $P = 0.0510$) by day 6 after infestation (Table 1). Mean number of larvae per plant for the three infestation times was 4.0 larvae per plant for first instar and 2.9 larvae per plant for third instar. We can infer, using the P value of the ANOVA, that first instar larvae were better for inoculations than third instar larvae when the numbers of larvae per plant for each of the two instars were pooled over time for analysis. By day 12, the effects of both time of day at infestation ($F = 7.30$; df = 2, 15; $P = 0.0061$) and instar ($F = 16.86$; df = 2, 15; $P = 0.0009$) for number of larvae per plant were significant (Table 1). With a significant P value for pooled times of infestation, we can recognize that a greater number of larvae were on plants inoculated at 7:00 a.m. than at 1:00 p.m. or 6:00 p.m. (2.1, 1.4 and 1.6 larvae per plant, respectively). The same general levels in pooled mean numbers of larval per plant at day 12, as at day 6, after infestation were observed for first and third instars (2.0 and 1.4 mean number of larvae per plant, respectively).

Sorghum in whorl stage can be artificially infested in early morning with first instar or third instar FAW larvae reared on either sorghum foliage or wheat germ diet to obtain acceptable insect survival and establishment of infestations for field experiments.

Effect of food source. There were no significant differences in number of third instar FAW larvae on sorghum at days 1, 2, 6 or 12 after bazooka inoculations for insects fed sorghum or artificial diet prior to inoculation (Table 2). The relative ease of rearing FAW larvae on artificial diet compared with rearing larvae on sorghum plant material suggests that larvae reared on artificial diet would be the choice for use in scientific studies as they are suitable for use in the bazooka/inoculator to infest test plants.

Acknowledgments

The authors thank Drs. Richard Brown, Joe Mulrooney and Jack Reed for their reviews of this manuscript. Mississippi Agricultural and Forestry Experiment Station Publication No. J11089. This research was supported in part by the United States Agency for

International Development (USAID) through the PL 480 Title XII Program Agreement, the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP), and USAID development grant LAG-G-OO-96-90009-00. The views and interpretations in this publication are those of the authors and should not be attributed to USAID.

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[1]Received 25 April 2008; Accepted for publication 10 May 2008.
[2]Corresponding author (hnp1@msstate.edu).
Department of Entomology and Plant Pathology, Mississippi State, Mississippi 39762 US.

ISSN 1523-5475

Journal of Agricultural and Urban Entomology

Contents

Volume 24, No. 4 October 2007

THE JOURNAL OF AGRICULTURAL AND URBAN ENTOMOLOGY

http://entweb.clemson.edu/scesweb/jaue.htm

Volume 24 • Number 4 • 2007

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Long Range Planning: Gerry Carner, Clemson University, 318 Long Hall, Clemson, SC 29634; phone 864.656.5040; fax 864.656.5065

Membership: Pat Zungoli, Clemson University, Urban Entomology Lab, 114 Long Hall, Clemson, SC 29634; phone 864.656.3137; fax 864.656.5065

Nomination: D. Michael Jackson, USDA/ARS /Veg. Lab., 2700 Savannah Highway, Charleston, SC 29414; phone 843.402.5308; fax 843.571.4654

Program: Jeremy K. Greene, Clemson University Edisto REC, 64 Research Rd., Blackville, SC 29817; phone 864.284-3343; fax

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Resolutions: Paula Mitchell, Winthrop Univ. Biology Dept., Rock Hill, SC, 29733; fax 803-323-3448

Evaluation of Clays and Fluorescent Brightener upon the Activity of the Gypsy Moth (Lepidoptera: Lymantriidae) Nucleopolyhedrovirus[1]

Martin Shapiro[2,3] and B. Merle Shepard[4]

J. Agric. Urban Entomol. 24(4): 165–175 (October 2007)

ABSTRACT In the past we have examined different components (i.e., sunlight protectants, virus enhancers) that would be essential in a viral pesticide formulation. In this study, we investigated the effects of silicon-containing materials (bentonite, diatomeaceous earth, kaolin) on the activity of the gypsy moth nucleopolyhedrovirus LdMNPV, as well as the effect of these materials on the viral enhancement activity of a fluorescent brightener Tinopal LPW. Bentonite (1%), diatomaceous earth (1%), and kaolin (1%) had little effect upon the activity of LdMNPV. Moreover, the addition of bentonite, diatomaceous earth or kaolin to LdMNPV/Tinopal had little effect upon the enhancement activity of Tinopal LPW.

KEY WORDS *Lymantria dispar*, gypsy moth, nucleopolyhedrovirus, clays, brightener

Insect pathogenic viruses are attractive alternatives to chemical pesticides for subduing plant pests. Their specificity for the host pest (Kondo and Maeda 1991, Miller and Lu 1997, Rahman and Gopinathan 2003, Simon et al. 2004) minimizes their environmental impacts as pollutants. However, entomopathogens used as biocontrol agents suffer from instability after exposure to solar radiation, especially in the ultraviolet (UV) portion of the spectrum (Bullock 1967, Jaques 1968, Morris 1971, Timans 1982). The resulting loss of biological activity prolongs the rate at which insect pests are killed. In many instances, the pathogens lose >90% of their original activity within days (David et al. 1968, Broome et al. 1974, Ignoffo et al. 1977, Jones and McKinley 1986). Another important constraint to the widespread use of insect viruses for managing insect pests is that these microbes are inherently slow acting even without the loss of activity due to UV radiation. Thus several days or more are required to achieve insect mortality (Shapiro et al. 1992a, Moscardi 1999, Szewczyk et al. 2006). Larvae continue to feed and damage crops until shortly before death; therefore, field applications may not provide adequate crop protection.

During the past 40+ years, much research has been conducted to maximize the efficacy of insect pathogens by: (1) the addition of sunlight protectants (Shapiro

[1]Accepted for publication 26 Jun 2008.

[2]Current address: Clemson University, Coastal Research and Education Center, Charleston, South Carolina 29414, USA.

[3]Insect Biocontrol Laboratory, USDA-ARS, Henry A. Wallace Agricultural Research Center, Beltsville, MD 20705.

[4]Clemson University, Coastal Research and Education Center, Charleston, South Carolina 29414, USA.

1989, Tamez-Guerra et al. 2000, Filho et al. 2001, Farrar et al. 2003), (2) the use of adjuvants to increase host susceptibility, thereby increasing viral activity (Shapiro et al. 1992b, Dougherty et al. 1996, Vail et al. 1999, Dougherty et al. 2006, Mukawa and Goto 2007), and (3) genetically modifying the virus genome to increase host susceptibility, thereby increasing virus activity (Maeda et al. 1991, Zlotkin et al. 2000, Szewczyk et al. 2006). The purpose of our research is to obtain a formulation that consists of UV protectant(s), as well as virus enhancers. To this end, we investigated the effects of silicon-containing materials (bentonite, diatomaceous earth, kaolin) and a fluorescent brightener (Tinopal LPW), singly and in combination, upon the activity of an insect baculovirus from the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae).

Materials and Methods

Insects, virus inocula, bioassays. The New Jersey strain of the gypsy moth (USDA-APHIS, Otis ANGB, MA) was used. Insects were reared on a wheat germ-based diet (Bell et al. 1981). The gypsy moth nucleopolyhedrovirus (LdMNPV) was the Hamden isolate LDP-226 (U.S. Forest Service, Hamden, CT) (Shapiro 2002). Viral occlusion bodies (OBs) were diluted in distilled water, in a clay suspension (l% wt:wt), in a fluorescent brightener (Tinopal LPW; 1%) or in a clay (l%)/brightener (1% suspension) to produce virus concentrations ranging from 10^2 to 10^6 OBs per ml (=0.02 *209.6 OBs* / mm^2). One ml of inoculum was pipetted onto the diet surface in individual 180-ml containers (=4,470 mm^2; Sweetheart Cup, Chicago, IL). Ten second instars (7 d old; mean ± SE weight, 35 ± 6.8 mg per larva) were placed in each container and were maintained for 21 d at 28°C, 50% RH and a photoperiod of 12:12 (L:D). Treatments included LdMNPV, LdMNPV/clays, LdMNPVl/brightener, and LdMNPV/clay/brightener combinations and were replicated eight times. Each replicate consisted of 30 larvae per virus concentration per treatment, 30 untreated larvae, 30 clay-only-treated larvae per replicate, and 30 brightener-only-treated larvae per replicate.

Clays, brightener. Bentonite (CAS #1302-78-9), Diatomaceous Earth (CAS #68855-54-9), Kaolin (CAS #1332-58-7), Tinopal LPW (CAS #4404-43-7), and Calcofluor M2R (Fluorescent Brightener 28) were obtained as technical-grade powders from Sigma-Aldrich (St. Louis, MO). Settling rates were determined after 90, 120, 330, 420 and 1440 min using 1 g samples of bentonite, diatomaceous earth, and kaolin diluted in 99 g distilled water or in 1 g of Tinopal LPW in 98 g distilled water, placed in individual 100 mL graduated cylinders.

Statistical Methods. Concentration-mortality regressions were calculated to determine the effects of clays, the brightener, and clay/brightener combinations on LdMNPV activity. Slopes and LC_{50s} were obtained with the probit option of POLO (LeOra software 1987). Means were separated for significance according to Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ (Steel and Torrie 1960).

Results

None of the treatments were toxic to gypsy moth larvae and none adversely affected larval growth and development. Kaolin, bentonite, and diatomaceous earth had little effect upon the activity of LdMNPV (Table 1). The addition of Tinopal LPW

Table 1. LCso (95% CL) of the gypsy moth nucleopolyhedrovirus with and without a clay, a brightener or a clay-brightener combination.

Treatment[a]	LC_{50} (95% CL)[b]	Slope (± SE)	AR[s]
LdMNPV	8.58 (5.51–13.15)	1.54 ± 0.01	1.00
LdMNPVIKaolin	6.16 (3.56–10.05)	1.62 ± 0.03	1.39
LdMNPV*IBentonite*	6.78 (3.18–13.57)	1.37 ± 0.03	1.27
LdMNPVIDiatomaceous Earth	7.88 (4.82–12.62)	1.49 ± 0.02	1.09
LdMNPV/Tinopal LPW	0.009 (0.006–0.015)	1.46 ± 0.02	953.33
LdMNPV/Kaolin Tinopal LPW	0.005 (0.003–0.008)	1.42 ± 0.003	1716.00
LdMNPV/*Bentonite* Tinopal LPW	0.008 (0.004–0.011)	1.58 ± 0.03	1072.50
LdMNPV/Diatomaceous Earth Tinopal LPW	0.010 (0.007–0.016)	1.35 ± 0.01	858.00

[a]Kaolin, Bentonite, Diatomaceous Earth and Tinopal LPW were tested at 1% (wt:wt).
[b]LCsos were expressed as *OBs*/mm^2 of diet surface; eight replicates; five virus concentrations per virus treatment per replicate; 30 larvae per concentration per treatment per replicate; 30 untreated larvae per replicate; 30 clay-only-treated larvae per replicate; 30 brightener-only-treated larvae per replicate. Gypsy moth virus was suspended either in distilled water (LdMNPV alone) or in a clay suspension (l%), brightener (l%), or in a clay (l%)/brightener (l%) combination. Activity ratio (AR) was calculated by dividing the LC_{50} for LdMNPV by LCsos for LdMNPV/clay, LdMNPV/brightener, or LdMNPV/clay/brightener combinations.

Activity ratio. Tinopal LPW (1%) significantly decreased the LC_{50} by ≈950-fold. The addition of kaolin (1%), bentonite (1%) or diatomaceous earth (1%) to LdMNPV/Tinopal LPW had little effect on the enhancement activity of LdMNPV/Tinopal LPW alone (Table 1).

Neither kaolin, bentonite nor diatomaceous earth are soluble in water and these materials settled to the bottom of the graduated cylinders within 90 minutes. Due to differences in the size and weights of particles, complete settling of these materials occurred between 7 h and 24 h. When bentonite/Tinopal LPW and diatomaceous earth/Tinopal LPW mixtures were examined, complete settling of bentonite and diatomaceous earth also occurred between 7 h and 24 h. During the 24 h test period, kaolin remained in suspension in the kaolin/Tinopal LPW mixture, and did not form a sediment.

Discussion

The overall goal of our research is to develop efficient strategies and technologies for controlling pest populations using insect pathogenic viruses. Two essential components of this research have included virus enhancers (e.g., stilbene fluorescent brighteners) (Shapiro and Robertson 1992, Shapiro et al. 1992b, Dougherty et al. 1996, Shapiro and Argauer 2001) and radiation protectants (Shapiro 1992, Shapiro and Domek 2002, Farrar et al. 2003). Whereas some brighteners have proven to be virus enhancers under laboratory conditions (Hamm 1999, Arakawa et al. 2000, Wang et al. 2007, Zhu et al. 2007), they have been unproven as UV protectants under field conditions (Martinez et al. 2000, Tamez-Guerra et al. 2000, Farrar et al. 2003). Therefore, in order to

maximize viral activity and persistence under natural conditions, it will be necessary to include other radiation protectants in the overall formulations. In other words, we feel that the brightener cannot act as both a UV protectant and a virus enhancer; thus an effective UV protectant is needed in addition to the brightener enhancer.

During the past 30+ years, many materials have been tested as UV protectants for insect pathogens and have shown some success in the laboratory and the field. These materials have included UV absorbers (Ignoffo and Batzer 1971, Martignoni and Iwai 1985, Jones and McKinley 1986, Felton 1999, Cohen et al. 2001), and UV reflectants (Bull et al. 1976, Foster 2000, Farrar et al. 2003, Deleo 2005). The present investigation was conducted to determine whether silicate-containing test materials such as bentonite, diatomaceous earth, and kaolin were compatible with the gypsy moth nucleopolyhedrovirus LdMNPV and the virus enhancer Tinopal LPW (=Fluorescent brightener 28; Calcofluor M2R) before commencing any UV experiments.

In this study, silicates (e.g., bentonite, kaolin and diatomaceous earth) were chosen because (l) silicate-containing clays have been utilized in wettable powder formulations (Tapp and Stotzky 1995, Medungo et al. 1997, Filho et al. 2001), and (2) microbes, toxins and UV-protectants are adsorbed to clays and soil-forming minerals (Moore et al. 1981, 1982, Lipson and Stotzky 1983, 1985, Cohen et al. 1991, Venkateswerlu and Stotzky 1992, Christian et al. 2006), and (3) they act as sunblockers that reflect or scatter UV rays (Jones and McKinley 1986, Margulies et al. 1987, Menzel and Nickel 1993, Glenn et al. 1999).

As a result of studies "on the fate of viruses in aquatic and soil environments" (Moore et al. 1982), it has been known for more than 20 years that viruses such as poliovirus, echoviruses, coxsackieviruses, hepatitis A virus, reoviruses, rotaviruses, and adenoviruses are adsorbed by clay minerals and soils (Moore et al. 1982, Schiffenbauer and Stotzky 1982, Lipson and Stotzky 1985). Subsequently, investigators demonstrated that such bacteria as *Pseudomonas putida* (Trevisan) (Jiang et al. 2007), and toxins from *Bacillus thuringiensis* Berliner subspecies *kurstaki* and *tenebrionis* were adsorbed and bound on clays and clay minerals (montmorillonite or kaolinate) (Tapp and Stotzky 1995). The toxins were "rapidly adsorbed" (at equilibrium) and tightly bound (after "ultimate washing") on the clay minerals montrmorillonite and kaolinite (Tapp and Stotzky 1995). Equilibrium adsorption occurred within 30 min and was lower on kaolinite than on montmorillonite (Lee et al. 2003). Adsorption of bacteriophage PBS 1 of *Bacillus subtilis* Cohn was maximal for montmorillionite and kaolinite after 30 min (Vettori et al. 1999). In 2006 Christian et al. reported that the adsorption of three different insect pathogenic viruses (i.e., *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) single nucleopolyhedrovirus HaSNPV, cricket paralysis virus CrPV, and invertebrate iridescent virus 6 IIV-6) differed on ferric oxide, attapulgite, and kaolinite and that "soils with a high content of iron oxides or kaolinite would likely represent highly effective reservoirs for insect-pathogenic viruses."

The relevance of these research findings to our investigation is that aqueous suspensions of insect pathogenic viruses are used primarily in wettable powder (WP) formulations (Young and Yearian 1986). These formulations, in general, consist of active ingredient, inert carriers, and surface active compounds and "must allow uniform distribution of the pathogen" (Filho et al. 2001). If the virus

is not uniformly distributed, non-uniform applications and non-uniform performance of the virus may occur. One challenge with this approach is settling of the active ingredient (=virus), as well as other formulation components. Settling of the active ingredient is a concern, whether the active ingredient is a microbial insecticide (Studdert et al. 1990, Mullen and Hinkle 1988, Stevens et al. 2005) or a chemical insecticide, fungicide or herbicide (Morcombe et al. 1995, Shafer and Hudson 2002, Vogt and Hutetz 2003). In our study, when bentonite (l%), diatomaceous earth (1%) or kaolin (l%) were suspended in distilled water in a graduated cylinder, 75–80% of these materials settled within 90 minutes and settling continued for the next 330 min. When suspended in 1% Tinopal LPW, bentonite (l%) and diatomaceous earth (1%) these materials settled within 90 minutes but kaolin (1%) did not form a sediment within the 24 h observation period. The result obtained with the kaolin/brightener combination was unexpected, as we assumed that this combination would behave in a similar fashion as the kaolin/water suspension (i.e., formation of a kaolin sediment). Since the sedimentation study indicated differences in behavior, would there also be differences in virus activity when SeMNPV was "formulated" with bentonite, diatomaceous earth, or kaolin in water and brightener combinations?

Assays of virus activity in the presence of mineral suspensions revealed that certain minerals altered the infectivity of the nucleopolyhedrovirus and the iridovirus but did not affect CrPV activity (Christian et al. 2006). For example, the activity of HaSNPV was reduced by bentonite and kaolinate samples but not by attapulgite, ferric oxide or talc. Whereas the *H. armigera* NPV was adsorbed (>98%) by all the minerals tested, adsorption of the cricket paralysis virus CrPV depended upon mineral type. In other words, interactions are both mineral and virus specific (Christian et al. 2006) and inferred that a mineral, whether used as an inert carrier or as a UV protectant, should be tested for its effect upon the virus, as well as on the insect. In the former case (i.e., effect upon virus), kaolin appeared to inactivate HaSNPV immediately after mixing (Christian et al. 2006) and AgMNPV after 8 months in storage at 24.5°C (Filho et al. 2001). Whereas AgMNPV formulations containing attapulgite, bentonite and amorphous silica were wettable, kaolinate formulations were less wettable and were not stabile after storage (Medugno et al. 1997). On the other hand, toxin activity of *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* against the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), was maintained, or even increased after adsorption by montmorrillonite or kaolinite (Tapp and Stotzky 1995). In the latter case (i.e., effect upon insects), both kaolin and diatomaceous earth possess biological activity against insects. Kaolin, also known as china clay, has been the subject of much research as a kaolin-based particle film formulation (Glenn et al. 1999, Puterka et al. 2000, Eigenbrode et al. 2006). The kaolin particle film does not appear to act directly as an insecticide, but acts as a repellent (Liang and Liu 2002, Barker et al. 2006), suppresses oviposition (Lapointe 2000, Unruh et al. 2000, Sisterson et al. 2003), and adversely affects insect behavior and development (Knight et al. 2000, Barker et al. 2006, Hall et al. 2007). Diatomaceous earth is a natural siliceous compound (>96% SiO_2) from the skeletal remains of diatoms with insecticidal (Arthur and Throne 2003, Athanassiou et al. 2005, Ulrichs et al. 2006) and acaricidal (Palyvos et al. 2006) properties. In addition, diatomaceous earth has also been used in conjunction with insect pathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin (Lord

2001, Akbar et al. 2004, Vassilakos et al. 2006) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Kavallieratos et al. 2006). Diatomaceous earth not only increased the efficacy of *B. bassiana* (Akbar et al. 2004), but acted as a synergist against such stored grain beetles as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) (Lord 2001).

In our study, neither bentonite, diatomaceous earth nor kaolin had any detrimental effects upon the growth and development of gypsy moth larvae. Moreover, at the concentration used (l%), no mortality occurred. In terms of virus activity (i.e., LCsos), the addition of bentonite, diatomaceous earth or kaolin to LdMNPV had little effect upon virus activity (Table 1). When Tinopal LPW was added to a virus suspension, the LC_{50} was reduced by ~950-fold (Table 1). These results were expected, as Tinopal LPW (=Calcofluor M2R) had previously been shown to act as a viral enhancer for LdMNPV (Dougherty et al. 1996, Shapiro and Robertson 1992, Shapiro et al. 1992b), as well as for other insect pathogenic viruses (Hamm 1999, Boughton et al. 2001, Murillo et al. 2003, Mukawa et al. 2007). When bentonite, diatomaceous earth or kaolin was added to an LdMNPV/Tinopal LPW combination, little differences in LCso were observed for these treatments and the LCso for LdMNPV/Tinopal LPW. In other words, the addition of the silicate-containing materials had little effect upon the enhancement activity of LdMNPV, regardless of the solubility of the brightener/silicate combination. At this time it is not known whether the efficacies of the three virus/brightener/clay formulations would be different in the field because of differences in settling of the brightener/clay combinations. In other words, would a non-sedimented brightemer/kaolin formulation be more efficacious than sedimented brightener/bentonite or sedimented/diatomaceous earth formulations? This question will be answered in the field, using the nucleopolyhedrovirus from the beet armyworm SeMNPV.

The research reported represents the first half of our ultimate goal (i.e., development of an effective formulation that maximizes virus activity). The next part of our research will be the evaluation of these silicate-containing materials bentonite, diatomaceous earth, and kaolin as UV protectants for SeMNPV, with and without the addition of a brightener. We believe that a formulation containing an effective UV protectant plus a viral enhancer would maximize the performance of an insect pathogenic virus and result in effective insect control.

Acknowledgments

The research was conducted (2002–2003) when Martin Shapiro was a Research Entomologist, Insect Biocontrol Laboratory, USDA-Agricultural Research Service, Beltsville, MD. Technical Contribution No. 5450 of the Clemson University Experiment Station.

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Evaluation of Clays and Fluorescent Brightener upon the Activity of the Gypsy Moth (Lepidoptera: Lymantriidae) Nucleopolyhedrovirus[1]

Martin Shapiro[2,3] and B. Merle Shepard[4]

J. Agric. Urban Entomol. 24(4): 165–175 (October 2007)

ABSTRACT In the past we have examined different components (i.e., sunlight protectants, virus enhancers) that would be essential in a viral pesticide formulation. In this study, we investigated the effects of silicon-containing materials (bentonite, diatomeaceous earth, kaolin) on the activity of the gypsy moth nucleopolyhedrovirus LdMNPV, as well as the effect of these materials on the viral enhancement activity of a fluorescent brightener Tinopal LPW. Bentonite (1%), diatomaceous earth (1%), and kaolin (1%) had little effect upon the activity of LdMNPV. Moreover, the addition of bentonite, diatomaceous earth or kaolin to LdMNPV/Tinopal had little effect upon the enhancement activity of Tinopal LPW.

KEY WORDS *Lymantria dispar*, gypsy moth, nucleopolyhedrovirus, clays, brightener

Insect pathogenic viruses are attractive alternatives to chemical pesticides for subduing plant pests. Their specificity for the host pest (Kondo and Maeda 1991, Miller and Lu 1997, Rahman and Gopinathan 2003, Simon et al. 2004) minimizes their environmental impacts as pollutants. However, entomopathogens used as biocontrol agents suffer from instability after exposure to solar radiation, especially in the ultraviolet (UV) portion of the spectrum (Bullock 1967, Jaques 1968, Morris 1971, Timans 1982). The resulting loss of biological activity prolongs the rate at which insect pests are killed. In many instances, the pathogens lose >90% of their original activity within days (David et al. 1968, Broome et al. 1974, Ignoffo et al. 1977, Jones and McKinley 1986). Another important constraint to the widespread use of insect viruses for managing insect pests is that these microbes are inherently slow acting even without the loss of activity due to UV radiation. Thus several days or more are required to achieve insect mortality (Shapiro et al. 1992a, Moscardi 1999, Szewczyk et al. 2006). Larvae continue to feed and damage crops until shortly before death; therefore, field applications may not provide adequate crop protection.

During the past 40+ years, much research has been conducted to maximize the efficacy of insect pathogens by: (1) the addition of sunlight protectants (Shapiro

[1]Accepted for publication 26 Jun 2008.

[2]Current address: Clemson University, Coastal Research and Education Center, Charleston, South Carolina 29414, USA.

[3]Insect Biocontrol Laboratory, USDA-ARS, Henry A. Wallace Agricultural Research Center, Beltsville, MD 20705.

[4]Clemson University, Coastal Research and Education Center, Charleston, South Carolina 29414, USA.

Seed Treatment Effects on Early-Season Pests of Corn and on Corn Growth and Yield in the Absence of Insect Pests[1]

Gerald Wilde,[2] Kraig Roozeboom,[3] Aqeel Ahmad,[2] Mark Claassen,[4] Barney Gordon,[5] William Heer,[6] Larry Maddux,[7] Victor Martin,[8] Patrick Evans,[9] Ken Kofoid,[10] James Long,[11] Alan Schlegel,[12] and Merle Witt[13]

J. Agric. Urban Entomol. 24(4): 177–193 (October 2007)

ABSTRACT Second-generation neonicotinoid insecticides are being used to protect seeds and seedlings against injury by early season insects on a wide variety of crops. Seed-applied insecticides have recently been commercialized in the USA for early season insect control in corn. The systemic insecticides clothianidin (Poncho®) and thiamethoxam (Cruiser®) applied as seed treatments were evaluated for their effect on corn yield in the absence of noticeable insect attack over a three year period at a number of locations representing diverse growing environments in Kansas. No consistent effect on yield was detected for either compound at either high or low rates in locations where insects were not observed at damaging populations. Controlled-environment studies detected no differences in early season growth in the absence of insect pests in response to the same two rates of both insecticides. Both compounds were evaluated for their effect on early season corn pests and were found to be effective at high and low rates on wireworm, white grub, flea beetles and chinch bugs. Higher rates of both compounds were needed to reduce feeding damage caused by black cutworm. Seed treatments with either compound would be useful where early season pests are chronic problems.

KEY WORDS corn, growth, yield, seed treatment, neonicotinoid, *Melanotus cribulosus*, *Agrotis ipsilon*, *Phyllophaga* sp., *Chaetocnema pulicaria*, *Blissus leucopterus*

Introduction

Protecting the seed and young plants from insect pests is increasingly important for establishing a healthy, vigorous corn stand with increases in total

[1]Accepted for publication 11 Jun 2008.
[2]Department of Entomology, Kansas State University, Manhattan, Kansas.
[3]Department of Agronomy, Kansas State University, Manhattan, Kansas.
[4]Department of Agronomy, Kansas State University, Harvey County Experiment Field, Hesston, Kansas.
[5]Department of Agronomy, Kansas State University, North Central and Irrigation Experiment Fields, Scandia, Kansas.
[6]Department of Agronomy, Kansas State University, South Central Experiment Field, Hutchinson,
[7]Department of Agronomy, Kansas State University, Kansas River Valley Experiment Field, Topeka,
[8]Department of Agronomy, Kansas State University, Sandyland Experiment Field, St. John, Kansas.
[9]Kansas State University Northwest Research-Extension Center, Colby, Kansas.
[10]Kansas State University Agricultural Research Center – Hays, Hays, Kansas.
[11]Kansas State University Southeast Research Center, Parsons, Kansas.
[12]Kansas State University Southwest Research-Extension Center, Tribune, Kansas.
[13]Kansas State University Southwest Research-Extension Center, Garden City, Kansas.

corn acreage and with more corn-on-corn situations. The second-generation neonicotinoid insecticides applied as seed treatments are being used to protect seeds and seedlings against injury by early season insects on a wide variety of crops through contact, stomach and systemic activity (Almand 1995, Burd et al. 1996, Mckirdy and Jones 1996, Graham 1998, Wilde et al. 1999, Wilde et al. 2004). Seed-applied insecticides have recently been commercialized in the USA for early season insect control in corn (Andersch and Schwarz 2003). Cruiser® is the trade name for thiamethoxam, a second generation chloronicotinyl insecticide (CNI) developed by Syngenta Crop Protection (Greensboro, North Carolina), and Poncho® is the trade name for clothianidin, another CNI developed by Bayer Crop Science, Monheim, Germany. It is estimated that about 75% of the U.S. corn acreage was treated with one of these two seed-applied insecticides in 2007 (C. Nichols, Syngenta Crop Protection, personal communication).

In addition to controlling a broad spectrum of insect pests, it has been suggested that these compounds may afford plants a "stress shield" (Thielert 2006) or have a "vigor effect" (Schade et al. 2007) that result in higher crop yields even in the absence of insect attack, especially if plants are under some abiotic stress such as drought. Wilde et al. (2004) evaluated the effect of Cruiser® and Gaucho® on a variety of pests and on yield in the absence of pests in corn and sorghum, respectively. They concluded that positive yield responses from seed treatment in both crops were usually associated with noticeable insect pest activity. More recently, Cox et al. (2007) reported that Poncho® seed treatments were inconsistent in their effect on corn forage yield following soybean.

The purpose of our study was to (1) evaluate the effect of Poncho® and Cruiser® seed treatments on corn yield in the field in the absence of noticeable insect pest attack, (2) evaluate the effect of Poncho® and Cruiser® on early plant development under controlled conditions in the greenhouse, and (3) obtain further information on the efficacy of these treatments on some common early season insect pests, i.e., black cutworm, wireworms, chinch bugs, white grubs, and flea beetles.

Materials and Methods

Comparison of yields from Cruiser® and Poncho® treated corn seed. Field tests were conducted during the 2004 growing season at 19 locations (Table 1) associated with Kansas State University Corn Crop Performance Tests. A single seed lot of an adapted commercial corn hybrid (Midland 798) was subdivided, with one portion retained as an untreated check and the remaining two portions treated with Poncho® and Cruiser® at 0.25 mg/seed by their respective companies. Yields from plots planted with the treated seed samples were compared with that of plots planted with the untreated check seed at each location. The test comparison was nested in an experiment along with varying numbers of hybrids at each location. Plots consisted of two rows spaced 76 cm apart and 6.2 m long replicated 4 times.

In 2005 and 2006, tests were conducted at 9 and 8 locations, respectively. Uniform seed lots of two corn hybrids (Pioneer Brand 33R78 and DEKALB DKC60-16) were subdivided with one portion retained as untreated and the other portions treated with different rates of Poncho® and Cruiser® by their respective companies. In 2005 each of the two hybrids were treated with two rates of

Table 1. Annual precipitation and agronomic information for seed treatment field evaluations on corn in Kansas.

Location	October to September precipitation				Previous crop	Soil series
	Normal	2004	2005	2006		
	inches					
Topeka (I*)	33.2	34.9	46.9	34.0	Soybean	Eudora silt loam
Clay Center (I)	32.3	28.4	31.7		Soybean	Muir silt loam
Scandia (I)	27.6	34.4	39.4	33.3	Soybean	Crete silt loam
Inman (I)	30.7	35.4			Soybean	Crete silt loam
Hutchinson (I)	27.4	33.8			Soybean	Punching silt loam
St. John (I)	24.2	25.4			Corn	Naron loamy fine sand
Colby (I)	17.4	16.8			Sunflower or Soybean	Keith silt loam
Tribune (I)	15.0	20.0			Sorghum	Ulysses silt loam
Garden City (I)	17.7	22.8	17.4	18.5	Soybean	Keith silt loam
Severance	35.8	33.5			Soybean	Monana silt loam
Centralia	35.3	33.3			Alfalfa	Wymore silt loam
Belleville	27.6	26.5	32.3		Soybean	Crete silt loam
Manhattan	32.3	33.7	35.8	27.0	Soybean	Reading silt loam
Topeka	33.2	37.2			Soybean	Eudora silt loam
Ottawa	32.8	41.6	57.5	27.0	Soybean	Woodson silt loam
Hays	22.0	22.5			Soybean	Harney clay loam
Colby	17.4	16.8	21.0		Wheat	Keith silt loam
Hesston	30.7	30.4	40.7	23.3	Wheat	Smolan silt loam
Parsons	39.6	36.9			Soybean	Parsons silt loam

*I, Irrigated.

Poncho® (0.25 and 1.25 mg/seed) and two rates of Cruiser® (0.125 and 0.25 mg/seed). In 2006 both Poncho® and Cruiser® were treated with the same rates of Poncho® and Cruiser® (0.25 and 1.25 mg/seed). All seed was treated with two fungicides (Apron® and Maxim®) and both hybrids contained the *Bt* gene for European corn borer resistance.

In all years, corn was planted after a crop other than corn to exclude western corn rootworm (*Diabrotica virgifera virgifera* Leconte) infestations as a factor that could affect yield in the tests. The one exception was at St. John in 2004. Hybrids and seed treatments were arranged in a factorial treatment structure that was replicated eight times in a randomized complete block design. Each hybrid–seed treatment combination was evaluated in plots consisting of two rows spaced 76 cm apart and 6.2 m long. At most locations, tests were planted in reduced-tillage cropping systems designed to maintain sufficient surface residue to conserve soil and moisture. Commercially available herbicides were used to control weeds as dictated by the rotation constraints and weed species present at each location. All of the agronomic practices (fertilization, planting dates, seeding rates etc.) were carried out in accordance with recommendations from Kansas State University Research and Extension (Roozeboom et al. 2007). Soil moisture

at planting was adequate for seed germination and emergence. Annual precipitation and agronomic information are given in Table 1.

Various parameters were measured to evaluate corn response to the insecticide treatment. Days to half silk were determined as the number of days from planting until half the ears in a plot were fully silked. Plots were harvested with a modified Gleaner K2 combine (AGCO Corp., Duluth, Georgia) equipped with a Grain Gage (Harvest Master, Inc., Logan, Utah) data collection system to determine yield, test weight, and grain moisture. Plots were examined at two-week intervals for the presence of insects.

Greenhouse tests. Corn seeds of two hybrids (Pioneer Brand 33R78 and Dekalb DKC60-16) treated with two rate of Poncho® (0.25 and 1.25 mg/seed) and Cruiser® (0.125 and 0.25 mg/seed) were planted in the greenhouse (1 seed/pot). Sunshine Mix #1, which contains peat moss, perlite, major and minor nutrients, gypsum, and dolomitic lime was obtained from SunGro Horticulture, Bellevue, WA. Plants were grown in 0.5 × 0.5 m pots in a mixture of Sunshine soil in a greenhouse bay maintained at 21°C, with a photoperiod 14:10 (L:D) and 40–50% relative humidity for the duration of the study. Plants were watered regularly and fertilized twice per week with a 100 ppm mix (Peters 20-20-20 General). Light intensity in the greenhouse at the canopy level under a clear sky at midday was 900 umol photons/m^2/s, recorded using a quantum sensor (model LI-190; LI-Cor, Lincoln, NE). There were four pots or replications of each treatment. Plants were destructively sampled at the one, two and five leaf stages. Leaf area (mm^2/plant) and root fresh weight (g/plant) were measured at the one and two leaf stages and plant fresh and dry weight (g/plant) were measured at the one, two and five leaf stages. Because there was no interaction between hybrids and treatments, hybrids were combined for treatment mean comparisons.

Insecticide efficacy. Wireworm control. The effects of various seed treatments and in-furrow applied insecticides were evaluated in two fields infested with wireworms (*Melanatus cribuloses*). The first test was near Silver Lake, KS in Shawnee County. Corn hybrid (Pioneer Brand 33R78) was planted and treatments applied with a v-belt seeder on 24 May 2005. Plots were one row 9.1 m in length with four replications. Plant populations and a vigor rating were determined on June 16 and yields were estimated by hand harvesting plots on Oct 10, shelling the ears, and calculating the yield of shelled grain in kg/ha.

A second test was planted near Horton, KS in Shawnee County on 13 April 2004. Treatments were applied with a v-belt seeder in plots 9.1 m in length replicated 3 times. Stand counts were made on May 12 and vigor ratings made on May 12 and June 3 using a 0–2 scale where 0 equals severe stunting and 2 equals no stunting. Yield estimates were made on September 14 by hand harvesting plots.

A greenhouse test using wireworms collected from the field was conducted in 2005. Five pots (6.35 × 6.35 cm size) filled with soil collected from an untreated area of the Silver Lake field were planted with 1 seed/pot. Pots were infested with 1 late-instar wireworm/pot after planting. A damage rating was made on June 12, 14 days after planting using a scale of 0 to 10 (0 = no damage, 10 = severe damage and stunting).

Chinch bug control. The effect of various seed treatments on chinch bugs (*Blissus leucopterus*) was evaluated in a field heavily infested with chinch bugs near Randolph, KS in Riley County, KS. Corn hybrid (N67-T4) was planted on 21 May

Table 2. Seed treatment evaluations on corn in Kansas, 2004.

Locations	Untreated	Poncho	Cruiser
Clay center (I*)	207.4 a**	196.2 ab	185.0 b
Tribune (I)	157.7 ab	141.7 b	176.5 a
Inman (I)	241.6 a	234.0 a	244.7 a
Hutchinson (I)	241.5 a	224.9 a	231.0 a
Scandia (I)	249.6 a	245.4 a	217.3 b
Topeka (I)	246.0 a	235.1 a	221.0 a
Colby (I)	271.2 a	240.2 b	257.1 ab
St. John (I)	200.5 a	197.5 a	197.6 a
Garden City (I)	243.4 a	243.2 a	243.3 a
Severance	221.6 a	202.9 b	213.8 ab
Hays	84.8 ab	102.3 a	78.6 b
Ottawa	168.2 a	177.6 a	167.5 a
Ottawa	155.7 a	161.9 a	162.1 a
Centralia	166.3 a	171.4 a	173.0 a
Manhattan	197.7 a	202.7 a	204.3 a
Belleville	113.6 b	115.0 ab	122.1 a
Topeka	215.9 a	214.0 a	224.3 a
Colby	38.9 a	46.2 a	45.9 a
Hesston	168.0 a	163.0 a	163.0 a
Average	188.9 a	185.0 a	185.7 a

*I, Irrigated.
**Means in the same row followed by the same letter are not significantly different, $\alpha = 0.05$.

2004 in 1 row plots 9.1 meter in length with a hand planter in a randomized complete block with 4 replications. The number of chinch bug adults and nymphs were counted on 2 plants per plot on June 3 (2 leaf stage) and 1 plant per plot on June 10 (4 leaf stage). Yield estimates were made by hand harvesting plots on September 1.

White grub control. The effect of various treatments on white grubs was evaluated in a brome grass field heavily infested with white grubs (*Phyllophaga* sp.) near Hanover in Washington County, KS. Corn (Pioneer Brand 33R78) was hand planted in hills (2 seeds/hill) on 4 April 2005. There were 4 hills per treatment in a randomized complete block design spaced 1 m apart. Treatments were evaluated by counting the number of plants/hill on April 24 and June 26 and by assessing stunting using a rating scale of 0 to 10 (0 = no stunting, 10 = severe stunting) on April 24. No yield data was taken because of a severe drought.

A similar test was planted on 22 April 2004 in a heavily infested brome grass pasture near Lyndon, KS in Osage County. Again, corn (Pioneer Brand 33R78) was planted in hills (2 seeds/hill) replicated 5 times. The number of emerging plants/hill was counted on 16 May 2004. No yield data was taken because animals were allowed to enter the pasture.

Black cutworm control. The effect of various seed treatments on black cutworm (*Agrotis ipsilon*) was evaluated in a field test on the Kansas State University Agronomy farm in Riley County. Corn (Pioneer Brand 33R78) was planted on 21 April 2005 with a v-belt seeder in one row plots 9.1 m in length with 4 replications. Two second-instar larvae were caged (cages 5 cm diameter)

Table 3. Seed treatment evaluations on corn in Kansas, 2005.

Location	*P*-values Population	*P*-values Yield	Treatments	Population plants/acre	Yield bu./acre
Colby			Untreated	30084 a*	251.3 a
Trt	0.20	0.23	Cruiser 5FS 0.125	30519 a	249.6 a
Hybrid	0.35	0.17	Cruiser 5FS 0.25	29730 a	241.2 a
Trt × Hybrid	0.58	0.97	Poncho 250	30084 a	248.5 a
			Poncho 1250	30465 a	240.2 a
Garden City			Untreated	26041 a	160.2 a
Trt	0.38	0.58	Cruiser 5FS 0.125	26894 a	158.2 a
Hybrid	0.13	0.00	Cruiser 5FS 0.25	26018 a	161.6 a
Trt × Hybrid	0.41	0.58	Poncho 250	27438 a	155.2 a
			Poncho 1250	26325 a	158.8 a
Scandia			Untreated	33193 a	236.5 a
Trt	0.92	0.38	Cruiser 5FS 0.125	33694 a	236.8 a
Hybrid	0.58	0.27	Cruiser 5FS 0.25	33803 a	238.0 a
Trt × Hybrid	0.12	0.06	Poncho 250	33541 a	237.5 a
			Poncho 1250	33759 a	238.8 a
Topeka			Untreated	25344 a	181.3 a
Trt	0.09	0.05	Cruiser 5FS 0.125	25681 a	182.9 a
Hybrid	0.15	0.00	Cruiser 5FS 0.25	25720 a	183.3 a
Trt × Hybrid	0.07	0.07	Poncho 250	25324 a	177.4 ab
			Poncho 1250	24473 a	164.1 b
Hesston			Untreated	14865 c	97.4 a
Trt	0.02	0.77	Cruiser 5FS 0.125	15645 abc	92.4 a
Hybrid	0.00	0.19	Cruiser 5FS 0.25	15246 bc	95.5 a
Trt × Hybrid	0.43	0.91	Poncho 250	16281 a	96.2 a
			Poncho 1250	16135 ab	94.8 a
Manhattan			Untreated	24281 a	126.9 b
Trt	0.19	0.04	Cruiser 5FS 0.125	24308 a	126.8 b
Hybrid	0.02	0.07	Cruiser 5FS 0.25	25007 a	134.6 ab
Trt × Hybrid	0.56	0.52	Poncho 250	24630 a	132.4 ab
			Poncho 1250	25948 a	137.5 a
Belleville			Untreated	24416 a	102.3 a
Trt	0.94	0.62	Cruiser 5FS 0.125	24677 a	101.8 a
Hybrid	0.22	0.00	Cruiser 5FS 0.25	24590 a	102.8 a
Trt × Hybrid	0.99	0.67	Poncho 250	24634 a	103.5 a
			Poncho 1250	24590 a	102.7 a
Parsons			Untreated	24851 a	128.3 b
Trt	0.67	0.02	Cruiser 5FS 0.125	25417 a	136.0 a
Hybrid	0.02	0.00	Cruiser 5FS 0.25	24742 a	135.3 a
Trt × Hybrid	0.33	0.40	Poncho 250	24677 a	131.2 ab
			Poncho 1250	25309 a	135.2 a
Ottawa**			Untreated × P33R78	22829 a	142.8 abc
Trt	0.07	0.22	Cruiser 5FS 0.125 × P33R78	21195 a	140.9 bc

Table 3. Continued.

Location	*P*-values Population	*P*-values Yield	Treatments	Population plants/acre	Yield bu./acre
Hybrid	0.00	0.73	Cruiser 5FS 0.25 × P33R78	21437 a	149.7 ab
Trt × Hybrid	0.35	0.01	Poncho 250 × P33R78	22445 a	149.3 ab
			Poncho 1250 × P33R78	22405 a	142.2 bc
			Untreated × DKC60-16		154.4 a
			Cruiser 5FS 0.125 × DKC60-16		138.2 bc
			Cruiser 5FS 0.25 × DKC60-16		134.4 c
			Poncho 250 × DKC60-16		142.1 abc
			Poncho 1250 × DKC60-16		151.1 a

*Means within a column for a location followed by the same letter are not significantly different, α = 0.05.
**Significant treatment × hybrid interaction for yield.

on two plants per plot on May 3 and damage ratings were made on May 10 using a 0 to 10 scale where 0 = no damage and 10 = plant cut or leaves severely damaged. In 2006, a similar test was conducted using the same protocol. Corn was planted on April 22, artificially infested on May 11 and evaluated on May 15 using the same rating scale.

Flea beetle control. The effect of Poncho® and Cruiser® seed treatments on flea beetle (*Chaetocnema pulicaria*) was assessed in a field on the Kansas State University Experiment field near Hesston, KS in Harvey county in 2006. Corn (Pioneer Brand 33R78) was planted with a v-belt seeder in 1 row 9.1 m plots replicated 4 times in a randomized complete block design. Damage ratings were assessed on May 20 using a 0 to 5 scale where 0 = no feeding and 5 = 50% of the leaf tissue scarred. Yield was estimated by hand harvesting plots on 15 September 2006.

Statistical analysis. Data from the Poncho®–Cruiser® comparisons were subjected to a mixed model analysis using PROC MIXED (Little et al. 1996). Replications were considered random and hybrids and seed treatment were considered fixed. Means were separated using pair-wise *t*-tests with a probability level of 0.05. Data for all insecticide efficacy tests were analyzed using analysis of variance (ANOVA) and means were compared with the LSMEANS procedure (P = 0.05) of PROC GLM (SAS Institute 2003).

Results

Comparison of Poncho® and Cruiser®. Results of the 2004 comparisons of the 0.25 rate of Poncho® and Cruiser® with an untreated check across 19 locations in Kansas are presented in Table 2. No noticeable insect activity occurred at any location. There was no significant difference between treatments in most locations. Occasionally, the yield in untreated plots was significantly higher than in plots treated with Cruiser® (Clay Center and Scandia) and in untreated plots compared to plots treated with Poncho® (Severance and Colby).

Table 4. Seed treatment evaluations on corn in Kansas, 2006.

Location	*P*-values Population	*P*-values Yield	Treatments	Population plants/acre	Yield bu./acre
Colby			Untreated	27377 a*	228.1 b
Trt	0.32	0.01	Cruiser 5FS 0.25	26202 a	223.2 b
Hybrid	0.81	0.19	Cruiser 5FS 1.25	26789 a	238.0 ab
Trt × Hybrid	0.69	0.54	Poncho 250	28345 a	255.6 a
			Poncho1250	26630 a	228.5 b
Garden City			Untreated	27059 a	143.7 a
Trt	0.00	0.01	Cruiser 5FS 0.25	25544 b	127.0 b
Hybrid	0.02	0.00	Cruiser 5FS 1.25	23437 c	140.3 a
Trt × Hybrid	1.00	0.26	Poncho 250	26373 ab	140.9 a
			Poncho 1250	26444 ab	126.7 b
Scandia			Untreated	33256 a	188.5 b
Trt	0.42	0.03	Cruiser 5FS 0.25	33061 a	191.8 ab
Hybrid	0.37	0.00	Cruiser 5FS 1.25	32810 a	194.8 a
Trt × Hybrid	0.53	0.13	Poncho 250	32586 a	194.2 a
			Poncho 1250	32614 a	192.9 a
Topeka**			Untreated × P33R78	24845 ab	231.9 a
Trt	0.67	0.93	Cruiser 5FS 0.25 × P33R78	24765 ab	226.9 ab
Hybrid	0.06	0.00	Cruiser 5FS 1.25 × P33R78	24160 b	225.6 ab
Trt × Hybrid	0.02	0.04	Poncho 250 × P33R78	23958 b	224.2 ab
			Poncho 1250 × P33R78	24160 b	208.5 bc
			Untreated × DKC60-16	24603 ab	184.0 de
			Cruiser 5FS 0.25 × DKC60-16	24160 b	189.4 cde
			Cruiser 5FS 1.25 × DKC60-16	24482 ab	179.0 e
			Poncho 250 × DKC60-16	25450 a	192.1 cde
			Poncho 1250 × DKC60-16	25329 a	205.9 bcd
Hesston			Untreated	17569 a	40.0 a
Trt	0.47	0.58	Cruiser 5FS 0.25	17587 a	39.6 a
Hybrid	0.24	0.00	Cruiser 5FS 1.25	17678 a	39.4 a
Trt × Hybrid	0.11	0.82	Poncho 250	17878 a	38.9 a
			Poncho 1250	17914 a	41.7 a
Manhattan			Untreated	25793 a	170.3 a
Trt	0.83	0.92	Cruiser 5FS 0.25	25612 a	170.7 a
Hybrid	0.00	0.09	Cruiser 5FS 1.25	25531 a	169.7 a
Trt × Hybrid	0.54	0.92	Poncho 250	25934 a	173.1 a
			Poncho 1250	25713 a	171.9 a
Parsons			Untreated	— #	63.4 a
Trt		0.73	Cruiser 5FS 0.25	—	62.5 a
Hybrid		0.19	Cruiser 5FS 1.25	—	66.2 a
Trt × Hybrid		0.89	Poncho 250	—	69.4 a
			Poncho 1250	—	67.5 a

Table 4. Continued.

Location	*P*-values Population	*P*-values Yield	Treatments	Population plants/acre	Yield bu./acre
Ottawa			Untreated	23797 c	33256 a
Trt	0.00	0.25	Cruiser 5FS 0.25	24462 bc	33061 a
Hybrid	0.00	0.00	Cruiser 5FS 1.25	24119 bc	32810 a
Trt × Hybrid	0.27	0.17	Poncho 250	24624 b	32586 a
			Poncho 1250	25370 a	32614 a

*Means within a column for a location followed by the same letter are not significantly different, α = 0.05.
**Significant treatment × hybrid interaction for plant population and yield.
#Not observed.

At Hays, Poncho®-treated plots yielded significantly more than Cruiser®-treated plots. Cruiser®-treated plots yielded significantly more than the untreated check plots at Belleville. When treatments were analyzed across all 19 locations, there was no significant difference between treatments.

No noticeable insect activity was noted at any location in 2005 or 2006. A combined analysis was not performed because Bartlette's chi-square test (Gomez and Gomez 1984) rejected the null hypothesis of homogenous variances for all response variables in both years (P = 0.05, df = 8 in 2005, 7 in 2006). Tables 3 and 4 list the P values associated with F tests of corn seed treatment and hybrid effects and their interactions in 2005 and 2006. Grain test weight, plant height, and lodging showed no significant interaction or seed treatment effects in any environment (location × years). The hybrid effect was the only source of variation to display significant differences for these characters (data not shown).

In 2005, seed treatment and hybrid interactions occurred only at the Ottawa location for yield (Table 3) and days to silk, and for percent moisture at the Garden City location (data not presented). Therefore, seed treatment main effect means across two hybrids are presented except as noted (Table 3). Days to silk and grain moisture showed no significant treatment or interaction effects except as noted above and are not presented. There was no significant effect of treatment on plant populations at 8 of the 9 locations. At Hesston, plots treated with the 0.25 mg/seed rate of Poncho® had a significantly greater population than the Cruiser® at 0.25 mg/seed treatment and the untreated plot. Poncho®-treated plots at the 1.25 mg/seed rate also had a significantly greater population that the untreated plots. There was no significant difference in yield between seed treatments at 5 of the 8 locations. At Manhattan, plots treated with the 1.25 mg/seed of Poncho® yielded significantly more than those treated with Cruiser® at the 0.125 mg/seed and the untreated plots. At Parsons, all treatments except Poncho® at the 0.25 mg/seed yielded significantly more than the untreated check. At Topeka, the high mg/seed of Poncho® yielded significantly less than the untreated plots and plots treated with both rates of Cruiser®. At Ottawa, where a significant interaction between hybrid and treatment was detected, there was no significant treatment effect for one hybrid (Pioneer Brand 33R78). For hybrid DKC60-16, the untreated plots yielded significantly more than plots treated with Cruiser® at both rates.

Table 5. Leaf area and root fresh weight of plants treated with two rates of Poncho® and Cruiser® at one and two leaf stages.

Treatment	Leaf area (mean ± SE)		Root fresh weight (mean ± SE)	
	1-leaf stage	2-leaf stage	1-leaf stage	2-leaf stage
	mm^2/plant		g/plant	
Untreated	20.50 ± 1.01a	88.44 ± 4.40a	0.68 ± 0.08a	2.93 ± 0.33a
Cruiser @ 0.125 mg/seed	22.10 ± 0.83a	90.16 ± 4.35a	0.58 ± 0.07a	2.71 ± 0.18a
Cruiser @ 0.25 mg/seed	20.45 ± 0.95a	83.65 ± 5.42a	0.59 ± 0.09a	2.84 ± 0.37a
Poncho @ 0.25 mg/seed	19.63 ± 1.14a	92.68 ± 4.44a	0.52 ± 0.03a	2.86 ± 0.40a
Poncho @ 1.25 mg/seed	20.76 ± 0.79a	83.46 ± 3.67a	0.64 ± 0.04a	2.73 ± 0.36a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

In 2006, seed treatment × hybrid interactions occurred only at the Topeka location for yield and plant population (Table 4) and at Garden City for days to silk (data not presented). Therefore, seed treatment effect means across two hybrids are presented except as noted (Table 4). There was no significant treatment effect on grain moisture or days to silk at any location (data not presented) except for days to silk at Garden City where plots treated with the high rate of Cruiser (0.25 mg/seed) reached silk almost a day sooner than the other treatments or the untreated plots for one hybrid (P33R78) and plots treated with Poncho® (1.25 mg/seed) were significantly sooner (3 days) from Cruiser® (1.25 mg/seed) and Poncho® (0.25 mg/seed) for the other hybrid (DKC60-16). There was no significant difference in plant population at 4 of the 8 locations. At Garden City, plant populations were significantly greater in the untreated plots than in plots treated with both rates of Cruiser®. At Ottawa, plant populations in the untreated check were significantly less than in the plots treated with the two rates of Poncho®. At Topeka, where a significant interaction between treatment and hybrid was detected, populations in plots treated with Cruiser® at the 0.25 mg/seed were significantly less than populations in the plots treated with two rates of Poncho® for hybrid DKC60-16. For yield, no significant difference occurred at 4 (Hesston, Manhattan, Ottawa, Parsons) of the 8 locations. At Colby, Poncho® at the 0.25 mg/seed yielded significantly more than Poncho® at 1.25 mg/seed, Cruiser® at 0.25 mg/seed and the untreated check. At Garden City, the untreated check yielded more than Cruiser® at 0.25 and Poncho® at 1.25. At Scandia, Poncho® at 1.25 yielded significantly more that the untreated check. At Topeka, where hybrids interacted with treatments, the untreated check yielded significantly more than Poncho® at 1.25 on one hybrid (P33R78) and Poncho® at 1.25 yielded significantly more than Cruiser® at 1.25 on the other hybrid (DKC60-16).

Greenhouse test. There were no significant differences between the untreated plants and those treated with Poncho® or Cruiser® for any of the parameters tested, including leaf area, fresh weight, dry weight or root fresh weight

Table 6. Fresh and dry weight of plants treated with two rates of Poncho® and Cruiser® at one, two and five leaf stages.

Treatment	Fresh weight (mean ± SE)			Dry weight (mean ± SE)		
	1-leaf stage	2-leaf stage	5-leaf stage	1-leaf stage	2-leaf stage	5-leaf stage
	g/plant			g/plant		
Untreated	0.85 ± 0.06 a	3.15 ± 0.30 a	72.87 ± 5.70 a	0.05 ± 0.01 a	0.16 ± 0.02 a	5.09 ± 0.53 a
Cruiser @ 125 mg/seed	0.93 ± 0.04 a	3.04 ± 0.26 a	58.72 ± 3.99 a	0.05 ± 0.01 a	0.14 ± 0.02 a	4.81 ± 0.48 a
Cruiser @ 250 mg/seed	0.88 ± 0.03 a	2.98 ± 0.38 a	68.63 ± 6.89 a	0.05 ± 0.01 a	0.13 ± 0.03 a	5.80 ± 0.79 a
Poncho @ 250 mg/seed	0.87 ± 0.04 a	3.14 ± 0.32 a	74.29 ± 8.67 a	0.05 ± 0.01 a	0.15 ± 0.02 a	6.34 ± 0.90 a
Poncho @ 1250 mg/seed	0.89 ± 0.05 a	2.88 ± 0.24 a	67.92 ± 2.07 a	0.04 ± 0.01 a	0.14 ± 0.02 a	4.72 ± 0.41 a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

Table 7. Wireworm control on corn with planting time treatments.

Treatment/ product name	Damage * (mean ± SE)	Plants/plot # (mean ± SE)	Plant vigor # (mean ± SE)	Yield # (mean ± SE)
2004				
Untreated	—@	17.7 ± 1.8 c	0.3 ± 0.3 b	85.2 ± 14.6 c
Cruiser 5FS @ 0.25 mg/seed	—	23.7 ± 2.4 abc	1.7 ± 0.3 a	166.8b ± 26.9 ab
Cruiser 5FS @ 1.25 mg/seed	—	24.0 ± 1.8 abc	1.7 ± 0.3 ab	165.7 ± 14.6 ab
Poncho 250 5SC @ 0.25 mg/seed	—	29.3 ± 2.3 ab	1.7 ± 0.3 a	170.3 ± 31.5 ab
Poncho 1250 5SC @ 1.25 mg/seed	—	26.0 ± 1.7 ab	1.0 ± 0.0 a	169.2 ± 9.9 ab
Lorsban 15G @ 8 oz./1000 ft	—	21.7 ± 5.2 bc	0.7 ± 0.3 b	147.0 ± 23.3 ab
Aztec 2.1G @ 6.7 oz./1000 ft	—	29.3 ± 2.4 ab	1.7 ± 0.3 a	149.3 ± 21.2 ab
Force 3 G @ 4.0 oz./1000 ft	—	23.7 ± 1.2 abc	1.0 ± 0.0 ab	157.5 ± 5.4 ab
Fortress 5G @ 3.0 oz./1000 ft	—	23.0 ± 3.2 abc	1.7 ± 0.3 a	180.8 ± 20.2 a
Lorsban 4F @ 2.4 fl. oz./1000 ft	—	30.8 ± 4.4 a	1.0 ± 0.0 ab	123.7 ± 9.9 bc
Capture 2E @ 0.37 fl. oz./1000 ft	—	28.7 ± 4.4 ab	1.0 ± 0.0 ab	173.8 ± 16.2 ab
Baythroid 2E @ 2.0 fl. oz./acre	—	26.0 ± 2.1 abc	1.0 ± 0.0 ab	151.7 ± 12.9 ab
Regent @ 0.24 fl. oz./1000 ft	—	22.7 ± 1.3 abc	1.0 ± 0.0 ab	165.7 ± 5.1 ab
2005				
Untreated	10.0 ± 0.0 a	11.3 ± 0.5 c	0.5 ± 0.3 c	119.0 ± 16.4 a
Cruiser 5 FS @ 0.25 mg/seed	0.0 ± 0.0 b	15.5 ± 1.3 ab	1.5 ± 0.3 ab	138.0 ± 10.9 a
Cruiser 5 FS @ 1.25 mg/seed	2.0 ± 2.0 b	15.0 ± 0.7 ab	1.5 ± 0.3 ab	122.5 ± 9.0 a
Force 3G @ 0.56 gm/100 m row	2.0 ± 2.0 b	18.0 ± 0.8 a	1.5 ± 0.3 ab	142.5 ± 19.1 a
Poncho 1250 ST @ 1.25 mg/seed	0.0 ± 0.0 b	15.0 ± 1.1 ab	2.0 ± 0.0 a	133.0 ± 14.9 a
Poncho 1250 ST @ 0.25 mg/seed	0.0 ± 0.0 b	13.5 ± 0.9 bc	1.8 ± 0.3 a	112.0 ± 20.2 a
Latitude @ 0.89 oz. ai/cwt	4.0 ± 2.4 b	13.8 ± 1.6 bc	1.0 ± 0.0 bc	106.0 ± 6.2 a

*Greenhouse study.
#Field study.
@Not observed in 2004.
Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

Table 8. Chinch bug control on corn with planting time treatments in 2006.

Treatment/ product name	Chinch bugs/ plant 13 DAP* (mean ± SE)	Chinch bugs/ plant 20 DAP (mean ± SE)	Yield (mean ± SE)
Untreated check	32.5 ± 5.2 a	17.5 ± 2.5 a	60.0 ± 5.2 b
Cruiser 5FS @ 0.125 mg/seed	0.0 ± 0.0 b	1.5 ± 1.5 b	106.0 ± 6.8 a
Cruiser 5FS @ 0.18 mg/seed	0.0 ± 0.0 b	2.0 ± 1.4 b	108.0 ± 10.1 a
Cruiser 5FS @ 0.25 mg/seed	0.0 ± 0.0 b	0.0 ± 0.0 b	106.0 ± 3.8 a
Cruiser 5FS @ 0.125 mg/seed	0.0 ± 0.0 b	0.0 ± 0.0 b	122.0 ± 6.8 a
Poncho 1250 ST @ 0.25 mg/seed	0.1 ± 0.1 b	0.0 ± 0.0 b	110.0 ± 5.0 a
Poncho 1250 ST @ 1.25 mg/seed	0.0 ± 0.0 b	0.0 ± 0.0 b	116.0 ± 5.1 a

*Days after planting.
Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

(Tables 5–6). In this test seed treatments did not stimulate early plant growth (root and shoot development at V1, V2, or V5).

Effects on wireworms. In 2005 most treatments significantly increased plant stands at Silver Lake, KS ($F = 3.95$; df = 6, 21; $P = 0.0084$) and resulted in significantly greater plant vigor or less stunting ($F = 4.37$; df = 6, 21; $P = 0.0051$) when compared to the untreated check (Table 7). These seedling and stand establishment effects resulted in greater numerical yields in most cases but there were no significant differences in yield between any of the treatments and the untreated check ($F = 0.86$; df = 6, 21; $P = 0.5369$) (Table 7). In 2004, similar results were obtained at Horton, KS where stand counts were greater in all treatments but because of variability differences were not significant ($F = 1.62$; df = 12, 26; $P = 0.1458$) (Table 7). Most treatments significantly affected plant growth as reflected in the vigor ratings ($F = 3.29$; df = 12, 26; $P = 0.0054$) (Table 7). Likewise, results from the greenhouse wireworm study in 2005 indicated that seed treatments have insecticidal activity on this pest. All treatments significantly reduced feeding damage caused by wireworms ($F = 6.48$; df = 6, 28; $P = 0.0002$) (Table 7). These results also suggest that wireworm insecticidal activity tests can be conducted successfully by collecting wireworms from the field and artificially infesting plants in the greenhouse.

Effect on chinch bugs. All insecticide treatments significantly reduced chinch bug numbers 13 days ($F = 38.93$; df = 6, 21; $P < 0.0001$) and 20 days ($F = 27.72$; df = 6, 21; $P < 0.0001$) after planting (Table 8). This plant protection resulted in significant ($F = 9.98$; df = 6, 21; $P < 0.0001$) increases in yield for all insecticide treatments with a near doubling of yield. The four rates of Cruiser® evaluated in this test were equally effective for controlling chinch bugs in seedling corn.

Effect on white grubs. In 2006, all five insecticide treatments resulted in significantly greater plant stands ($F = 2.81$; df = 6, 21; $P = 0.0363$) and reduced plant damage ($F = 5.25$; df = 6, 21; $P < 0.0019$) in Marshall County (Table 9). All plants were killed or failed to emerge in untreated hills by the second sampling date. Seed treatments compared favorably with an in-furrow application of

Table 9. White grub control on corn with planting time treatments.

Treatment/ product name	Plants/hill (mean ± SE)	Damage rating (mean ± SE)
2005		
Untreated	0.40 ± 0.40 b	— *
Cruiser 5FS @ 0.125 mg/seed	2.00 ± 0.00 a	—
Cruiser 5FS @ 0.25 mg/seed	2.00 ± 0.00 a	—
Cruiser 5FS @ 1.125 mg/seed	2.00 ± 0.00 a	—
Poncho 1250 ST @ 0.25 mg/seed	1.40 ± 0.40 a	—
Poncho 1250 ST @ 1.25 mg/seed	2.00 ± 0.32 a	—
2006		
Untreated	0.8 ± 0.5 b	7.0 ± 1.8 a
Force 3G @ 1.12 GA/100 m row	1.5 ± 0.5 ab	2.5 ± 2.5 b
Cruiser 5FS @ 0.125 mg/seed	2.0 ± 0.0 a	0.0 ± 0.0 b
Cruiser 5FS @ 0.25 mg/seed	2.0 ± 0.0 a	0.0 ± 0.0 b
Cruiser 5FS @ 1.125 mg/seed	1.8 ± 0.3 a	0.0 ± 0.0 b
Poncho FS @ 0.25 mg/seed	2.0 ± 0.0 a	0.0 ± 0.0 b
Poncho FS @ 1.25 mg/seed	2.0 ± 0.0 a	0.0 ± 0.0 b

*Not observed in 2005.
Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

Force® in providing plant protection from white grub attack. Similar results were obtained in the test conducted in 2005 in Osage County, although drought limited yields so much that treated plots were no different than untreated (Table 9). All five seed treatments resulted in significantly higher plant populations and differences between treatments were not significant ($F = 6.04$; df = 5, 24; $P = 0.0009$).

Effect on black cutworm. The effect of seed treatments on black cutworms in 2005 is presented in Figure 1a. There was no significant difference in damage between the corn from untreated seed and that treated with the two low rates of

Table 10. Corn flea beetle control on corn with seed applied insecticides in 2006.

Treatment/ product name	Damage rating (mean ± SE)	Yield (mean ± SE)
Untreated check	2.6 ± 0.4 a	35.10 ± 3.86 a
Cruiser 5 FS @ 0.25 MGA/seed	0.0 ± 0.0 b	37.05 ± 3.77 a
Cruiser 5 FS @ 0.25 MGA/seed + Force 20 CS @ 5.0 GA/100 Kg seed	0.2 ± 0.1 b	37.70 ± 2.65 a
Poncho 250 5 SC @ 0.25 MGA/seed	0.0 ± 0.0 b	41.60 ± 5.01 a
Poncho 600 @ 0.25 MGA/seed	0.1 ± 0.1 b	43.88 ± 3.16 a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; PROC GLM; Mean comparison by LSD [SAS Institute 2003]).

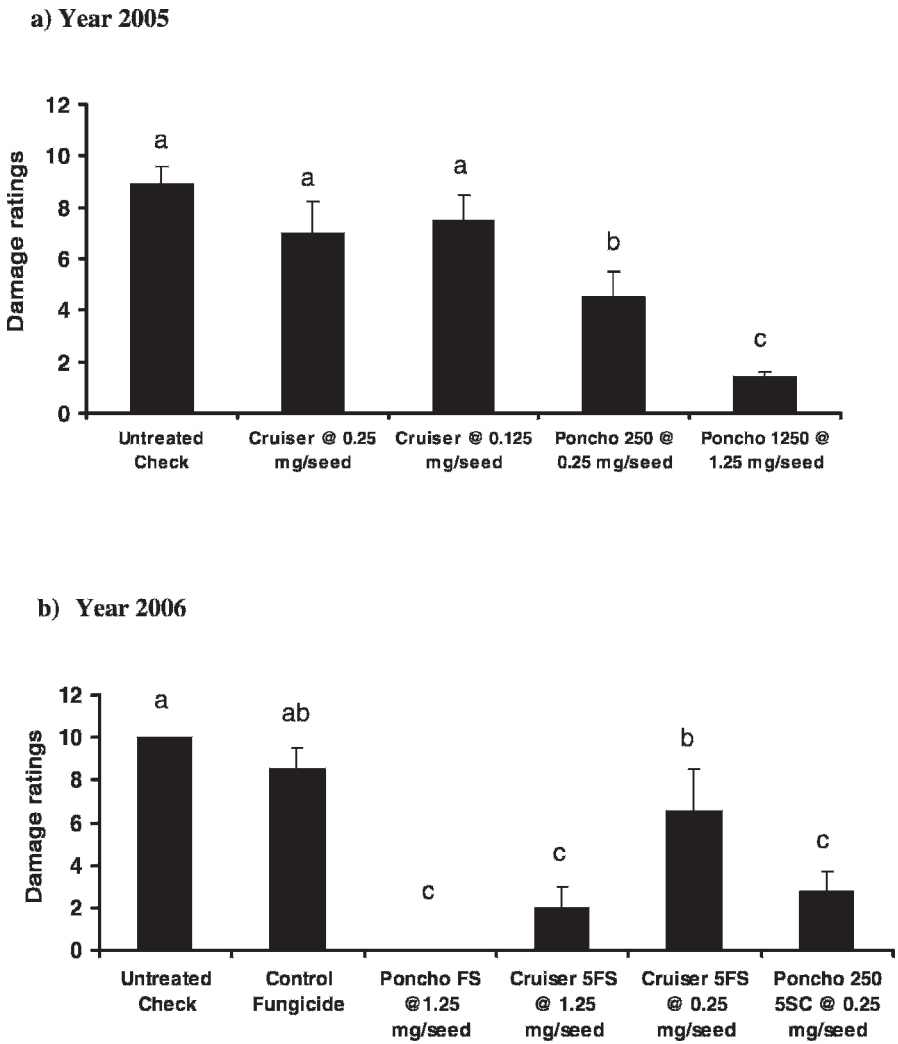

Fig. 1. Black cutworm control on corn with seed applied insecticides in (a) year 2005 and (b) year 2006. Vertical bars indicate standard errors of means. Means followed by the same letter (letters placed above groups of bars are not significantly different (P = 0.05; PROC GLM; LSMEANS [SAS Institute 2003].

Cruiser®. Poncho® at both rates tested significantly reduced black cutworm damage compared to the untreated with significantly less damage for the high rate of Poncho® (1.25 mg/seed) than that treated at the lower rate. (0.25 mg/seed) (F = 11.01; df = 4, 15; $P < 0.0001$). In 2006, Poncho® at the low and high rate and Cruiser® at the high rate (1.25 mg/seed) significantly reduced black cutworm feeding (F = 14.01; df = 5, 18; $P < 0.0001$) compared to the untreated check and the fungicide-only control. However, feeding on the low rate of Cruiser® was not significantly different from the untreated check (Figure 1b).

Effect on flea beetles. All seed and furrow treatment combinations significantly ($F = 41.86$; df = 4, 15; $P < 0.0001$) reduced flea beetle feeding damage (Table 10). Yields were extremely low because of a severe drought and there was no significant difference between treatments ($F = 0.90$; df = 4, 15; $P = 0.4891$).

Discussion

In these tests Poncho® and Cruiser® at low and high rates were effective and exhibited insecticidal activity against chinch bug, white grub, wireworm, and flea beetle. These results compare favorably with those reported by Wilde et al. (2004) and suggest great promise for growers dealing with a variety of early-season soil inhabiting and foliar pests. Seed treated with Poncho® appeared to be more effective than Cruiser® at equivalent rates in reducing black cutworm damage. There was a definite rate response, with the high rate of Poncho® (1.25 mg/seed) being the most effective against this pest. These results agree with a study reported by Rice et al. (2006) where they found a similar rate response and difference in efficacy of Poncho® and Cruiser® against this pest.

In the field tests involving Poncho® and Cruiser® at 19 locations in 2004, 8 locations in 2005, and 7 locations in 2006, no consistent effect of seed treatment at any rate on grain moisture, days to silk, plant population, or yield of two corn hybrids was detected. Cox et al. (2007) also found Poncho® seed treatments inconsistently affected corn forage yield when corn followed soybeans. Wilde et al. (2004), in another 3-year study involving imidacloprid and thiomethoxam at four locations, found that yield increases from seed treatment were usually associated with noticeable insect activity rather than a growth or vigor effect. Likewise, our greenhouse study did not detect such an effect on corn root or shoot development. These results are different than that of Thielert (2006) who reported that field trial analysis indicated improved plant growth on a variety of plant species when imidacloprid was applied as a spray, soil drench, or stem application. Schade et al. (2007) also reported that seed treated with Cruiser® produced plants with greater plant vigor, resulting in increased tolerance to drought and other biotic and abiotic stresses. Our tests over a diverse range of environments, including various amounts of rainfall and different temperature regimes and soil types, did not detect significant differences in plant growth of corn that resulted in consistent increases in yield.

Acknowledgments

We thank Dr. Jeff Whitworth for reviewing the manuscript. This is contribution 08-276-J from the Kansas Agriculture Experimental Station, Kansas State University and represents work sponsored by Bayer Crop Science and Syngenta Crop Protection.

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Seed Treatment Effects on Early-Season Pests of Corn and on Corn Growth and Yield in the Absence of Insect Pests[1]

Gerald Wilde,[2] Kraig Roozeboom,[3] Aqeel Ahmad,[2] Mark Claassen,[4] Barney Gordon,[5] William Heer,[6] Larry Maddux,[7] Victor Martin,[8] Patrick Evans,[9] Ken Kofoid,[10] James Long,[11] Alan Schlegel,[12] and Merle Witt[13]

J. Agric. Urban Entomol. 24(4): 177–193 (October 2007)

ABSTRACT Second-generation neonicotinoid insecticides are being used to protect seeds and seedlings against injury by early season insects on a wide variety of crops. Seed-applied insecticides have recently been commercialized in the USA for early season insect control in corn. The systemic insecticides clothianidin (Poncho®) and thiamethoxam (Cruiser®) applied as seed treatments were evaluated for their effect on corn yield in the absence of noticeable insect attack over a three year period at a number of locations representing diverse growing environments in Kansas. No consistent effect on yield was detected for either compound at either high or low rates in locations where insects were not observed at damaging populations. Controlled-environment studies detected no differences in early season growth in the absence of insect pests in response to the same two rates of both insecticides. Both compounds were evaluated for their effect on early season corn pests and were found to be effective at high and low rates on wireworm, white grub, flea beetles and chinch bugs. Higher rates of both compounds were needed to reduce feeding damage caused by black cutworm. Seed treatments with either compound would be useful where early season pests are chronic problems.

KEY WORDS corn, growth, yield, seed treatment, neonicotinoid, *Melanotus cribulosus*, *Agrotis ipsilon*, *Phyllophaga* sp., *Chaetocnema pulicaria*, *Blissus leucopterus*

Introduction

Protecting the seed and young plants from insect pests is increasingly important for establishing a healthy, vigorous corn stand with increases in total

[1]Accepted for publication 11 Jun 2008.
[2]Department of Entomology, Kansas State University, Manhattan, Kansas.
[3]Department of Agronomy, Kansas State University, Manhattan, Kansas.
[4]Department of Agronomy, Kansas State University, Harvey County Experiment Field, Hesston, Kansas.
[5]Department of Agronomy, Kansas State University, North Central and Irrigation Experiment Fields, Scandia, Kansas.
[6]Department of Agronomy, Kansas State University, South Central Experiment Field, Hutchinson,
[7]Department of Agronomy, Kansas State University, Kansas River Valley Experiment Field, Topeka,
[8]Department of Agronomy, Kansas State University, Sandyland Experiment Field, St. John, Kansas.
[9]Kansas State University Northwest Research-Extension Center, Colby, Kansas.
[10]Kansas State University Agricultural Research Center – Hays, Hays, Kansas.
[11]Kansas State University Southeast Research Center, Parsons, Kansas.
[12]Kansas State University Southwest Research-Extension Center, Tribune, Kansas.
[13]Kansas State University Southwest Research-Extension Center, Garden City, Kansas.

Observations on the Peritrophic Membrane of Tortricid and Noctuid Insects and its Role in Susceptibility and Enhancement[1]

Said A. El Salamouny[2,3]

J. Agric. Urban Entomol. 24(4): 195–204 (October 2007)

ABSTRACT Although stilbene fluorecent brighteners have been demonstrated to increase the susceptibilities of many lepidopterous insects to insect viruses, little information exists on the effects of a brightener on the susceptibility of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and the false codling moth, *Cryptophlebia leucotreta* Meyrick (Lepidoptera: Tortricidae). In the present study, we tested the effects of the fluorescent brightener 28 (UNPA-GX) on increasing of the insects' susceptibility to a granuloviruses. The bioassay tests showed that brightener did not increase the susceptibility of codling moth neonate larvae and false codling moth larvae to the granulovirus (CpGV). The same result was obtained when the false codling moth GV was tested in presence of brightener against its homologous host. In addition, brightener failed to change the susceptibility of the codling moth to ClGV.

In histological sections from the codling moth's midgut, the peritrophic membrane (PM) was found only in the anterior part of the midgut of fifth instar larvae, while the middle and posterior part of the midgut was PM free. Thus, the food and/or virus is in direct contact with the microvilli. No membrane could be detected in the first and third instar larvae. Use of the electron microscope confirmed our findings by light microscope. In contrast, light and electron microscopic examination showed that the PM is much thicker in the turnip cutworm, *Agrotis segetum* (Denis & Schiff.), larvae and lines the entire midgut.

The present findings may explain why the tortricid species were highly susceptible and could not be enhanced by brightener but susceptibility of the turnip cutworm can be enhanced by brightener.

KEY WORDS *Agrotis segetum*, *Cryptophlebia leucotreta*, *Cydia pomonella*, enhancement, granulovirus, midgut, noctuidae, peritrophic membrane, tortricidae

Baculoviruses are naturally occurring biocontrol agents that are safe, environmentally friendly, effective and host specific (Burges et al. 1980). Many efforts have been devoted to use these promising bioagents in order to reduce dependence on chemical pesticides.

[1]Accepted for publication 8 Aug 2008.

[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

[3]Institute for Biological Control, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Heinrichstr. 243, 64287 Darmstadt, Germany.

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), and the false codling moth, *Cryptophlebia leucotreta* Meyrick (Lepidoptera: Tortricidae), are important pests of fruits such as apple, citrus, etc. Codling moth larvae are highly susceptible to their homologous granulovirus. Also, the larvae of the false codling moth are susceptible to their homologous granulovirus as well as to the *C. pomonella* granulovirus (Weber 1984). Infection of insect larvae by granulovirus occurs by oral ingestion of the viral occlusion bodies (OBs). The OBs dissolve in the alkaline digestive juices of the midgut, releasing enveloped virus particles. These particles pass the peritrophic membrane (PM), which lines the midgut before infecting the midgut cells (Granados & Lawler 1981). In insects such as the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), the midgut cells are not infected but the addition of a fluorescent brightener to the virus suspension results in infection (Adams et al. 1994, Dougherty et al. 2006). PM lines the gut of many arthropods and other animals, separates ingested food from the gut epithelium and provides important protection against microorganisms and parasites (Bolognesi et al. 2001, Eisemann & Binnington 1994, Tellam 1996, Terra 2001, Wang & Granados 1998, Jayachandran et al. 2000, Wang & Granados 2001). Wang & Granados (1998) studied the presence of the PM in *Trichoplusia ni* larvae *in vivo* and its role in limiting baculovirus infection. Brightener destroyed the PM, which increased the larval susceptibility. El Salamouny et al. (2003), Mukawa et al. (2003) and Zhu et al. (2007) confirmed that the PM is composed of chitin and protein (Peters 1992), thus the activity of baculovirus can be enhanced by altering the chitin with chitinase (Shapiro et al. 1987) or the protein with protease (Lepore et al. 1996, Wang & Granados 1998, Peng et al. 1999).

In order to obtain effective formulation of baculovirus under field conditions, the additives should increase the virus's efficacy. Previous studies have shown that the efficacy of baculoviruses is increased by enhancers in noctuid and tortricid insects. Brighteners can increase the susceptibility of noctuid insects to nucleopolyhedrovirus but they cannot change unsusceptible host to susceptible ones (El Salamouny et al. 1997). It is known that brighteners increased larval susceptibility of several noctuid species to NPV infections by 2200 fold (Okuno et al. 2003), 1800 fold (El Salamouny et al. 2001), 461 (El Salamouny et al. 1997), and 160 fold (Boughton et al. 2001). Brighteners disrupt the PM that lines the midgut and facilitates NPV infection of midgut epithelial cells, thus the PM loses its integrity and tears in the matrix structure (Okuno et al. 2003). *In vitro* studies showed that protein in the PM could be dissociated *in vitro* by calcofluor (Wang & Granados 2000). On the other hand, brighteners provide baculovirus activity enhancement and UV radiation protection (Dougherty & Shapiro 1996).

Enhancement of baculoviruses activity in tortricid species was less than the rate obtained in noctuid species. The rate of increased susceptibility of the tortricid spruce bud worm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) to its homologous MNPV was 83.9 fold and only 4.1 fold against the heterologous host *C. occidentalis* (Li & Otovos, 2001). Also, Li & Otovos (1999a) reported only 13.08 fold enhancement when compared to the LC_{95} and only 3.55 fold when compared to LC_{50} of the *C. fumerana* MNPV against *C. occidentalis*. Brightener enhanced the activity of *Cf*MNPV against a field strain of *C. occidentalis* more than (7.6–11.0 fold) in the laboratory strain (2.5–3.5 fold) (Li & Otovos 1999b).

In the present study, we tested brightener against two granuloviruses; *C. pomonella* GV and *C. leucotreta* GV as examples for the high virulent granulovirus in comparison with previously published data on *A. segetum* GV. Cross infection tests using the homologous virus of codling moth, false codling moth and turnip cutworm were carried out.

The role of the presence of PM in susceptibility and ability of enhancement by brightener of codling moth in comparison with turnip cutworm was histologically investigated.

Material and Methods

Test insects. Neonate larvae of a noctuid, the turnip cutworm, *Agrotis segetum* were tested and compared to the codling moth and the false codling moth larvae. The turnip cutworm larvae were reared on a semi artificial diet described by Hassani (2000), however tortricid larvae were reared on another diet described by Ivalid-Sender (1974).

Viruses. The viruses tested included the codling moth granulovirus (*Cp*GV) and the false codling moth granulovirus (*Cl*GV). These were obtained from the Institute for Biological Control, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Darmstadt, Germany. For comparison, the previously published data by El Salamouny et al. (2003) on enhancing the activity of *A. segetum* GV by brightener (Calcoflour, UNPA-GX, Sigma-Aldrich) were used.

Synergisitic additive. Fluorescent brightener 28 (Calcoflour, UNPA-GX, Sigma-Aldrich).

Diet-incorporation bioassay. The viruses were incorporated into the semi-synthetic diet in the presence or absence of brightener additive (Huber 1981, El Salamouny et al. 2003). Viruses were tested at concentrations ranging from 10^2 to 10^7 capsules/ml diet. UNPA-GX was used as a 0.05% and 0.1% final concentration (wt/vol). The mixture of the diet (40 ml) and virus (5 ml) (or plus brightener at 5 ml) was placed in special bioassay plates containing 50 cells (LICEFA, Bad-Salzuflen, Germany). Fifty neonate larvae were tested per replicate. The bioassay plate was covered with a layer of tissue paper and a polyethylene sheet that was fixed with rubber bands and incubated at 26°C, 60–70% RH and 16 hours light and 8 hours dark. Mortality due to viral infection was recorded every two days for 8 days in case of tortricid insects. Three replicates of each treatment were made.

Weight of larvae. For studying the effect of brightener on the weight of codling moth larvae were fed continuously on brightener concentrations of 0.05, 0.1 and 1% (wt/vol) in the diet. Ten fourth instars of each treatment replicate were weighed by using a digital balance to investigate the effect of brightener. Data has been taken at the last day of the bioassay (Day 8).

Light microscopy. *Dissection and isolation of the peritrophic membrane.* Fifth instars of codling moth were dissected in Ringer's solution buffer for isolation of the PM.

Serial thin sections. For the serial sections of the midgut or the whole codling moth larvae, 7, 13 and 25 larvae were used for the tested first, third and fifth instar, respectively. However, 19, 20 and 8 *Agrotis segetum* larvae were used for the same instars, respectively. The fixation and embedding process used the same method used by Kleespies et al. (2001) and El Salamouny et al. (2003). First, third

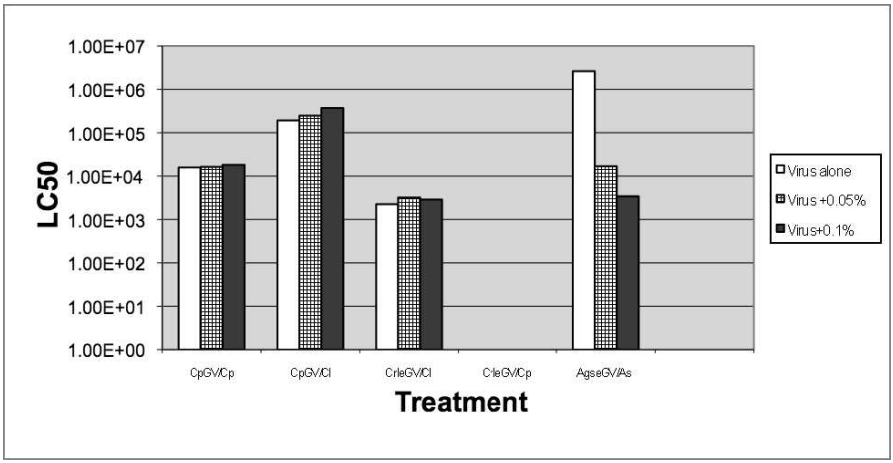

Fig. 1. Effect of Fluorescent brightener 28 on the virulence of granuloviruses tested against tortricid and noctuid species (Data on susceptibility of *Agrotis segetum* has previously been published (El Salamouny et al. 2003).

and fifth instar larvae were fixed with Dubosq-Brazil's alcoholic Bouin's and embedded in Histosec (Merck, Darmstadt, Germany). For the third and fifth instar larvae, the sections were cut at 9 µm and stained with Heidenhain's iron haematoxylin and counterstained with erythrosine or with Giemsa's stain (both are from Merck, Darmstadt, Germany). A Leica DMRB (Leica, Bensheim, Germany) photomicroscope with phase contrast and bright field equipment was used to examine the histological sections at 50, 200 and 400 magnifications.

Electron microscopy. *Negative staining*. The dissected PM of turnip cutworm larvae was kept in buffer, cut to small pieces, stained with sodium-phosphotungstate (2%) and examined by electron microscopy.

Ultrathin sections. First instars as well as small pieces of the isolated PM and the midgut were fixed overnight at 4°C in 3.0% glutaraldehyde in Veronal buffer (pH 7.2) and post fixed in 2.0% osmium tetroxide in the same buffer for 2 hours. Subsequently, midgut and PM samples were stained enbloc in 2% aqueous uranyl acetate for 5 hours, dehydrated through increasing concentrations of ethanol and embedded in methacrylate. Ultrathin histological sections were obtained with a Leica Ultracut-S microtome and stained with 6% lead citrate and 2% aqueous uranyl acetate (Kleespies et al. 2001). The stained sections were examined by transmission electron microscopy (TEM) at 7,000, 12,000, and 20,000 magnification.

Statistical analysis. LC_{50} values were calculated using a computer program to calculate the linear regression of log concentration against Probit mortality (Finney, 1971).

Results

Effect of fluorescent brightener 28 on the susceptibility of tortricid larvae. Data graphically presented in Fig. 1 show that no enhancement effect was recorded at

the tested brightener concentrations of 0.05%, and 0.1% when *Cp*GV was tested against its homologous host, the codling moth. LC_{50} values were 1.58×10^4, 1.63×10^4 and 1.82×10^4 granules/ml diet for the virus-alone treatment, and 0.05% and 0.1% brightener, respectively. Also, no enhancement effect was obtained in the case of the heterologous host, the false codling moth. The obtained LC_{50} values were close to each other with a value of 1.92×10^5, 2.47×10^5, and 3.71×10^5 G/ml diet for virus alone treatment, 0.05%, and 0.1% brightener, respectively (Fig. 1).

When *Cl*GV was tested against its homologous host *C. leucotreta*, no change in the susceptibility was recorded. The LC_{50} values were 2.25×10^3, 3.21×10^3, and 2.89×10^3 capsules/ml diet for virus alone treatment, and 0.05% and 0.1% brightener treatments, respectively. When *Cl*GV was used against *C. pomonella* as a heterologous host, brightener did not cause the insect to become susceptible to this virus (Fig. 1).

In contrast, the published data by El Salamouny et al. (2003) on the turnip cutworm (*Agse*GV) that was tested against *A. segetum* larvae for comparison showed that a reasonable enhancement effect was obtained. LC_{50} value was estimated by 2.62×10^6 capsules/ml without brightener, decreased sharply to 1.69×10^4 and 3.43×10^3 capsules/ml for concentrations of 0.05% and 0.1% brightener, respectively (Fig. 1).

Effect of brightener on the weight of the tested larvae. Data demonstrated that the brightener did not reduce the weight of the codling moth larvae at the concentrations of 0.05% and 0.1%. On the larvae of the codling moth, however a slight increase in weight was observed. The average weight per larva was 0.019, 0.024 and 0.026 g/larva for the untreated control (H_2O), 0.05 and 0.1%, respectively.

Histological studies on peritrophic membrane. In order to determine whether or not the susceptibility of the codling moth and the turnip cutworm GVs and with and without UNPA-GX is different, we compare the structure of the midgut of both species.

Isolation of PM. Isolation of fifth stage codling moth larval PM was not successful. Therefore, histological studies by fixation of the whole midgut or the whole larva were carried out.

Serial longitudinal sections. Histological sections of codling moth of the first and 3rd instar midgut showed no PM was detected and the food was in direct contact with microvilli (Fig. 2). However, in case of the fifth instar larval sections, PM was thinner, found in the anterior part of the midgut and did not line the entire midgut.

In contrast, structure of first stage larvae was the membrane consisted only of one layer but still lined the entire midgut. However, third and fifth instar larvae of turnip cutworm were multilayered and lined the whole midgut (Fig. 3).

Electron microscopy. Negatively stained preparations of PM of the fifth instar larvae of turnip cutworm showed that it has no special structure (Fig. 4a) and ultrathin sections showed that PM is multilayered (Fig. 4b).

In case of codling moth fifth instar sections, the membrane had a similar structure to that of turnip cutworm PM and assumed that this section was made at the anterior part of the midgut. Other sections showed a very thin layer on the microvilli. In neonate larvae no membrane was detected and the food was in direct contact with the microvilli.

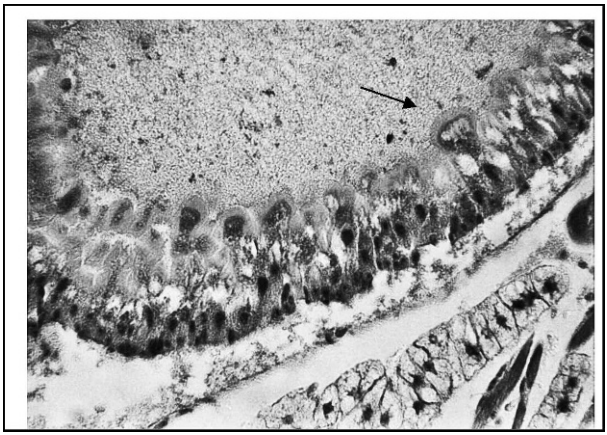

Fig. 2. Histological section in the midgut of *Cydia pomonella* third instar larvae shows no membrane and the food is in direct contact of microvilli.

Discussion

In the present study, we demonstrated that brightener did not increase the susceptibility of the codling moth and the false codling moth. The PM was thick in the turnip cutworm larvae and lined the whole midgut, thus the virus had to pass through the PM in order to cause infection. We compared the relationship between presence and thickness of the PM and the susceptibility rate of tortricid

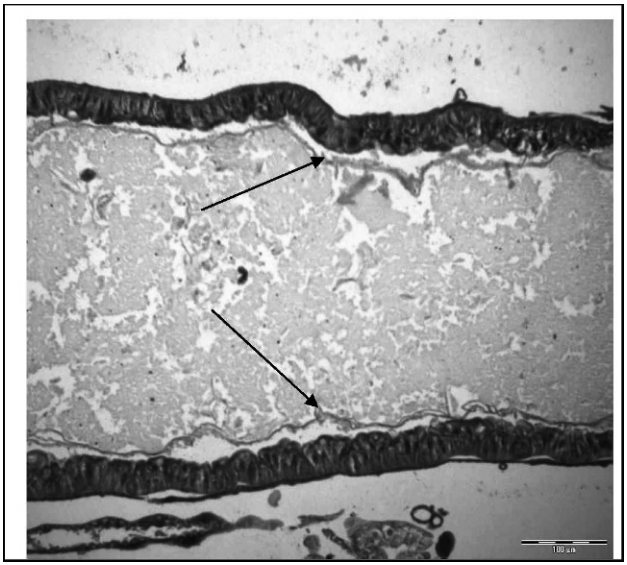

Fig. 3. Histological section in the midgut of *Agrotis segetum* third instar larvae shows that the peritrophic membrane lines the whole midgut and separate the food and microvilli.

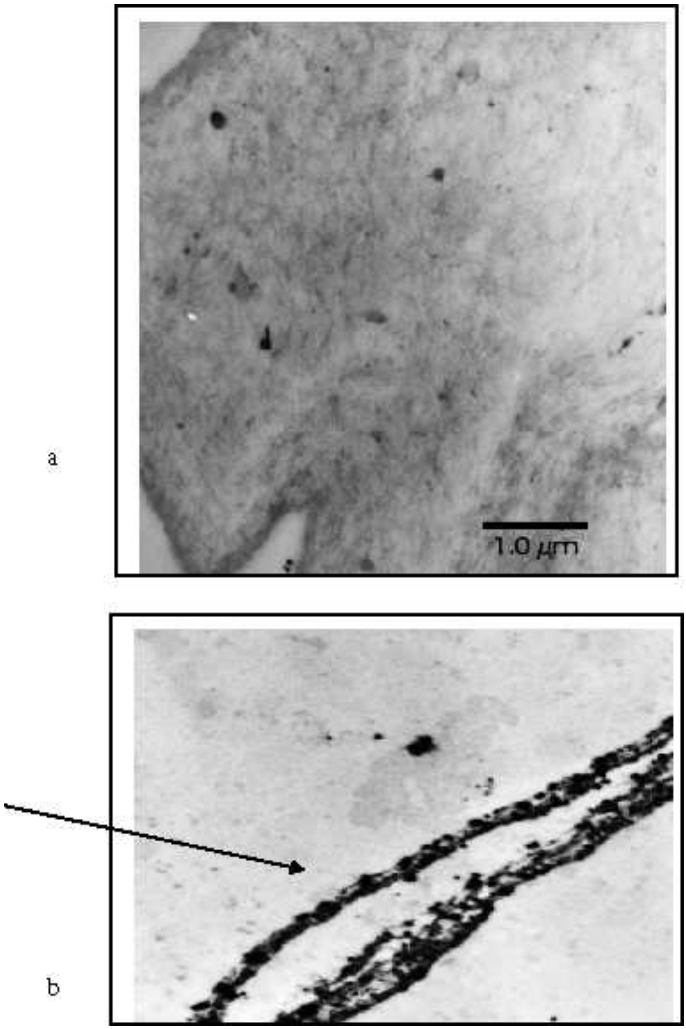

Fig. 4. Electron micrograph of *Agrotis segetum* peritrophic membrane shows no special structure (a) and ultilayered (b).

and noctuid insects. The relationship between the synergistic effect of brightener on susceptibility of the turnip cutworm and presence or absence of the site of action (PM) appears to play an important role in insect susceptibility. Shapiro (1992) speculated that brightener was to act as a chitin syntheses inhibitor which would allow a large number of virions to infect epithelial cells. El Salamouny et al. (2003) reported a complete disruption of the PM of the turnip cutworm as a result of maintaining the larvae diet containing 0.1% of UNPA-GX for 12 days. Also, exposing the fifth stage larvae to the diet with a 10× concentration of UNPA-GX for 24 hours, produce a similar disruption of the PM. Destruction of the PM was corresponded to a high rate of enhancement, which could explain the

mechanism for increased susceptibility of insects by addition of brighteners. This result is similar to that found by Wang & Granados (2000), and Okuno et al. (2003). Absence of the PM in the midgut in case of *C. pomonella*, could allow the virus particles in midgut to be in direct contact with the epithelial cells. The confirmation by the longitudinal sections by light microscopy for all investigated instars except that the anterior part of the midgut as well as by the ultrathin sections by the transmission electron microscopy showed that the food was in direct with the microvilli with no physical barrier in case of the first and third codling moth instar larvae. Our findings may explain why the tortricids *C. pomonella* and *C. leucotreta* are highly susceptible each to its homologous virus either with or without brightener.

The obtained low rate of enhancement of brightener on tortricid insects is compatible with the low rate of enhancement of other tortricid insects found by Li and Otvos (1999a,b & 2001). The result of brightener could not induce codling moth larvae toward susceptiblity to *Cl*GV. This agreed with results of El Salamouny et al. (1997) in the case of NPV's and their highly susceptible hosts. The absence of a PM in codling moth larvae may explain the absence of the synergistic action of UNPA-GX which, is due to absence of the site of action (PM).

No explanation for the slight increase in the larval weight of codling moth due to addition of brightener which is contrary with the previous decrease in weight due to brightener reported on turnip cutworm by El Salamouny et al. (2003).

The obtained results support the hypothesis that the stilbene brightener UNPA-GX facilitates baculovirus infection of larvae by dissolving the PM (Wang & Granados 2000) which serves as a physical barrier to virus entry. The reason of the non response of tortricid larvae to UNPA-GX could be due to its action as carbonic anhydrase inhibitors.

Acknowledgment

The author thanks deeply the ICSC-World Laboratory, Lausanne, Switzerland for providing the fund. Deep thanks to Dr. Jürg Huber, Dr. Regina Kleespies, Institute for Biological Control, Darmstadt, Germany for the scientific support and for providing the work facility. Thanks to Dr. Doreen Winstanley and Dr. Martin Andermatt for providing the *Cryptophlebia leucotreta* test insects and to Dr. Martin Shapiro and Dr. Merle Shepard for reviewing the manuscript. Also, many thanks to Mrs. Helga Radke, Mrs. Beverung, Mrs. Silvia Schneider and Mr. Eckard Gabrys for the excellent technical assistance.

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Observations on the Peritrophic Membrane of Tortricid and Noctuid Insects and its Role in Susceptibility and Enhancement[1]

Said A. El Salamouny[2,3]

J. Agric. Urban Entomol. 24(4): 195–204 (October 2007)

ABSTRACT Although stilbene fluorecent brighteners have been demonstrated to increase the susceptibilities of many lepidopterous insects to insect viruses, little information exists on the effects of a brightener on the susceptibility of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and the false codling moth, *Cryptophlebia leucotreta* Meyrick (Lepidoptera: Tortricidae). In the present study, we tested the effects of the fluorescent brightener 28 (UNPA-GX) on increasing of the insects' susceptibility to a granuloviruses. The bioassay tests showed that brightener did not increase the susceptibility of codling moth neonate larvae and false codling moth larvae to the granulovirus (CpGV). The same result was obtained when the false codling moth GV was tested in presence of brightener against its homologous host. In addition, brightener failed to change the susceptibility of the codling moth to ClGV.

In histological sections from the codling moth's midgut, the peritrophic membrane (PM) was found only in the anterior part of the midgut of fifth instar larvae, while the middle and posterior part of the midgut was PM free. Thus, the food and/or virus is in direct contact with the microvilli. No membrane could be detected in the first and third instar larvae. Use of the electron microscope confirmed our findings by light microscope. In contrast, light and electron microscopic examination showed that the PM is much thicker in the turnip cutworm, *Agrotis segetum* (Denis & Schiff.), larvae and lines the entire midgut.

The present findings may explain why the tortricid species were highly susceptible and could not be enhanced by brightener but susceptibility of the turnip cutworm can be enhanced by brightener.

KEY WORDS *Agrotis segetum*, *Cryptophlebia leucotreta*, *Cydia pomonella*, enhancement, granulovirus, midgut, noctuidae, peritrophic membrane, tortricidae

Baculoviruses are naturally occurring biocontrol agents that are safe, environmentally friendly, effective and host specific (Burges et al. 1980). Many efforts have been devoted to use these promising bioagents in order to reduce dependence on chemical pesticides.

[1]Accepted for publication 8 Aug 2008.

[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

[3]Institute for Biological Control, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Heinrichstr. 243, 64287 Darmstadt, Germany.

Epidemiology and Spatial Relationships of Bacteria Associated with *Periplaneta americana* (Blattodea: Blattidae) in Central Texas[1]

Jennifer L. Pechal,[2] James Austin, Roger Gold, and Jeffery K. Tomberlin

J. Agric. Urban Entomol. 24(4): 205–216 (October 2007)

ABSTRACT Identifying cockroach (Order: Blattodea) populations is important to understanding the ability of surrogate species indirectly affecting humans by their ability to vector disease-causing organisms including bacteria. These interactions may have potentially deleterious health consequences on animal and/or human populations. In this study, American cockroaches, *Periplaneta americana* were sampled from 12 locations throughout College Station, Texas from January through May 2008. Cockroach distribution was examined as well as prevalence of *Escherichia coli* including the O157:H7 strain and *Campylobacter* spp. on their external surfaces.

Bacteria isolated from total populations collected indicated a high prevalence (92.3%) of microbes carried on the exoskeleton of *P. americana*. Gram-negative bacteria acquisition and dissemination of organisms such as *E. coli* was prevalent throughout the campus. Screening for *E. coli* O157:H7 and *Campylobacter* spp. resulted in no positive colony growth. The lack of *Campylobacter* spp. growth from cuticular surfaces may have resulted from undesirable conditions required to sustain colony growth. Data from this study corroborate the potential ability of cockroaches to mechanically transmit pathogens.

KEY WORDS *Periplaneta americana*, *Campylobacter* spp., *Escherichia coli*, central Texas

Cockroaches (Order: Blattodea) are important vectors of pathogens due in part to their unsanitary lifestyle. Cockroach cuticle can harbor several *Enterobacteriaceae* species including *Salmonella* spp., *Klebsiella* spp., and *Escherichia* spp. (Mpuchane et al. 2006). A few medically important pathogens that can be vectored by the American cockroach, *Periplaneta americana* (Linnæus) (Blattodea: Blattidae), include: *Campylobacter* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Toxoplasma gondii* (Barcay 2004). Cockroaches are also able to transmit pathogens such as anthrax, cholera, diphtheria, pneumonia, tetanus, and tuberculosis (Baumholtz et al. 1997). Many of which could be used as bioterrorism agents targeting animal or human populations (Lane et al. 2001, Moran 2002).

Understanding the nature of pathogen transmission from urban insect pests to humans could clarify the epidemiology of many illnesses. The epidemiology of

[1]Accepted for publication 17 December 2008.
[2]Corresponding author (jenpechal18@tamu.edu).
Department of Entomology, Texas A&M University, College Station, TX, USA.

these pathogens needs to be thoroughly examined as they relate to cockroaches. Certain disease causing pathogens commonly associated with cockroaches result in gastro-intestinal related illnesses. Pathogens, such as *E. coli* and *Campylobacter* spp., commonly transmitted by cockroaches may be overlooked during diagnosis of sudden ailments with symptoms being similar to food-borne illnesses, including abdominal cramping, diarrhea, nausea, and fever.

Campylobacter spp. are not part of a normal bacterial fauna in humans but have been found in individuals displaying symptoms such as diarrhea and fever (Blaser et al. 1979). In human patients with symptoms of diarrhea, *C. jejuni* has been isolated and causes diarrhea-like symptoms more than *Shigella* spp., *Salmonella* spp., and *E. coli* O157:H7 (Blaser et al. 1979, Blaser 1997).

Diseases associated with *Campylobacter* spp. result from ingesting undercooked poultry, or mishandling raw poultry and cross-contaminating surfaces. *Campylobacter jejuni* is enteric in livestock such as cattle, swine, poultry, companion animals (i.e., dogs and cats), and wild animals such as rodents and raccoons (Blaser 1997, Sahin et al. 2002). *Campylobacter jejuni* is susceptible to atmospheric desiccation and oxygen can inhibit growth in locations such as livestock feed and water (Sahin et al. 2002). However, human interactions with livestock increase the potential risk of contamination.

In human cases, there are several strains of *E. coli* that produce varying effects, ranging from mild fevers to hospitalizations and even death. *Escherichia coli* titers in the environment corresponded with levels of fecal contamination (Le Guyader et al. 1989, Rivault et al. 1994). Transmission of these organisms can follow an unsuspected fecal-oral interaction, such as using a contaminated hand towel and then touching food or the mouth area.

Escherichia coli 0157:H7 is a medically important strain initially reported in 1982 (McGee et al. 2004). It can cause bloody diarrhea, hemolytic uremic syndrome (HUS), and death (McGee et al. 2004). *Escherichia coli* O157:H7 had reported outbreaks in the United States, Great Britain, and Canada, with 20,000 infections and 100 deaths in the United States (Michino et al. 1999).

The objective of this study was to analyze spatial distributions of *E. coli* and *Campylobacter* spp. in relationship to different cockroach populations. This information may determine the spatial distribution of bacterial fauna and identify locations with high bacterial titers.

Materials and Methods

Sampling technique for cockroaches. *Periplaneta americana* (L.) were collected from February 2007–May 2008 within 50 m of neighboring urban structures in the Texas A&M University campus, College Station, Texas. Collecting sites on campus were selected from locations with the highest cockroach populations during preliminary trapping conducted the previous year. Once locations were established, three collecting containers were placed within a 1.83 km^2 square at each trapping location. The north quadrant was approximately 0.29 km^2. The central quadrant was approximately 0.40 km^2. The south quadrant was approximately 0.32 km^2, and the west quadrant had an area of approximately 0.58 km^2. Coordinates of each site were determined with a Garmin, Blaser 1997 eTrex® Vista Cx GPS unit (Garmin Ltd., Olathe, KS, USA) and data points uploaded to Google Earth™.

Containers used for collecting roaches were glass mason jars (430 mL) coated with Elmer's Acid Free Craft Bond© (Elmer's Products, Inc., Columbus, Oh, USA) and rolled in Quickrete® Playsand (Quickrete® International, Inc., Atlanta, GA, USA), according to Granovsky (1983). The top 2 cm of the jar opening was lined with H-E-B brand petroleum jelly (H-E-B, San Antonio, TX, USA) and baited with approximately 51.76 mL beer (Miller Brewing Co., Milwaukee, WI, USA), and 7.04 g of H-E-B brand white bread (H-E-B, San Antonio, TX, USA) for attracting and collecting cockroaches (Barcay 2004). Baited containers were placed in the field at dusk immediately after adding the beer/bread mixture and collected from the field after 8–12 h. Cockroaches collected were stored in a freezer at −20°C until analysis.

Adult cockroaches were collected from each jar and stored in individual plastic bags (16.5 × 14.9 cm), with up to three plastic bags containing roaches from each site. This method should not negatively influence bacterial colony growth (Szalanski et al. 2004). Voucher specimens were placed in the Texas A&M University insect collection.

Screening for *Escherichia coli* activity. Media used for screening *Escherichia coli* followed the CHROMagar™ ECC media manufacture's recipe (CHROMagar, Paris, France). *Escherichia coli* O157:H7 specific media was made using CHROMagar™ 0157 (CHROMagar, Paris, France) ratio.

Agar was poured into sterile petri dishes (100 × 15 mm, VWR International, West Chester, PA, USA). Petri dishes were divided into thirds and appropriately labeled for the specimen. Working under sterile conditions, forceps were flame sterilized using 95% ethanol and cooled prior to touching the cockroach to be plated. Dorsal and ventral sides of each cockroach were plated within their designated areas. Once the cockroach was plated it was moved to an isolated area, the forceps were sterilized using the aforementioned flaming technique. The process was repeated for all *P. americana* collected.

Escherichia coli samples plated on CHROMagar ECC and CHROMagar 0157 were incubated in a Percival Environmental Chamber Model I36LLVL (Percival Scientific, Inc., Perry, IA, USA) at 37°C for 24–48 h. Blue colored colonies were identified as *E. coli*, red colonies were coliform forming bacteria, and colorless colony forming units were non-coliform forming gram-negative bacteria and counted. Screening for *E. coli* O157:H7 followed the same technique, but with positives indicated by a mauve coloration

Colonies that were positive for *E. coli* were stored in sterile 1.5 mL microtubes with snap caps (VWR International, West Chester, PA, USA) in a 60% Tryptic soy agar (Fisher Scientific, Pittsburg, PA, USA)/40% glycerin (Fisher Scientific, Fair Lawn, NJ, USA), and frozen at −80°C, according to Hanahan et al. (1995).

Screening for *Campylobacter* species activity. *Campylobacter* specific media was made using the following procedure: 25 mL defibrinated sheep blood (Colorado Serum Co, Denver, CO, USA), one tube of antibiotic premix, 21.5 g BBL™ Brucella agar (BD, Becton, Dickinson and Co., Sparks, MD, USA), and 500 ml distilled water. Antibiotic premix was made by suspending 159.0 mg Cephalothin (MP Biomedicals, LLC., Solon, OH, USA), 50.0 mg Trimethoprim Lactate (Research Products International Corp., Prospect, IL, USA), 100.0 mg Vancomycin hydrochloride (Acros Organics, Morris Plains, NJ, USA), 3.22 mg Polymyxin B (InvivoGen, San Diego, CA, USA), and 20.0 mg Amphotericin B (Acros Organics, Morris Plains, NJ, USA) into 100 mL distilled, sterile water.

The total antibiotic premixture was divided into 20 tubes each containing 5 mL aliquots, covered with parafilm (American National Can™, Greenwich, CT, USA), and stored in a −20°C freezer. Plating methods previously described for screening for the presence of *E. coli* were also used when screening for *Campylobacter* spp.

Campylobacter spp. specific media was grown in an anaerobic environment for 96 h prior to checking for growth. An anaerobic environment was achieved by placing a BD BBL™ CampyPak™ Plus Microaerophilic system envelope with Palladium catalyst (Becton, Dickinson and Company, Sparks, MD, USA) in an acrylic canister (17.8 × 12.7 cm, Oggi Co., Anaheim, CA, USA) with a chrome locking clamp with a silicone gasket that sealed air tight. *Campylobacter* spp. selective media were removed from the anaerobic environment after 96 h followed by identification and prevalence of colonies.

Statistical analysis. JMP Statistical Discovery software version 5.1 (SAS Institute Inc., Cary, North Carolina) was used for the analysis of all results. Oneway ANOVA ($\alpha = 0.05$) was performed to analyze the mean total population numbers collected and quadrant counts. A Tukey-Kramer HSD was used to separate means. A linear regression was performed mean daily temperature and cockroach collected. Oneway ANOVA ($\alpha = 0.05$) was performed to analyze the mean bacteria colony forming units for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative, and quadrant counts. Oneway ANOVA ($\alpha = 0.05$) was performed to analyze the mean number of bacteria colony forming units for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative; cockroach stage of development; and quadrant counts.

Results

Periplaneta americana (L.) (n = 687), were collected from four designated areas, north, central, south and west, from the Texas A&M University campus College Station, Texas (Fig. 1). The mean number of cockroaches collected from January–May 2008 was 3.67 ± 4.23 (3.10 ± 3.31 nymphs and 0.56 ± 1.73 adults) per day. The north quadrant had the lowest mean of cockroaches collected with 1.86 ± 1.60 total (1.86 ± 1.25 nymphs and 0.00 ± 0.65 adults). The central quadrant had a mean of 2.21 ± 1.13 cockroaches with 2.14 ± 0.88 nymphs and 0.07 ± 0.46 adults. The south quadrant had a mean of 4.05 ± 0.94 total (3.25 ± 0.74 nymphs and 0.80 ± 0.39 adults). The mean number of cockroaches collected in the west quadrant was 7.29 ± 1.60 total (5.86 ± 1.25 nymphs and 1.43 ± 0.65 adults). There was no significant difference ($F = 2.746$; df = 4, 160; $P = 0.0542$) between population means within quadrants (north, central, south, and west).

There were five categories (Table 1) for building and/or structures from which cockroach populations were collected adjacent to: administration (primarily offices, some classrooms, and vending machines); lecture buildings (primarily lecture or research areas, some offices, and vending machines); dining halls (food establishments on campus with the primary purpose of food and beverage distribution); water tower; and garage. The prevalence of bacteria on cockroaches for each building type indicated that administration buildings had the highest positive rate of cockroaches (Table 1), while the dining hall maintained the lowest rate of prevalence on *P. americana* populations.

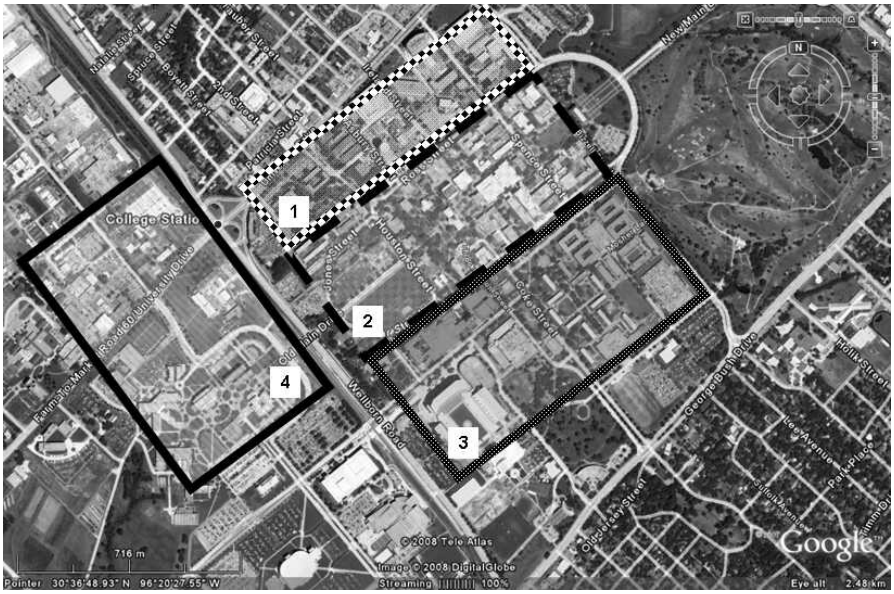

Fig. 1. The Texas A&M University campus, College Station, Texas, divided into four areas, north (checkered) 1, central (dashed) 2, south (dots) 3, and west (solid) 4, used for sampling cockroach populations. Images taken from Google™ Earth Plus v. 4.3.

Microbes isolated from total populations collected indicated a high prevalence (92.3%) of bacteria on the exoskeleton of *P. americana* (Table 2). Bacterial screening for *E. coli* resulted in a significant difference ($F = 2.468$; df = 4, 694; $P = 0.0437$) between quadrants (Table 3). There were also cockroaches that after plating had too many bacterial colony forming units to count. The north quadrant had one *E. coli*, seven coliform forming colonies, and zero non-coliform forming

Table 1. Positive rates of bacterial (*E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative) prevalence for *P. americana* populations collected on the Texas A&M campus, College Station, Texas, as categorized by building function.

Building type	Cockroach population[a]
Administration	427/687 (62.2%)
Lecture building	103/687 (15.0%)
Dining hall	2/687 (0.3%)
Water tower	75/687 (10.9%)
Garage	80/687 (11.6%)

[a]Percentages based on the number of cockroaches collected at each building type compared to the total number of cockroaches collected from February 2007–May 2008.

Table 2. Prevalence of bacteria (*E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative) from the total cockroach population collected on the Texas A&M University campus, College Station, Texas.

Location[a]	*E. coli*[b]	Coliform (G-)	Non-coliform (G-)	Total
North	46/104 (44.2%)	102/104 (98.1%)	87/104 (83.7%)	103/104 (99.0%)
Central	105/155 (67.7%)	145/155 (93.5%)	126/155 (81.3%)	154/155 (99.4%)
South	169/354 (47.7%)	271/354 (76.6%)	225/354 (63.6%)	310/354 (87.6%)
West	31/74 (41.9%)	54/74 (73.0%)	50/74 (67.6%)	64/74 (86.5%)
College Station	23/37 (62.2%)	36/37 (97.3%)	37/37 (100.0%)	37/37 (100.0%)
Total	374/724 (51.7%)	608/724 (84.0%)	525/724 (72.5%)	668/724 (92.3%)

[a]Collections from north, central, south, and west were quadrants on the Texas A&M University campus, College Station, Texas, and College Station specimens were from undisclosed locations in College Station, Texas.
[b]Percentages based on number of cockroaches with colony forming units compared to total number of cockroaches collected in each quadrant.

Table 3. Bacterial counts from cockroach populations screened from each quadrant on the Texas A&M University campus, College Station, Texas.

Bacteria	Location[a]	n	Bacterial mean ± SE[b]	95% Mean Upper	95% Mean Lower
E. coli	North	118	24.14 ± 3.86 ab	16.56	31.72
	Central	150	24.45 ± 3.42 ab	17.73	31.18
	South	318	25.75 ± 2.35 a	21.14	30.37
	West	72	19.51 ± 4.92 ab	9.81	29.22
	College Station	37	3.76 ± 6.89 b	−9.78	17.29
Coliform	North	106	93.92 ± 6.24 c	81.67	106.16
	Central	152	26.91 ± 5.21 e	16.68	37.14
	South	305	69.36 ± 3.67 d	62.14	76.58
	West	70	85.44 ± 7.67 cd	70.37	100.52
	College Station	37	22.35 ± 10.56 e	1.62	43.08
Gram-	North	120	17.73 ± 3.08 f	11.68	23.77
	Central	149	21.87 ± 2.76 f	16.44	27.29
	South	308	24.90 ± 1.92 f	21.13	28.67
	West	71	15.93 ± 4.00 f	8.08	23.78
	College Station	37	14.41 ± 5.54 f	3.53	25.29

[a]Collections from north, central, south, and west were quadrants on the Texas A&M University campus, College Station, Texas, and College Station specimens were from undisclosed locations in College Station, Texas.
[b]Same letters following means within a column were not significantly different ($P < 0.05$, Tukey-Kramer HSD).

colonies. Central quadrant had four *E. coli*, two coliform forming colonies, and five non-coliform forming colonies. The south quadrant had the most with 28 *E. coli*, 14 coliform forming colonies, and 11 non-coliform forming colonies. The west quadrant had no *E. coli*, two coliform forming colonies, and one non-coliform forming colony. Various locations on the Texas A&M University campus resulted in zero plates with too many to count (Table 4). Coliform forming bacteria were significantly different ($F = 24.728$; df = 4, 665; $P < 0.001$) between quadrants, while non-coliform forming gram-negative bacteria had no significant difference ($F = 2.0573$; df = 4, 680; $P = 0.0848$) (Table 3).

There was no significant difference ($F = 0.0420$; df = 2, 205; $P = 0.8379$) between mean number of adult and nymph cockroaches collected and *E. coli* forming units (Table 5). There was no significant difference ($F = 3.0748$; df =2, 216; $P = 0.0809$) between adult and nymph stages of cockroaches collected and coliform-forming bacteria units (Table 5). There was no significant difference ($F = 0.0003$; df = 2, 216; $P = 0.987$) between adult and nymph cockroaches collected and non-coliform forming bacteria units of (Table 5).

Screening for *E. coli* O157:H7 and *Campylobacter* spp. yielded no positive colony forming units for all of the samples screened (n = 724).

Discussion

The purpose of this study was to determine the amount and viability of bacteria harbored by *P. americana* in an outdoor, urban environment by observing commonly occurring and ubiquitous bacteria such as *E. coli* and *Campylobacter*. Outdoor collecting sites on campus provided insight into American cockroach population within an artificial environment. There were no significant differences between collecting sites in each quadrant and

Table 4. Prevalence of cockroach specimens plated for *E. coli*, coliform forming gram-negative, and non-coliform forming gram-negative that resulted in too many bacteria colony forming units to count for cockroaches collected on the Texas A&M University campus, College Station, Texas and various undisclosed locations in College Station, Texas.

Location[a]	*E. coli*[b]	Coliform (G-)	Non-coliform (G-)	Total
North	1/104 (0.009%)	7/104 (0.067%)	0/104 (0%)	8/104 (0.077%)
Central	4/155 (0.026%)	2/155 (0.013%)	5/155 (0.032%)	11/155 (0.071%)
South	28/354 (0.079%)	14/354 (0.040%)	11/354 (0.031%)	53/354 (0.150%)
West	0/74 (0%)	2/74 (0.027%)	1/74 (0.014%)	3/74 (0.041%)
College Station	0/37 (0%)	0/37 (0%)	0/37 (0%)	0/37 (0%)
Total	33/724 (0.046%)	25/724 (0.035%)	17/724 (0.023%)	75/724 (0.102%)

[a]Collections from north, central, south, and west were quadrants on the Texas A&M University campus, College Station, Texas, and College Station specimens were from undisclosed locations in College Station, Texas.

[b]Percentages based on the number of specimens with too many bacteria colony forming units to count compared to the total number of specimens collected from each location.

Table 5. Comparison of bacteria counts for adults and nymphs in all quadrants collected on the Texas A&M University campus, College Station, Texas.

Bacteria	Stage	n	Bacteria Mean ± SE[a]	95% Mean Upper	95% Mean Lower
E. coli	Adult	77	18.64 ± 5.31 a	8.16	29.11
	Nymph	131	20.01 ± 4.07 a	11.98	28.04
Coliform	Adult	83	88.02 ± 21.34 a	45.97	130.08
	Nymph	136	135.51 ± 16.67 a	102.65	168.36
Non-coliform	Adult	83	85.98 ± 32.68 a	21.56	150.39
	Nymph	136	86.65 ± 25.53 a	36.33	136.97

[a]Same letters following means within a column were not significantly different ($P < 0.05$, Tukey-Kramer HSD).

populations of *P. americana* collected. Specific areas of campus did appear to yield higher populations based on observations.

Haines & Palmer (1955) determined that *P. americana* was a predominant species in sewer systems with low population densities indoors and around the home; although the restrooms of indoor facilities maintained the highest population numbers. Overall, building type does not play a significant role in the population densities of cockroaches. The assumption can be made that the same applies for an area such as a university where cockroaches were ubiquitous in the environment.

Pai et al. (2003) determined that adult populations of *P. americana* and *Blatella germanica* L. were significantly higher than nymph populations collected in hospitals, which fails to correspond with data found in this study. There were no significant differences between adult and nymph populations collected around campus. The difference between studies may result from a difference in collection techniques or that the Pai et al. (2003) study was conducted indoors, from a single structure (hospitals) type. Our study exploited various collecting locations and their outdoor structures.

Spatial distribution of natural population is typically patchy. Resource levels fluctuate over time in individual locations, thus population numbers will also change over time indicating a patchy distribution (Roughgarden, 1977). Population fluxes are normal because collecting cockroaches from outside coincided with weather. Population surges may result from rainfall, food availability, an overabundance of water in sewer systems, and/or external weather conditions.

Campylobacter spp. are not part of a normal bacterial fauna in humans but has been found in individuals displaying symptoms such as diarrhea and fever (Blaser et al. 1979). In human patients with symptoms of diarrhea, *C. jejuni* has been isolated to cause diarrhea-like symptoms more than *Shigella* spp., *Salmonella* spp., and *E. coli* O157:H7 (Blaser et al. 1979, Blaser 1997).

Cockroaches could be competent carriers of nosocomial infection agents, especially to patients in neonatal units, intensive care, and immunocompromised patients (Elgderi et al. 2006, Fotedar et al. 1991, Gliniewick et al. 2003,

Salehzadeh et al. 2007). Nosocomial infections may result from pathogens on food; a contaminated water supply; and/or unsanitary facilities, like bathrooms (Lemos et al. 2006). Salehzadeh et al. (2007) described cockroaches collected in hospitals to have greater bacterial counts than cockroaches found in residential areas. Hospital environments may be more conducive to bacterial acquisition from numerous contaminated sources such as water, food, and/or harborage thus resulting in higher rates of bacteria prevalence. Multiple drug-resistant bacterial strains of medical importance have also been isolated from cockroaches in several hospitals (Elgderi et al. 2006, Fotedar et al. 1991, Gliniewick et al. 2003, Salehzadeh et al. 2007).

Escherichia coli can be found on both internal and external surfaces of cockroaches (Rivault et al. 1994). The current study concurred with the Le Guyader al. (1989) study of gram-negative bacteria amounts not having a significant difference between adults and nymphs. Despite the stigma of cockroaches being filth laden, Bell et al. (2007) indicated cockroaches spending at least half of their time grooming and removing foreign objects from their body. The amount of time spent cleaning is inadequate because of contamination of the habitat and the capability to become re-inoculated with pathogens present in the environment. The ability to harbor bacteria on internal and external surfaces provides multiple means of pathogen transmission. In addition to direct contact with surfaces, cockroaches can disseminate internal organisms via defecation and/or regurgitation.

Compared to previous studies made indoors, the presence of bacteria on cockroaches appears to correlate with other studies with positives rates of bacteria in Ghana, France, and Taiwan (Agbodaze & Owusu 1989, Pai et al. 2004, Rivault et al. 1994). Overall, 92.3% of cockroaches collected from outdoor locations on campus carried gram-negative bacteria on their cuticular surfaces. Pai et al. (2005) determined there was no significant difference between *P. americana* and *B. germanica* incident rates of positive growth of bacterial colonies on the integument and the gut. Although, *P. americana* had significantly higher rate of gram-negative colonies than *B. germanica* (Pai et al. 2005). A previous study indicated cockroaches harbored bacteria present in the surrounding environment, as opposed to introducing new pathogens into the environmental fauna (Rivault et al. 1993).

During this study, it was assumed cockroaches were mechanically transmitting pathogens obtained in the environment and were capable of traveling while harboring these bacteria. This creates a public health concern if cockroaches inoculated with bacteria from outside migrated indoors and transmitted pathogens to sterile surfaces, such as areas in the kitchen. Chaichanawongsaroj et al. (2004) indicated *E. coli* levels on cockroaches coincided to *E. coli* levels in the environment. Rivault et al. (1993) discussed that not all bacteria would be able to survive on surfaces that a cockroach contacted.

Contamination rates of cockroaches compounded with their gregarious behavior could provide a mode for pathogens to spread to surfaces having direct contact with food. During this study, 51.7% of all cockroaches trapped were contaminated with *E. coli*. This was the lowest percentage of positive bacteria out of all the cockroaches screened for colony forming units. Despite having the lowest percentage of prevalence, one out of every two cockroaches on campus was carrying *E. coli*. A comparison was made to determine if the life stage (adult or

nymph) made an impact on bacteria associations with the cockroaches and found there to be no significant difference.

Data indicated collection locations as related to *E. coli*, coliform forming gram-negative bacteria were significantly different while there was no significant difference between non-coliform forming gram-negative bacterial species. It was interesting to note differences among collected populations and prevalence of bacteria, despite collecting sites being up to 1.44 km apart. A significant difference may indicate the environment of various collecting locations having differing compositions of bacteria. It is possible that the values for *E. coli* were not significantly difference for each quadrant even though the *P*-value indicated a significant difference. There were 75 specimens that resulted with too many bacteria colony forming units to count. These numbers should not have affected the overall significant difference between populations, quadrants, and bacteria species because the number of too many to count bacteria colony forming units were proportional to initial rates of prevalence among populations collected on campus. No differences for non-coliform forming gram-negative bacteria among collected populations implies that cockroaches may have obtained bacteria from common means throughout campus, such as soil in the flowerbeds or a common water source any of which may have been contaminated with bacteria.

A common water source that may have been easily accessible to all specimens is through the sewer systems. *P. americana* may have traveled from one area of campus to another through various methods of transportation. Cockroaches are capable of migration by ground movement, climbing vertical surfaces, swimming, and some limited flight capabilities (Bell et al. 2007). Jackson & Maier (1955) determined through capture and release experiments that cockroaches could travel through the sewer up to 107 m. It is possible cockroaches remained in locations until resources were depleted and then dispersed in search of food.

All specimens collected were negative for *E. coli* O157:H7. Presence of this pathogen usually occurs in livestock area because cattle and sheep act as reservoirs for the pathogen (McGee et al. 2004). There were no locations on campus that housed livestock which were regularly sampled for cockroach populations. This may have contributed to why there were no positives for *E. coli* O157:H7.

Overall, this study displayed the wide distribution of cockroach populations on campus and their ability to indiscriminately inhabit areas within an urban environment. Pathogen acquisition and dissemination of gram-negative bacteria, such as *E. coli*, was prevalent on campus but without detection of the highly pathogenic strain of *E. coli* O157:H7. Also, there was a lack of *Campylobacter* spp. growth from cuticular plating which may have resulted from undesirable conditions required to sustain colony growth.

Acknowledgment

The authors would like to thank Alan Szalanski for his input and review which improved an early version of the manuscript.

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Epidemiology and Spatial Relationships of Bacteria Associated with *Periplaneta americana* (Blattodea: Blattidae) in Central Texas[1]

Jennifer L. Pechal,[2] James Austin, Roger Gold, and Jeffery K. Tomberlin

J. Agric. Urban Entomol. 24(4): 205–216 (October 2007)

ABSTRACT Identifying cockroach (Order: Blattodea) populations is important to understanding the ability of surrogate species indirectly affecting humans by their ability to vector disease-causing organisms including bacteria. These interactions may have potentially deleterious health consequences on animal and/or human populations. In this study, American cockroaches, *Periplaneta americana* were sampled from 12 locations throughout College Station, Texas from January through May 2008. Cockroach distribution was examined as well as prevalence of *Escherichia coli* including the O157:H7 strain and *Campylobacter* spp. on their external surfaces.

Bacteria isolated from total populations collected indicated a high prevalence (92.3%) of microbes carried on the exoskeleton of *P. americana*. Gram-negative bacteria acquisition and dissemination of organisms such as *E. coli* was prevalent throughout the campus. Screening for *E. coli* O157:H7 and *Campylobacter* spp. resulted in no positive colony growth. The lack of *Campylobacter* spp. growth from cuticular surfaces may have resulted from undesirable conditions required to sustain colony growth. Data from this study corroborate the potential ability of cockroaches to mechanically transmit pathogens.

KEY WORDS *Periplaneta americana*, *Campylobacter* spp., *Escherichia coli*, central Texas

Cockroaches (Order: Blattodea) are important vectors of pathogens due in part to their unsanitary lifestyle. Cockroach cuticle can harbor several *Enterobacteriaceae* species including *Salmonella* spp., *Klebsiella* spp., and *Escherichia* spp. (Mpuchane et al. 2006). A few medically important pathogens that can be vectored by the American cockroach, *Periplaneta americana* (Linnæus) (Blattodea: Blattidae), include: *Campylobacter* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Toxoplasma gondii* (Barcay 2004). Cockroaches are also able to transmit pathogens such as anthrax, cholera, diphtheria, pneumonia, tetanus, and tuberculosis (Baumholtz et al. 1997). Many of which could be used as bioterrorism agents targeting animal or human populations (Lane et al. 2001, Moran 2002).

Understanding the nature of pathogen transmission from urban insect pests to humans could clarify the epidemiology of many illnesses. The epidemiology of

[1]Accepted for publication 17 December 2008.
[2]Corresponding author (jenpechal18@tamu.edu).
Department of Entomology, Texas A&M University, College Station, TX, USA.

Detection of *Cochlosoma anatis* (Kotlan) in *Musca domestica* L. (Diptera: Muscidae) Collected from Commercial Turkey Farms in Arkansas[1]

Sheri M. Brazil,[2] C. Dayton Steelman,[2] Allen L. Szalanski,[2] and Edward E. Gbur[3]

J. Agric. Urban Entomol. 24(4): 217–225 (October 2007)

ABSTRACT *Cochlosoma anatis* (Kotlan) is a flagellated protozoan that has been implicated in turkey enteritis. After sequencing and comparing a portion of the *C. anatis* 16S gene, species specific primers were previously developed and used for polymerase chain reaction detection of *C. anatis* DNA from house flies within 6 h after the flies had been collected in the field. In this study, filth flies were collected from six turkey production facilities in Arkansas during and between outbreaks of enteritis to determine the role of flies in the spread of *C. anatis* during 2002, 2003, and 2004 using *C. anatis* specific primers. Over the 3-yr-period we found that 181/1996 (9.1%) of the house flies collected from the farms were *C. anatis*-positive. There was a highly significant fly sex × month of collection interaction (X^2 = 9.83, df = 2, P = 0.0073) indicating that greater percentages of males than females were found to be *C. anatis*-positive in August and October, 2002, and May and July in 2004 (X^2= 12.84, df = 2, P = 0.0016) during enteritis outbreaks. *Cocholosoma anatis*-positive house flies were collected inside and outside the turkey facilities establishing that they could potentially move the protozoan parasite to or from adjacent poultry facilities on the same farm or to or from other turkey farms in the area.

KEY WORDS Enteritis, *Cochlosoma anatis*, PCR, molecular diagnostics, filth fly

Many microorganisms are present in the digestive systems of turkeys including pathogens that gain entrance into the poultry house environment. Enteritis generally occurs in turkey poults at 1–3 wk of age causing clinical signs of the intestinal infection that includes diarrhea, shrill chirping, and litter eating that results in decreased feed efficiency and weight gain, uneven flock growth, and excessive water consumption (Long Lin 1997). Generally, the disease causes high morbidity, but low mortality. There are many causes of turkey enteritis, and variable combinations of pathogens appear to initiate the disease.

The flagellated protozoan, *Cochlosoma anatis* (Kotlan) is a parasite of birds that was first described from the intestines of a duckling diagnosed with coccidiosis by Kotlan (1923). In 1945, an enteritis outbreak occurred in Scotland effecting 2–10 wk-old turkey poults that resulted in high mortality. *Cochlosoma anatis* was found in large quantities and was the only pathogen diagnosed from the intestinal tracts of the poults during the outbreak (Campbell 1945). This

[1]Accepted for publication 8 Aug 2008.
[2]Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.
[3]Agricultural Statistics Laboratory, University of Arkansas, Fayetteville, Arkansas, USA.

parasite is often associated with enteritis in turkeys and can cause stunting of both turkey poults and ducklings (Bermudez 2003). *Cochlosoma anatis* has been found within the entire intestinal tract of turkey poults and is usually associated with enteric bacterial pathogens and viruses present when infections occur. Studies have been conducted in which ducks with *C. anatis* infections also contained low numbers of *Hexamita* spp. and *Campylobacter jejuni* (Bermudez 2003) and combinations of *C. anatis* and Coronavirus were found more often in pathogenic infections of poults than either of the pathogens alone (Straight et al. 1999).

In 1992, a classic outbreak of turkey enteritis occurred in six flocks of poults in California. The poults showed signs of yellowish diarrhea, depression, and ruffled feathers resulting in a 50 bird-per-day mortality at the highest point of the outbreak. Feed containing antibodies was ineffective in stopping the outbreak and surviving turkeys had depressed body weights at processing. Necropsied poults were found to be infected with *C. anatis* (Cooper et al. 1995). Bollinger & Barker (1996) reported that *Cochlosoma* caused poor weight gain and delayed tail-feather development in Muscovy ducks and found that this parasite had a detrimental effect on the digestive function of the intestinal mucosa.

Lindsay et al. (1999) showed that turkeys became infected with *C. anatis* through consumption of contaminated feed or litter because the trophozoite stage of the pathogen was killed by immersion in water. They demonstrated that oral transmission occurred and that *C. anatis* infected turkeys successfully transmitted the protozoa to uninfected turkeys within 6-d-post inoculation. In addition, their data indicated that long term survival of trophozoites in the environment did not occur.

The study by Lindsay et al. (1999) indicating *C. anatis* transmission through consumption of contaminated feed or litter was substantiated by the report of Hu & McDougald (2003) wherein direct lateral transmission of *Histomonas meleagridis* occurred in turkeys by coprophagy. These studies stimulated the research involving the development of PCR-markers specific for *C. anatis* that could be used to detect the protozoans' DNA (McElroy et al. 2005). In addition, these authors reported the detection of *C. anatis* DNA in filth flies, turkey intestines, and fecal samples.

The present study was conducted to determine the seasonal occurrence of *C. anatis* DNA in filth flies collected during and between enteritis outbreaks on commercial turkey farms in Arkansas.

Materials and Methods

Commercial turkey farms. Six turkey farms located in Carroll County, Arkansas were selected for collection of filth fly specimens: five farms were within a 10 km radius of Green Forrest, Arkansas, and one farm was located approximately 12 km from Green Forrest near Berryville, Arkansas. Generally, each farm was composed of two or more finishing houses attached to brooder houses. All farms in this study were integrated by the same poultry company and turkey finishing facilities measured 121.92 m (400 ft) long and 12.19 m (40 ft) wide. The production system at each farm followed the same procedures for flock grow-outs in which approximately 15,000 poults were maintained in a brooder house for 6 wk then relocated to two finishing houses. The flock cycles consisted of a brood house period that lasted for 6 wk followed by 6 wk in the finishing

houses for light weight hens (8 kg live weight at processing), 8–10 wk for heavy hens (10–12 kg live weight at processing) or 12–14 wk for heavy toms (13–16 kg live weight at processing).

On each farm when the 6-wk-old turkeys were moved from the brooder house to the finishing houses, the turkeys were moved into one finishing house and the wood-shaving litter from the brooder house was moved to the second finishing house. New litter was placed in the brooder house for each flock. After the brooder house litter was distributed evenly throughout one finishing house, one-half of the 6-wk-old turkeys were moved to the second finishing house. The distribution of litter from the brooder house was alternatively placed in the finishing houses with each movement of 6-wk-old turkeys throughout the year. At least a 1 wk interval was allowed between each flock for the placement of new litter and preparation of the production equipment before placement of a new flock of 1-d-old poults. On most farms, five flock grow-outs were accomplished during each calendar year. This production cycle generally ended in February or March at which time the litter was removed from all houses and spread across pastures adjacent to the facilities. Beef cattle were maintained on pastures that surrounded all sides of each of the turkey production facilities. Cattle numbers ranged from 50 yearling heifers to 150 mature cows with calves.

Filth fly collection methods. Flies were collected from inside the finishing houses and immediately outside the areas surrounding the facilities using aerial nets. To prevent PCR amplification and possible contamination between collections of filth flies, the nets were autoclaved and exposed to UV light using a CL-1000 Crosslinker (UVP Inc., Upland, CA) prior to use. Killing jars containing potassium cyanide were used to kill flies immediately after collection in aerial nets. The flies were transported to the laboratory in zip-lock bags that contained one net, and all nets were stored in a chest cooler during transport to the laboratory.

Laboratory preparation and testing. A maximum of 18 male and 18 female adult filth flies were collected from each farm for each week, identified to species and sex in the laboratory, and each fly was placed in an Eppendorf tube and stored at −80°C until used for testing. Total genomic DNA from house flies and other flies was extracted using a Puregene DNA extraction kit (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μl Tris:EDTA pH 8.0 and frozen at −20°C until used for further testing. To minimize protozoan contamination in the laboratory, all isolations were performed in a Safety Class II hood cabinet.

Extracted DNA from the filth flies was subjected to PCR using the primers COCH-16S-F (5′-AAGGTTTGTCATTTCAAAAT-3′) and COCH-16S-R (5′-TCTTCCTCCTGCTTAAATAA-3′), per McElroy et al. (2005), which amplifies a 374 bp region of the 16S rRNA gene. PCR conditions consisted of an initial denaturation step of 94°C for 5 min followed by 40 cycles of 94°C for 45 s, 43°C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 5 min (McElroy et al. 2005). The *C. anatis* control was taken from turkey intestinal samples positive for the protozoan. To visualize the presence of *C. anatis*, 1% agarose gel electrophoresis was performed on the PCR product of each sample, and diagnostic PCR amplicons were visualized and recorded using a UVP biodoc-it system (UVP Inc., Upland, CA).

Table 1. Total filth flies, *M. domestica*, and *H. aenescens* collected from all farms positive for *C. anatis* in 2002, 2003, and 2004.

Year	Total filth flies	*C. anatis* no./%	Total *M. domestica*	*C. anatis* no./%	Total *H. aenescens*	*C. anatis* no./%
2002	830	103/12.4	630	102/16.2	200	1/0.5
2003	296	13/4.4	290	13/4.5	6	0/0.0
2004	1150	68/5.9	1076	66/6.1	74	2/2.7
Total	2276	184/8.1	1996	181/9.1	280	3/1.1

Statistical analysis. Formal statistical analysis was limited to those outbreaks for which a sufficient number of flies tested positive. Thus, only the 2002 outbreak on Farm 1 and the 2004 outbreaks on Farm 5 was analyzed.

The proportion of flies testing positive for each month-sex combination was assumed to follow a binomial distribution. Data were subjected to logit analysis (analog of normal theory analysis of variance). For significant effects, least square means were compared and the results were back-transformed to the percent scale for presentation. A significance level of 0.10 was used. All statistical analyses were carried out using SAS Version 9.1 (SAS Institute, Inc., Cary, NC).

Results and Discussion

During this study, filth flies were collected from the farms when outbreaks of enteritis had been diagnosed as well as from farms on which there were no outbreaks occurring, but these farms had high fly populations and the potential for an outbreak to occur existed. Overall, 184/2276 (8.1%) adult filth flies collected from the six commercial turkey farms were found to contain *C. anatis* using PCR, of which 181/1996 (9.1%) were house flies and 3/280 (1.1%) were black garbage flies, *Hydrotaea aenescens* (Wiedemann), were found to be positive for the protozoan (Table 1).

The results of this study indicated that a relatively high percentage of the house flies both inside and outside the turkey production facilities were carrying *C. anatis* during turkey enteritis outbreaks. Although some black garbage flies, *H. aenescens*, were collected and found to be positive, more house flies were detected with *C. anatis* than *H. aenescens*. Generally, house flies are more abundant around poultry facilities (Axtell & Arends, 1990) than other species of filth flies. Palmer (2004) reported that *M. domestica* and *H. aenescens* comprised 85.5 and 13.9% of the adult filth flies, respectively, with other species accounting for 0.3% using sticky ribbons to monitor seasonal abundance of filth flies in Arkansas turkey production facilities. In the present study 2276 adult flies were collected with aerial nets and tested for detection of *C. anatis*, of which 87.6% were *M. domestica* and 12.3% were *H. aenescens*. Only 1.1% of the adult *H. aenescens* were detected with *C. anatis* while 9.1% of *M. domestica* tested positive for the protozoan DNA.

House flies were collected inside and outside the turkey finishing facilities to establish that house flies carrying *C. anatis* could potentially move the protozoan parasite to or from adjacent poultry facilities on the same farm or to or from other

turkey farms in the area. In 2002, filth flies were collected from Farm 1 during an enteritis outbreak that lasted from August through October, wherein *C. anatis* was diagnosed from intestine samples collected from poults on the farm and transported to the University of Missouri, College of Veterinary Medicine (Dr. Alex Bermudez, Department of Veterinary Pathobiology, University of Missouri, Columbia, MO, personal communication). During this period, a total of 102/630 (16.2%) house flies were found to be positive for *C. anatis* DNA. Sixty-two of the flies were collected inside the turkey finishing houses of which 60.8% were detected with *C. anatis* DNA and 39.3% of the 40 flies collected outside of the facilities tested positive between the time the enteritis outbreak was diagnosed until the flock was shipped to processing (Table 2). Only 2 of 100 flies collected and tested from inside the facilities at Farm 1 in October 2003 were *C. anatis* positive.

In 2004, before the diagnosed enteritis outbreak at Farm 5, 25 house flies collected outside the facilities were found to be positive for *C. anatis* DNA. The outbreak was diagnosed in June, during which time 364 flies were collected at Farm 5, of which 10.6% of 198 flies collected inside the facilities and 21.1% of 166 flies collected outside the facilities were positive for *C. anatis* DNA. No enteritis outbreak was diagnosed at Farm 6 during 2004, however, of 287 house flies collected 9 (3.1%) were detected with *C. anatis* DNA. There was 3.9% of 181 flies collected inside the facilities that tested positive while 1.9% of 106 flies collected outside tested positive for *C. anatis* at Farm 6 (Table 2).

Overall, similar percentages of house flies determined to be positive for *C. anatis* were collected outside (8.2%) and inside (8.9%) the turkey facilities. Fotedar et al. (1992) reported that *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Viridans streptococci* were only found in *M. domestica* collected within the patient wards of a hospital compared to flies collected 5 km away from the hospital in a residential area. The presence of *Staphylococcus aureus* was significantly higher in the flies collected within the hospital than those collected in the residential area while no significant difference was found in the isolation of parasitic ova, cysts, or in *Candida* spp. isolated from both groups of house flies. In similar studies, Rady et al. (1992) reported that 21 species of bacteria were isolated from house flies collected inside four hospitals and from garbage sites outside the hospitals. They concluded that house flies could play an important role in the contamination of the hospitals by their movement of bacteria either from outside into the hospital or vice-versa as well as from one site to another within the same hospital. The relatively high percentage of house flies detected with *C. anatis* DNA combined with the report of house fly movement of up to 20 km (Lysyk & Axtell 1986) indicate the potential for flies to distribute *C. anatis* from within and among turkey farms.

In 2002, Farm 1 was experiencing an outbreak of enteritis in which *C. anatis* was found to be a contributing factor upon diagnosis of turkey intestines. House flies were found positive for *C. anatis* at this farm which corresponded with the time interval that the outbreak was occurring. House flies were also found to be positive for the protozoan in 2003, at three farms when outbreaks were not diagnosed (Dr. Alex Bermudez, personal communication). In 2004, large numbers of house flies were detected positive for *C. anatis* at Farm 5, which was the only farm that reported an enteritis outbreak; however the other farms had flies that tested positive although no outbreaks were occurring. Although infected flies were collected outside the turkey production facilities no data was obtained on

Table 2. Percentage house flies positive for *C. anatis* DNA collected inside and outside turkey production facilities during 2002, 2003, and 2004.

Year	Month(s)	Farm no.	Enteritis	No. flies collected	No. flies positive (%)	No. positive flies collected	
						Inside	Outside
2002	Aug, Sep, Oct	1	Outbreak	630	102 (16.2)	62	40
2003	Oct	1	No outbreak	100	2 (2.0)	2	0
	Oct	2	Symptoms	19	3 (15.8)	3	0
	Oct	3	Symptoms	171	8 (4.7)	7	1
2004	Apr, Jul	1	No outbreak	38	0	0	0
	Apr	2	No outbreak	24	0	0	0
	Apr–Jul	3	No outbreak	132	0	0	0
	Apr–Jul	4	Symptoms	231	1 (0.4)	1	0
	Apr–Jul	5	Outbreak	364	56 (15.4)	21	35
	Apr–Jul	6	Symptoms	287	9 (3.1)	7	2

Table 3. Percentage *C. anatis* DNA positive male and female house flies collected at Farm 1 in 2002 and Farm 5 in 2004, during enteritis outbreaks.

	Fly sex	
Month/year	Female	Male
Farm 1, 2002		
August	19.4 bc	36.1 a
September	10.8 cd	3.3 d
October	15.9 bcd	21.2 b
Farm 5, 2004		
May	<0.1 d	20.0 ab
June	17.2 ab	13.0 bc
July	6.0 c	25.1 a

Percentages followed by the same letter within years are not significantly different at $P = 0.10$.
For Farm 1, 2002, $X^2 = 9.83$, df = 2, $P = 0.0073$.
For Farm 5, 2004, $X^2 = 12.84$, df = 2, $P = 0.0016$.

whether these flies came from within the facilities or from other surrounding areas. However, the data did suggest that once outside the infected area flies could disperse in any direction as fly movement among farms had been reported previously by Hogsette & Jacobs (1999) and Lysyk & Axtell (1986). Relatively small numbers of *H. aenescens* were found in the facilities and the percentage detected was low compared to *C. anatis* detection in house flies.

Flies collected at Farm 1 during 2002, had a highly significant sex × month of collection interaction ($X^2 = 9.83$, df = 2, $P = 0.0073$) wherein greater percentages of males were found to be *C. anatis*-positive in August and October than in September (Table 3). No significant difference was found in percentage females that were positive during these months. In August 2002, significantly greater percentages of males collected were positive than females while no significant difference was found between the percentages of males and females collected in September and October, 2002, at Farm 1.

During the month of October, 2003, no enteritis outbreaks were diagnosed at Farms 1, 2, and 3, but it appeared that approximately 15–25% of the turkeys were voiding diarrhea-type feces generally characteristic of enteritis. Filth flies were collected at each farm on 31 October and although filth fly numbers were quite low at Farm 2, 3 of 19 flies (15.8%) were positive for *C. anatis*. The highest number of house flies were collected at Farm 3 (171) of which 8 (4.7%) tested positive for *C. anatis*. Only 2 of 100 flies collected at Farm 1 tested positive in October 2003. There was a greater proportion (6.46%) of *C. anatis* positive flies collected at the three farms on one day during this time period when the turkeys were exhibiting enteritis symptoms.

Filth flies were collected and tested for pathogen DNA during 2004 when no outbreaks occurred at Farms 1, 2, 4 and 6 and although turkeys at Farm 3 appeared to have clinical symptoms of enteritis, no outbreak was diagnosed when samples were sent to the University of Missouri (Dr. Bermudez, personal

communication). No house flies were detected with *C. anatis* DNA at Farms 1, 2, and 3, however one female house fly was *C. anatis*-positive in July that was collected at Farm 4. In addition, 9 flies were found to be positive at Farm 6, during the month of June. However, at this time Farm 5 was experiencing an outbreak, with turkeys showing clinical signs of enteritis and the samples sent to the University of Missouri were confirmed by Dr. Alex Bermudez (personal communication). There was a highly significant sex × month interaction (X^2 = 12.84, df = 2, P =0.0016) detected during May through July at Farm 5. Greater percentages of male flies were found to be positive for *C. anatis* while no significant difference was detected between male and female flies collected during June. There were significant differences between the percentages of *C. anatis*-positive female flies collected during the 3-month-period at Farm 5 (Table 3).

Male and female house flies collected inside and outside turkey production facilities were found to harbor *C. anatis*. Palmer (2004) found that the mean seasonal sex ratio collected on weekly intervals using sticky ribbons was 4:1 (M:F) for house flies and 3.8:1 for *H. aenescens* in Arkansas turkey production facilities. In the present study, using aerial nets, the overall male to female ratio was 1.2:1, 2.2:1, and 1.42:1 (M:F) in 2002, in 2003, and 2004, respectively. Mian et al. (2002) isolated several serotypes of *Salmonella enteritidis* from *M. domestica* collected on dairy and commercial egg farms and reported that the male to female ratio testing positive was 3:8 (M:F) pools indicating that more female flies collected on the dairy and commercial egg poultry farms were carrying the bacteria. Their data suggested that the female flies could have become infected through their attraction to accumulated animal manure for oviposition. During the present 3-yr study, 1165 male and 831 female house flies were tested of which *C. anatis* was detected in 9.7% and 8% of the males and females, respectively. These data suggest that male and female flies were attracted to the diarrhea-type fecal deposits voided by turkeys with enteritis for feeding purposes. This observation is supported by Palmer (2004) who indicated that *M. domestica* and *H. aenescens* were breeding outside the turkey facilities and adult flies of both species were moving into the facilities for feeding. He reported that only a mean of 0.4 (±0.2) larvae per core sample was found in the 3127 samples taken (1.5% positive) from inside the turkey facilities.

Fast detection of *C. anatis* is important to prevent economic losses that have been reported when enteritis outbreaks occur in turkey flocks. Cooper et al. (1995) reported that *C. anatis* infected flocks experienced a 16% decrease in body weight. Initiation of fly management tactics when *C. anatis* has been detected within and among the turkey production components of the Agro-Ecosystem could aid in preventing outbreaks of turkey enteritis. More research is needed to determine the distribution of filth flies within and among turkey production farms, and there is a need for an area wide control program based on the dispersal ability of house flies.

Acknowledgment

This research was supported in part by the University of Arkansas, Fayetteville, Arkansas, Agricultural Experiment Station. Special thanks are extended to Dr. Alex Bermudez, Department of Veterinary Pathobiology, University of Missouri College of Veterinary Medicine, Columbia, Missouri for providing *C. anatis* diagnosis.

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Detection of *Cochlosoma anatis* (Kotlan) in *Musca domestica* L. (Diptera: Muscidae) Collected from Commercial Turkey Farms in Arkansas[1]

Sheri M. Brazil,[2] C. Dayton Steelman,[2] Allen L. Szalanski,[2] and Edward E. Gbur[3]

J. Agric. Urban Entomol. 24(4): 217–225 (October 2007)

ABSTRACT *Cochlosoma anatis* (Kotlan) is a flagellated protozoan that has been implicated in turkey enteritis. After sequencing and comparing a portion of the *C. anatis* 16S gene, species specific primers were previously developed and used for polymerase chain reaction detection of *C. anatis* DNA from house flies within 6 h after the flies had been collected in the field. In this study, filth flies were collected from six turkey production facilities in Arkansas during and between outbreaks of enteritis to determine the role of flies in the spread of *C. anatis* during 2002, 2003, and 2004 using *C. anatis* specific primers. Over the 3-yr-period we found that 181/1996 (9.1%) of the house flies collected from the farms were *C. anatis*-positive. There was a highly significant fly sex × month of collection interaction (X^2 = 9.83, df = 2, P = 0.0073) indicating that greater percentages of males than females were found to be *C. anatis*-positive in August and October, 2002, and May and July in 2004 (X^2= 12.84, df = 2, P = 0.0016) during enteritis outbreaks. *Cocholosoma anatis*-positive house flies were collected inside and outside the turkey facilities establishing that they could potentially move the protozoan parasite to or from adjacent poultry facilities on the same farm or to or from other turkey farms in the area.

KEY WORDS Enteritis, *Cochlosoma anatis*, PCR, molecular diagnostics, filth fly

Many microorganisms are present in the digestive systems of turkeys including pathogens that gain entrance into the poultry house environment. Enteritis generally occurs in turkey poults at 1–3 wk of age causing clinical signs of the intestinal infection that includes diarrhea, shrill chirping, and litter eating that results in decreased feed efficiency and weight gain, uneven flock growth, and excessive water consumption (Long Lin 1997). Generally, the disease causes high morbidity, but low mortality. There are many causes of turkey enteritis, and variable combinations of pathogens appear to initiate the disease.

The flagellated protozoan, *Cochlosoma anatis* (Kotlan) is a parasite of birds that was first described from the intestines of a duckling diagnosed with coccidiosis by Kotlan (1923). In 1945, an enteritis outbreak occurred in Scotland effecting 2–10 wk-old turkey poults that resulted in high mortality. *Cochlosoma anatis* was found in large quantities and was the only pathogen diagnosed from the intestinal tracts of the poults during the outbreak (Campbell 1945). This

[1]Accepted for publication 8 Aug 2008.
[2]Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.
[3]Agricultural Statistics Laboratory, University of Arkansas, Fayetteville, Arkansas, USA.

Plant Parasitic Nematodes Associated with *Olea europea* L. Fauna of Turkey[1]

Cihan Cilbircioğlu

J. Agric. Urban Entomol. 24(4): 227–231 (October 2007)

ABSTRACT The olive (*Olea europea* L.) is characteristic of the culture in the Mediterranean region. Its origins are Anatolian in Turkey and neighboring Syria. Olive trees serve as host to a fairly high number of plant parasitic nematodes in the Mediterranean countries. Several of these nematodes are sedentary endoparasitic forms, which are recognized as pathogens to olives. In this study, records of plant parasitic nematodes associated with olive in Turkey were examined faunistically and taxominically. The records were compiled to create a current record of these fauna of plant parasitic nematodes of olive in Turkey. Their biology, distribution and associated host plants were described. This fauna consists of 19 Tylenchida plant parasitic nematode species. Since Turkish records are scant and poorly documented, this study documents the most current list of these important species.

KEY WORDS *Olea europea* L., plant parasitic nematodes, fauna, Turkey

Olive (*Olea europaea* sp. *europaea* L.) is grown extensively in the Mediterranean Basin, the subtropical regions of Australia, southern Africa, and North and South America. About 90% of 8 million hectares of olives grown worldwide in 2001 are located in the Mediterranean countries. At present, olives constitute a combination of uncultivated forms and cultivated varieties (Castillo et al. 2003). Its homeland origins are Eastern Mediterranean and it is an important asset to Turkey. Approximate 90 million olive trees are grown in Turkey. Turkey ranks fourth for countries producing olives according to tree numbers, and ranks sixth according to area (in hectares) worldwide. Turkey contributes almost eight percent of the world olive production and is second only to Spain according to food olive production (Bartolini et al. 2005). Olives are grown extensively in arid and rugged areas which encompass the Aegean, Marmara and Mediterranean regions of Turkey.

Plant parasitic nematodes cause yield losses for many crops, but the economic significance of the damage caused by these parasites is generally not well understood or recognized by growers. Olive roots can be infected by a range of plant-parasitic nematodes. Nematode species associated with olive trees include *Mesocriconema xenoplax* (Raski) Loof & De Grisse (=*Criconemella xenoplax* (Raski) Luc & Raski), *Helicotylenchus* spp., *Heterodera mediterranea* Vovlas, Inserra & Stone, *Meloidogyne* spp., *Pratylenchus* spp., *Xiphinema* spp., and *Rotylenchulus* spp. Also, several authors have reported impairment of cultivated

[1]Received 10 August 2008; Accepted 2 October 2008.
Ankara University Agricultural Faculty Plant Protection Department, Entomology Mainbranch 06100, Ankara/Turkey. E-mail: cihancilbirci@hotmail.com

olive due to root-knot nematodes *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood. However, to our knowledge, this is the first report of root-knot nematodes on wild olive. The accurate identification and pathogenic characterization of root-knot nematodes attacking wild and cultivated olives are needed as an initial step in designing effective control measures. This is especially important in the search for possible sources of host plant resistance against *Meloidogyne* spp. to reduce the initial nematode population density (Castillo et al. 2003).

Studies on nematodes associated with olives around the world have been previously reported by several authors (Diab & El-Eraki 1968; Scognamaglio et al. 1968, Lamberti 1969, 1981, Scognamiglio et al. 1971, Gallo & Jiménez 1976, Fiume 1978, Inserra & Vovlas 1981, Hashim 1982, 1983, Santiago 1990). Only basic information exists on most parasitic nematodes, their host associations and occurrences for different localities in Turkey as has been described by Ökten et al. (2000) and Kepeneki (2001) for some nematoda species associated with *Olea europea* L.

Materials and Methods

In this study, the list of plant parasitic nematodes associated with olive published by Kepeneki (2001) was examined faunistically and taxominically. A revised list of these and from other sources was compiled to update plant parasitic nematodes of olive in Turkey. Their biology and distribution in Turkey with their associated host plants are described.

Results and Discussion

A review of plant parasitic nematodes associated with olive fauna of Turkey was determined to consist of 19 plant parasitic nematode species: two species belong to the Tylenchidae family, 8 species to Dolichodoridae, 4 species to Hoplolaimidae, 2 species to Pratylenchidae, 1 specie to Criconematidae, 1 specie of Hemicycliophoridae, and 1 specie of Anguinidae. A breakdown of the morphological characters for these follow:

Morphology of Tylenchidae. Amphids mostly lateral in position. Stylet shaft mostly formed by metarhabdions, lacking innervation; basal knobs formed by telorhabdions, often well developed and marked off. Orifice of dorsal ossophageal gland in precorpus at the base of the stylet or a short distance behind it. Median oesophageal bulb, if present, without a muscular valve anterior to the central valve-like cuticular thickening. Anus inconspicuous, minute, pore-like, directed outward. Sperm usually small, nucleus not showing discrete chromosomes. Male caudal papillae absent; bursa lacking papillary ribs or rays, never present only at the tail tip. Spicules present only at the tail tip.

This family usually consists of slender, elongate, small species. Sexes similar. Stylet usually small, delicate. Small (from 0.33 mm) to medium (up to 1.3 mm) sized, a few much larger; most always slender, vermiform in shape. Body marked with many transverse striae (some species appear smooth when seen by light microscopy but some striae have been found in every case where study by SEM has been possible). Lateral field with 0, 2, 3, 4, 6 or multiple longitudinal lines (sometimes so delicate or shallow to be seen only by SEM). Lip region usually

elevated, rounded; transverse striae usually extend up onto labial region, some species with smooth labial region. Labial framework delicate, weakly developed (moderately developed in *Anturctenchus*). Stylet mostly small (3 pm–20 pm), delicate, with distinct basal knobs (rarely without knobs, a few species with long to very long stylet (Geraert and Raski, 1987).

Morphology of Dolichodoridae. *Female.* Body cylindrical, elongate, slightly arcuate. Lip region set off by a distinct constriction, bearing 7–9 (sometimes 11) fine annules. Perioral disc prominent, distinctly raised. Anterior portion of head with strong cephalic framework, basal plate heavily sclerotized. Cuticle coarsely and distinctly annulated. Anterior cephalids conspicuous, opposite, 4–5 annules posterior to constriction bearing 7–9 (sometimes 11) fine annules. Perioral disc prominent, distinctly raised. Anterior portion of head with strong cephalic framework, basal plate heavily sclerotized. Cuticle coarsely and distinctly annulated. Anterior cephalids conspicuous, opposite, 4–5 annules posterior to constriction at lip region. Deirids not seen. Lateral field areolated, 7.7–14.1 wide near midbody, incisures three. Stylet long, slender to stout, with strongly developed posteriorly sloping somewhat rounded knobs, conus longer than shaft. Nerve ring near middle of isthmus.

Male. Similar to the female in digestive tract, nerve ring and excretory pore position, annulation, and lateral field; but body slightly smaller and posterior end slightly hooked ventrally. Tail terminus bifurcate. Caudal alae trilobed striated, enveloping tail. Testis single, outstretched (Golden et. al. 1986).

Morphology of Hoplolaimidae. Tylenchoidea. Female vermiform to kidney-shaped; when vermiform, habitus often spiral. Lip region high, typically higher than 1/2 the diameter of the basal lip annulus; anterior end with rounded or trapezoidal outline in lateral view, annulated, sometimes with longitudinal striae on basal lip annulus, rarely striae on other lip annuli. Lateral field typically with four lines, sometimes regressed (some Hoplolaimus spp.). Phasmids typically near anus level, rarely on tail, sometimes migrated far anteriorly (Hoplolaimus), generally small pore-like structures, sometimes enlarged into scutella, rarely absent (Aphasmatylenchus). Tail typically short, less than two tail diameters long, rarely longer; generally more curved dorsally, sometimes regularly rounded, rarely conical. Caudalids and cephalids generally present; deirids absent (Fortuner 1987).

Morphology of Criconematidae. *Female.* Body sausage-shaped to cylindrical. Cuticle thick, lacking a typical lateral field (sometimes marked by irregularities in body annuli and/or superficial longitudinal lines extremely variable within the same species). Body annuli either retrorse, provided or not with lobation, crenation, scales or spines, or rounded and covered or not with an extra-cuticular layer. Labial area variously shaped; submedian lobes absent or variously developed. Labial sclerotization strong. Stylet massive; cone much longer than base plus knobs; stylet knobs anchor-shaped or sloping backwards. Isthmus very short; esophageal glandular bulb markedly reduced.

Male. No stylet. Spicules variously shaped. Caudal alae absent or well-developed (Raski & Luc 1987).

Morphology of Hemicycliophoridae. Female and juveniles with two cuticles, the outer one serving as a body sheath,which is not membranous. Cephalic annules of female not modified or separated (except in *Hemicycliophora hesperis*). Vulva a transverse slit over half a body-width long and marked by a

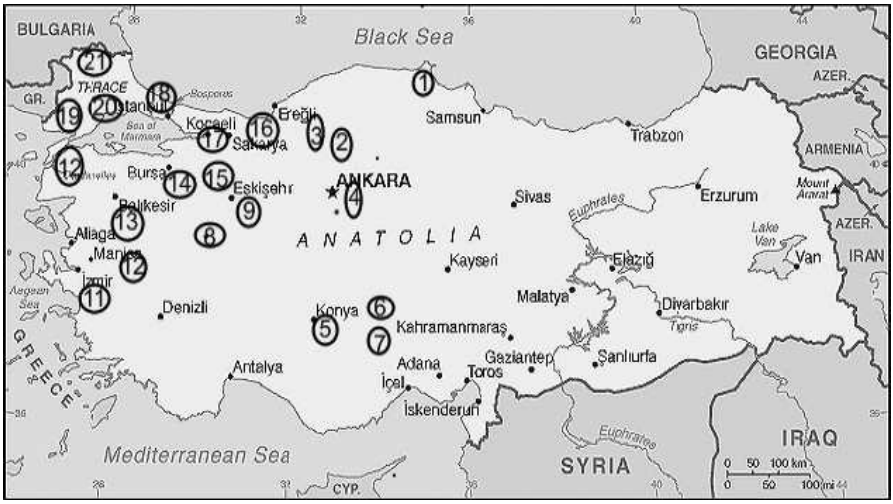

Fig. 1. Distribution of plant parasitic nematodes associated with olive *Olea europea* L. in Turkey. Localities identified by number include: 1=Adana, 2=Antalya, 3=Balıkesir, 4=Burdur, 5=Eskişehir, 6=Isparta, 7=İçel, 8=Samsun, 9=Trabzon, 10= Zonguldak. Nematode families and species associated with these localities (in parentheses) include: Tylenchidae *Coslenchus diversus* Lal & Khan (7), *Basiria dublexa* Hagemeyer & Allen (4, 6), Dolichodoridae *Neopsilenchus peshawarensis* Steiner (4), *Tylenchorhynchus claytoni* Steiner (2, 4, 7, 9, 10), *T. cylindricus* Cobb (2), *T. tritici* Golden, Maqbool & Hondoo (1), *Bitylenchus goffarti* Sturhan (1, 2), *Quinisulcius acutus* Allen (1, 3), *Scutylenchus lenorus* Brown (1, 2), Hoplolaimidae *Hoplolaimus galeatus* (Cobb) (6, 7), *Rotylenchus cypriensis* Antoniou (1, 2, 7), *H. tunisiensis* Siddiqi (7), *Pleciorotylenchus striaticeps* Volvas, Castillo & Lamberti (1, 2, 7), Pratylenchidae *Pratylenchus mediterraneus* Corbett (2), *Pratylenchoides erzurumensis* Yüksel (5), Criconematidae *P. ritteri* Sher (6, 7), *Hemicriconemoides gaddi* Loos (10), Hemicycliophoridae *Hemicycliophora sturhani* Loof (2, 7), and Anguinidae *Safianema anchilisposoma* Tarjan (6, 7, 8, 9).

discontinuity in ventral body tour (except in *Loofia*); vulva lips modified and projecting (except in *Loofia*). Vagina straight or curved but not sigmoid. Male cephalic region marked by a discontinuity in body annulation, usually offset; frame-work in lateral view appearing as "spectacle mark". Spicules arcuate, semi-cirdar, U- or hook-shaped (Siddiqi, 1980).

Morphology of Anguinidae. Vermiform nematodes; mature females sometimes enlarged, but never globose or kidney-shaped. Lip region low, anteriorly flattened, not or slightly offset, not annulated or with faint annuli. First lip annulus not divided into sectors; amphid apertures small, elliptical, directed towards the oral opening. Lateral field with either four or six and more lines. Deirids and phasmids generally absent. Tail long, slender, often with last third ventrally bent; sometimes shorter and rounded (Fortuner and Maggenti 1987).

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Plant Parasitic Nematodes Associated with *Olea europea* L. Fauna of Turkey[1]

Cihan Cilbircioğlu

J. Agric. Urban Entomol. 24(4): 227–231 (October 2007)

ABSTRACT The olive (*Olea europea* L.) is characteristic of the culture in the Mediterranean region. Its origins are Anatolian in Turkey and neighboring Syria. Olive trees serve as host to a fairly high number of plant parasitic nematodes in the Mediterranean countries. Several of these nematodes are sedentary endoparasitic forms, which are recognized as pathogens to olives. In this study, records of plant parasitic nematodes associated with olive in Turkey were examined faunistically and taxominically. The records were compiled to create a current record of these fauna of plant parasitic nematodes of olive in Turkey. Their biology, distribution and associated host plants were described. This fauna consists of 19 Tylenchida plant parasitic nematode species. Since Turkish records are scant and poorly documented, this study documents the most current list of these important species.

KEY WORDS *Olea europea* L., plant parasitic nematodes, fauna, Turkey

Olive (*Olea europaea* sp. *europaea* L.) is grown extensively in the Mediterranean Basin, the subtropical regions of Australia, southern Africa, and North and South America. About 90% of 8 million hectares of olives grown worldwide in 2001 are located in the Mediterranean countries. At present, olives constitute a combination of uncultivated forms and cultivated varieties (Castillo et al. 2003). Its homeland origins are Eastern Mediterranean and it is an important asset to Turkey. Approximate 90 million olive trees are grown in Turkey. Turkey ranks fourth for countries producing olives according to tree numbers, and ranks sixth according to area (in hectares) worldwide. Turkey contributes almost eight percent of the world olive production and is second only to Spain according to food olive production (Bartolini et al. 2005). Olives are grown extensively in arid and rugged areas which encompass the Aegean, Marmara and Mediterranean regions of Turkey.

Plant parasitic nematodes cause yield losses for many crops, but the economic significance of the damage caused by these parasites is generally not well understood or recognized by growers. Olive roots can be infected by a range of plant-parasitic nematodes. Nematode species associated with olive trees include *Mesocriconema xenoplax* (Raski) Loof & De Grisse (=*Criconemella xenoplax* (Raski) Luc & Raski), *Helicotylenchus* spp., *Heterodera mediterranea* Vovlas, Inserra & Stone, *Meloidogyne* spp., *Pratylenchus* spp., *Xiphinema* spp., and *Rotylenchulus* spp. Also, several authors have reported impairment of cultivated

[1]Received 10 August 2008; Accepted 2 October 2008.
Ankara University Agricultural Faculty Plant Protection Department, Entomology Mainbranch 06100, Ankara/Turkey. E-mail: cihancilbirci@hotmail.com