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AYHAN GÖKÇE

GEORGINA V. BINGHAM

MARK E. WHALON

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Impact of long-lasting insecticide-incorporated screens on *Colorado potato* beetle and plum curculio

Ayhan GÖKÇE[1,*], Georgina V. BINGHAM[2], Mark E. WHALON[3]
[1]Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, Niğde, Turkey
[2]Vestergaard S.A., Lausanne, Switzerland
[3]Department of Entomology, Michigan State University, East Lansing, MI, USA

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Abstract: The efficacy and behavior effects of long-lasting insecticide-incorporated screens (LLIS; polyolefin containing deltamethrin) were tested under laboratory conditions against two important pests (*Leptinotarsa decemlineata* and *Conotrachelus nenuphar*). Four Vestergaard ZeroFly® LLISs of different specifications were evaluated: trial batches JLCB061009, SPSA 6616, ZeroFly Greenhouse (B), and ZeroFly Greenhouse (G). Two control products were also included: ZeroFly Screen Greenhouse White with no insecticide and a standard commercial fiberglass insect screen. Contact bioassays revealed that all treated LLIS products, except JLCB061009, provided >90% mortality within 24 h of exposure for both susceptible (New Jersey, USA) and resistant (Long Island, NY, USA) *L. decemlineata* colonies. The LT_{50} values for the LLISs ranged from 30 to 51 min. Behavioral tests revealed that *L. decemlineata* adults were unable to feed on potato leaflets in all treatments except in the case of JLCB061009. In the contact bioassays conducted on *C. nenuphar*, the maximum mortality (98%) was observed with ZeroFly Greenhouse (B). There was no feeding scar on any apples in all treatments except JLCB061009. LLISs provided a high level of protection against Colorado potato beetle and plum curculio under laboratory conditions. Further studies with different species and under field conditions are needed to determine the full potential of LLISs for agricultural pest management.

Key words: *Conotrachelus nenuphar*, deltamethrin, insect behavior, *Leptinotarsa decemlineata*, preference

1. Introduction

Many strategies have been implemented in integrated pest management for the control of insect pests. For example, mechanical control (hand-picking), biological control (utilization of natural enemies), mass trapping, genetic control, trench traps, baits, attractants, push-pull strategies, host plant resistance, and conventional chemical control have all been used for the control of agricultural pests (Alyokhin et al., 2008; Boiteau et al., 2008; Jaleel et al., 2013; Saeed et al., 2014). However, the most commonly used method for insect pest management is still chemical control, primarily due to its efficient action on target pests and lower costs (Yang et al., 2005; Shi et al., 2011). However, the real costs of insecticide sprays include increasing costs of application, resistance development and pest resurgence, and adverse effects on nontarget organisms, human health, and the environment (Paranagama et al., 2003).

Effective alternative strategies should be devised to deal with the impacts of insecticide use and to support integrated pest management programs (Bell, 2000; Rajendran and Sriranjini, 2008). One recent advance in pest management is the development of insecticide-incorporated screens. Commercially available long-lasting insecticide-incorporated screens (LLISs) contain deltamethrin, the first introduced synthetic pyrethroid, which has been in use since 1978 (Zamojska et al., 2011). Deltamethrin is a highly potent insecticide that causes hypersensitivity, tremors, and paralysis in target organisms. It is known to modify the sodium channel in such a way as to prolong the tail current associated with step repolarization following a depolarizing pulse (Chalmers et al., 1987). The high efficacy and reduced impact of LLISs on nontarget organisms has been best demonstrated in their use for mosquito control in areas affected by malaria: LLISs have reduced cases of malaria by 62%, and child mortality has dropped to 29% in some areas (Lengeler, 2004). LLISs are now promoted as a key method to fight malaria and its vector (Atkinson et al.,

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* Correspondence: ayhan.gokce@ohu.edu.tr

2009). Although the efficacy and potential of LLISs for wide applications is undisputed, their use has mostly been limited to controlling household pests and insect vectors such as mosquitoes and flies (Baume and Marin, 2008; Okumu and Moore, 2011).

A few studies have evaluated LLISs for control of agricultural pests (Moreno et al., 2012; Dáder et al., 2015; Kuhar et al., 2017). In these studies, the applicability of LLISs for multiple economically important pests and their potential for reducing insecticide dependency in horticultural and open field agricultural systems were evaluated. In the current study, four different kinds of LLISs were tested against two serious insect pests of horticultural crops, Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), and plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera : Curculionidae). Due to the extensive use of insecticides applied as sprays, both of these pests have developed resistance to all the major categories of insecticides, as well as cross-resistance in *L. decemlineata* to many insecticide classes (Vincent et al., 1999; Alyokhin et al., 2006, 2008; Stankovic et al., 2012; Dader et al., 2015). Therefore, new strategies are urgently needed to control these insect species for economically viable crop production. Using LLISs for insect pest management would be a novel approach in the management of Colorado potato beetle and plum curculio, with this technology providing slow release of the insecticide (e.g., deltamethrin) as well as continuous contact with target insect species. This could lead to bioaccumulation of the active compound in a very short period of time in insects such as *L. decemlineata* larvae and *C. nenuphar* adults. The objectives of this study were to determine the effectiveness of LLISs containing deltamethrin for control of *L. decemlineata* and *C. nenuphar*, to establish the time-efficacy relationship for LLISs containing deltamethrin on *L. decemlineata*, and to determine behavioral effects of LLISs containing deltamethrin on *L. decemlineata* and *C. nenuphar*.

2. Materials and methods

2.1. Rearing and collection of insect species

The experiments were conducted at the Pesticide Alternatives Laboratory, Center for Integrated Plant Systems, Michigan State University, USA. Susceptible (New Jersey, USA) and resistant (Long Island, NY, USA) colonies of *L. decemlineata* were used. The susceptible colony had been reared without any pesticide treatment for more than 20 years. The resistant colony was obtained from an area where insecticides are frequently used and resistant populations to many commercially available insecticides have been reported (Mota-Sanchez et al., 2006).

Field-collected *C. nenuphar* adults were maintained on immature apples at 24 ± 2 °C, 60 ± 10% RH, and 16:8 h L:D photoperiod. The modified methodology of Gökçe et al. (2014) was used for the toxicological and behavioral studies of both of the insects.

2.2. Long-lasting insecticide-incorporated screens

Four types of LLISs (ZeroFly® Screens) and a control screen (without insecticide) were provided by Vestergaard S.A. (Lausanne, Switzerland) and a standard commercial fiberglass insect screen as a second control was sourced locally and used for these experiments. Treatments were as follows: **JLCB061009:** Vestergaard trial batch, 12 × 10 woven polyethylene monofilament, deltamethrin 4 g/kg; **SPSA 6616:** Vestergaard trial batch, 16 × 16 woven polyethylene monofilament, deltamethrin 4 g/kg; **ZeroFly Greenhouse (B):** SPSA 6619-Black, 25 × 50 woven polyethylene monofilament, deltamethrin 4 g/kg; **ZeroFly Greenhouse (G):** SPSA 6619-Grey, 25 × 50 woven polyethylene monofilament, deltamethrin 4 g/kg; **Control A:** ZeroFly Greenhouse white screen, 25 × 50 woven polyethylene monofilament, no insecticide incorporated; and **Control B:** fiberglass insect screen, mesh size 18 × 16, product code FCS8558-M, New York Wire, Hanover, PA, USA.

2.3. Bioassays

2.3.1. Efficacy of LLISs against *Leptinotarsa decemlineata*

2.3.1.1. Contact toxicity

Screens from all treatments were cut to fit in 90-mm petri dishes. Contact toxicity of each LLIS was tested separately against the susceptible and resistant colonies. Ten third-instar *L. decemlineata* larvae were transferred to a petri dish containing a disk of LLIS and then incubated for 72 h at 24 ± 2 °C, 60% RH, and 16:8 h L:D photoperiod. The two control screens were also assessed. Fresh potato leaflets were provided to the larvae every 24 h. Mortality was recorded every 24 h for 72 h. Each of the LLISs and controls were replicated six times.

2.3.1.2. Lethal time evaluation

Time-efficacy studies were carried out with *L. decemlineata* larvae (susceptible colony) using the same methodology. Third-instar larvae were allowed to crawl on the LLIS, and at set time intervals (15, 30, 60, 120, 240, 480, and 960 min) mortality was recorded. Insects were deemed dead if they were unable to right themselves when they were touched with a fine brush (Mota-Sanchez et al., 2006). Each time interval was replicated four times and consisted of the four LLISs and two controls.

2.3.1.3. Behavioral effects

Behavioral effects of LLISs were assessed under laboratory conditions in a choice setup. A fully grown potato leaflet was placed on one side of a 90-mm petri dish and then covered with a semicircle of LLIS half the size of the petri dish. Likewise, the opposite side of the dish was covered by another fully developed potato leaflet and covered with a semicircle of Control A mesh half the size of the petri dish. A 20-mm-wide strip separated the two sides of the dish. A susceptible *L. decemlineata* adult was transferred into each dish and its behaviors (walking, feeding, preening, and resting) were recorded for 10 min using Observer software (Noldus, Wageningen, the Netherlands). After an equilibration time of 10 min, 1-min video recordings were taken every 2 h for 24 h. The experiment was repeated ten times for each treatment.

2.4. Efficacy of LLISs against *Conotrachelus nenuphar*

2.4.1. Contact toxicity

An immature apple was glued to the bottom of a plastic cup (Dart Solo Ultra Clear TP16D, c. 473 mL, Dart Container Corporation, MI, USA) with hot glue. LLIS or control mesh was used to cover the apple preventing it from being eaten, but enabling the plant volatiles to escape through the mesh and attract *C. nenuphar*. In this way, study arenas were set up for each treatment. Ten *C. nenuphar* adults were transferred into each cup. A dental wick, soaked in distilled water, was provided to prevent test insects from dehydration. The insects were incubated for 7 days at 24 ± 2 °C, 60% RH, and 16:8 h L:D photoperiod. The experiment was laid out in a randomized complete block design. Each treatment was replicated six times. Mortalities were recorded every 24 h for 7 days.

2.4.2. Behavioral evaluation

Five immature apples were glued to the bottom of a rectangular plastic container (20 × 25 × 40 cm). Each apple was covered with a different mesh (i.e. four LLISs and Control A), preventing insect damage but allowing attraction of *C. nenuphar* adults. Twenty *C. nenuphar* adults were transferred into each container. The *C. nenuphar* adults were incubated at 26 ± 2 °C, 60% RH, and 16:8 h L:D photoperiod. Adult *C. nenuphar* behavior was monitored over 96 h and their distribution between treatments was recorded. Feeding scars and oviposition marks on each apple were assessed after 96 h. A randomized block design was used for the experiment using each container as a block. The whole experiment was repeated in four consecutive runs.

2.5. Statistical analysis

Results of the efficacy studies for *L. decemlineata* and *C. nenuphar* were corrected for mortality in the control (Control A) using Abbott's formula (Abbott, 1925) and then normalized using arcsine transformation (Zar, 1999). Transformed data were analyzed using analysis of variance (ANOVA) ($P < 0.05$) and Tukey's test ($P < 0.05$). Time-efficacy data were submitted to probit analysis (Finney, 1971) with POLO Plus software to determine LT_{50} and LT_{90} values (LeOra Software, 2002). Behavior (walking, feeding, preening, and resting) of *L. decemlineata* adults was analyzed using the Noldus Observer behavior analysis software (Noldus, 1991). The behavior of *L. decemlineata* was determined from 22 min of observations, and comparisons among treatments were conducted by ANOVA, followed by Fisher's protected least significant difference test. Feeding scars and behavioral data of *C. nenuphar* adults were subjected to the Kruskal–Wallis nonparametric test for biological effects.

3. Results

3.1. *Leptinotarsa decemlineata*

3.1.1. Toxicity

All LLISs tested caused significantly higher mortalities than the two controls in susceptible colonies ($F = 155$; df = 5,12; $P < 0.05$) and mortality rates were 100% for the susceptible larvae in all treatments. However, in the case of the resistant colony, only two types of LLIS, ZeroFly Greenhouse (G) and ZeroFly Greenhouse (B), caused 100% mortality, while JLCB061009 and SPSA 6616 mortalities were 80% and 97%, respectively (Table 1). The mortalities caused by LLISs in resistant *L. decemlineata* larvae were also significantly different from the two controls ($F = 88.8$; df = 5, 12; $P < 0.05$).

3.1.2. Lethal time

Time-efficacy evaluations of *L. decemlineata* with LLISs suggested that larvae died even after limited contact with the screens (Table 2). Among the LLISs, the ZeroFly Greenhouse (B) was the most efficient, killing 50% of the tested population in 30 min. The JLCB061009 screen took the longest time, 51 min, to kill half of the exposed population of *L. decemlineata*.

For LT_{90}, again the ZeroFly Greenhouse (B) screen was the most efficient (Table 2), killing 90 of the exposed *L. decemlineata* population in 53 min. The LT_{90} with ZeroFly Greenhouse (G), JLCB061009, and SPSA 6616 was 86, 83, and 70 min, respectively.

3.1.3. Behavioral effects

Leptinotarsa decemlineata adults spent most of their time on the potato leaflets covered with Control A mesh, about 33% of the time, but only 10% of the time on ZeroFly Greenhouse (B). There were significant differences between the time spent by *L. decemlineata* on the different LLISs ($F = 2.97$; df = 4, 45; $P < 0.05$). The beetle adults spent the most time (23 ± 7.0% of total time) on ZeroFly Greenhouse (G), while on JLCB061009, SPSA 6616, and ZeroFly Greenhouse (B) *L. decemlineata* adults spent 10 ± 4.9%, 12 ± 3.5%, and 14 ± 4.7% of total time, respectively.

Leptinotarsa decemlineata made the maximum number of feeding attempts (1.40 in all observations) on leaflets covered with ZeroFly Greenhouse (B), compared to no feeding attempts with SPSA 6616 mesh (Table 3).

Table 1. Mortality rates of third-instar *Leptinotarsa decemlineata* (susceptible and resistant colonies) exposed to four insecticide-incorporated screens and two control screens for 24 h.

Treatment	Susceptible colony	Resistant colony
	% Mortality ± SEM	% Mortality ± SEM
Control A	3.33 ± 2.35 b*	0.00 ± 0.00 b
Control B	0.00 ± 0.00 b	0.00 ± 0.00 b
JLCB061009	100.00 ± 0.00 a	80.00 ± 11.54 a
SPSA 6616	100.00 ± 0.00 a	96.67 ± 3.33 a
ZeroFly Greenhouse (B)	100.00 ± 0.00 a	100.00 ± 0.00 a
ZeroFly Greenhouse (G)	100.00 ± 0.00 a	100.00 ± 0.00 a

*Means within columns followed by the same letter are not significantly different ($P < 0.05$, Tukey's multiple comparison test).

Table 2. Time-efficacy result of Colorado potato beetle larvae (susceptible colony) exposed to four insecticide-incorporated screens.

Treatment	LT_{50} (min) (95% confidence interval)	LT_{90} (min) (95% confidence interval)	Chi-square*
JLCB061009	50.51 (43.54–58.38)	83.18 (70.15–109.80)	7.98
SPSA 6616	40.05 (34.37–46.82)	70.19 (58.15–95.12)	7.63
ZeroFly Greenhouse (B)	29.57 (25.55–34.68)	53.10 (43.70–73.28)	6.20
ZeroFly Greenhouse (G)	44.1 (37.25–52.19)	86.45 (70.43–119.09)	5.97

*Tabulated chi-square value (df = 10): 18.31 ($P < 0.05$).

Table 3. Mean percentage time spent on the screens and feeding attempts of Colorado potato beetle adult in behavioral tests (n = 10).

Treatment	% time spent (mean ± SEM)	Number of feeding attempts
Control A	32.70 ± 6.96 a*	1.30 a
JLCB061009	13.70 ± 4.72 ab	0.20 a
SPSA 6616	11.51 ± 3.53 ab	0.00 a
ZeroFly Greenhouse (B)	22.93 ± 6.96 ab	0.20 a
ZeroFly Greenhouse (G)	10.22 ± 4.87 b	1.40 a

*Means within columns followed by the same letter are not significantly different ($P < 0.05$, Tukey's multiple comparison test).

However, there was no feeding damage of *L. decemlineata* in all the treatments except JLCB061009, where an average of 0.5 leaflet scars were found after 96 h. The fewer feeding scars on the potato leaflets covered with LLISs could be also be explained by the smaller mesh size, which also provides a physical barrier.

3.2. *Conotrachelus nenuphar*

3.2.1. Toxicity and behavior analysis

Evaluation of the efficacy of LLISs against plum curculio revealed that there were significant differences between treatments (F = 179; df = 5, 30; $P < 0.05$). The ZeroFly Greenhouse (B) screen was the most effective screen tested with 98% mortality. This was followed by JLCB061009 with 33% mortality. The other LLISs, SPSA 6616 and ZeroFly Greenhouse (G), each caused 25% mortality. All *C. nenuphar* adults remained alive in control treatments (Table 4).

Data of the behavioral effects of the LLISs on *C. nenuphar* adults indicated that the screens tested had some behavioral effects on *C. nenuphar* adults. The number of adults observed on the screens was not significantly different among treatments, except after 16 h (H = 7.60; df = 4; $P \leq 0.10$). However, none of *C. nenuphar* adults were observed crawling on the ZeroFly Greenhouse (B) or ZeroFly Greenhouse (G) screens during the incubation period. Additionally, there were no feeding scars on apples covered with these screens, with the exception of JLCB061009, where 0.5 feeding scars were found even after 96 h, but this was not significantly different from the others (H = 6.12; df = 4; P = 0.19). These observations suggest that ZeroFly Greenhouse (B) and ZeroFly Greenhouse (G) screens have distinct behavioral effects on *C. nenuphar* adults that apparently prevent or inhibit ambulation and feeding.

4. Discussion

The need for new and effective strategies to support integrated pest management is clear. LLISs are likely to represent a new control strategy that could be extensively adopted in horticulture and agriculture. The associated high cost, economically and environmentally, of repeated insecticide use is a large issue, in addition to other problems related to chemical control. Particularly in developing countries, small-scale farmers have limited access to good quality synthetic insecticides (Paranagama et al., 2003). In addition to arable farming, livestock production in these countries is at high economic risk due to insect pests such as mosquitoes, stable flies, tsetse flies, and blackflies that are vectors of significant livestock and poultry diseases (Gubbins et al., 2008; Naqqash et al., 2016). Therefore, they urgently need alternative insect control options that do not have environmental and user risks. Treated screens have been shown to be durable and are effective even after a year of exposure to various environmental conditions (Bhatt et al., 2012).

In the current study, the contact toxicity bioassays revealed that LLISs are highly effective in controlling both the Colorado potato beetle and plum curculio, providing >80% mortality in 30 to 50 min. It could be argued that prolonged exposure of insects to deltamethrin could lead to resistance development, but the fast action of LLISs seen in this study as well as the limited ability of *L. decemlineata* larvae and *C. nenuphar* adults to move away from the LLIS, in comparison to mosquitoes, could encourage the use of this technology in agricultural pest management programs. Additionally, continuous exposure of beetles to slow-release deltamethrin could result in rapid bioaccumulation, which in turn can lead to high mortality of *L. decemlineata* larvae and *C. nenuphar* adults as seen in our biotests with mortality of 100% in the resistant colony. The susceptibility of *C. nenuphar* to sodium channel blockers is also well established, so traps incorporated with deltamethrin give satisfactory results (Leskey et al., 2013). Therefore, LLISs could provide an important addition to both integrated pest management and insecticide resistance management programs, and they should be further evaluated for such programs.

Chemical and visual cues in the behavior of insects are linked to a sequence of events that begins with their orientation to the plant from a distance and ends with their establishment on the plant for feeding and oviposition. By interfering with different links along this pathway, contact between the insect and the plant may be prevented (Antignus, 2000). The LLISs evaluated

Table 4. Percentage mortality of *Conotrachelus nenuphar* adults after exposure for 7 days to four insecticide-incorporated and two control screens.

Treatment	% Mortality ± SEM
Control A	1.67 ± 1.67 c
Control B	0.00 ± 0.00 c
JLCB061009	33.33 ± 4.94 b
SPSA 6616	25.20 ± 2.23 b
ZeroFly Greenhouse (B)	98.33 ± 1.67 a
ZeroFly Greenhouse (G)	25.00 ± 2.23 b

*Means within columns followed by the same letter are not significantly different ($P < 0.05$, Tukey's multiple comparison test).

produced a high level of behavioral effects on both species, reducing feeding scars to none with all but one LLIS. In the behavioral tests, *L. decemlineata* adults spent significantly less time on LLISs compared to the controls, which indicates that LLISs can have repellent effects on *L. decemlineata* adults. This repellence can be attributed to the deltamethrin incorporated into the LLISs. Similar repellent activity of LLISs against mosquitoes was reported by Kawada et al. (2014), who tested permethrin and deltamethrin repellency and reported that both gave significant repellence of *Anopheles* spp.

The behavioral tests' results also showed that the color of the LLIS can be important for feeding site selection of the insects tested. *Leptinotarsa decemlineata* is a diurnal insect that strongly relies on visual cues to guide its walk. It can be repelled by relatively dark colored mesh due to its positive phototactic behavior when stimulated with different wavelengths of light (Otálora-Luna and Dickens, 2011). In our studies, different preferences of *L. decemlineata* to LLISs were observed. Although the Colorado potato beetle spent more time on the black LLIS in our study, they made few attempts to feed on the covered leaflets, and this behavior could be attributed to the visual clues of the leaf rather than the insecticidal activity of the screens. *Conotrachelus nenuphar* is a nocturnal insect (Chouinard et al., 2002), active after sunset. It seems that the color of the LLISs tested did not cause any behavioral response in *C. nenuphar* as they were not affected by the dark colored mesh screens. These results are in accordance with those of Prokopy and Wright (1998), who reported that *C. nenuphar* adults climb black pyramid traps in the field. Different colors of traps have been utilized successfully as reflectors or repellents to insects (Harrison, 1984; Csizinski et al., 1995). Many insect species, e.g., aphids and whiteflies, are attracted to yellow (Chu et al., 2004). Additionally, light is as important as color in regards to an insect's search for an acceptable host plant (Otálora-Luna and Dickens, 2011). Therefore, prior to using LLISs in any control program, the color preferences of the target species should be evaluated for achieving the best possible control.

Further studies with LLISs should be conducted to determine their full potential in the control of important insect pests and the extent to which they can be used to reduce insecticide applications to horticultural and agricultural crops. The current studies were carried out under laboratory conditions, eliminating many environmental factors, e.g. rain, wind, and sunlight, but these factors are known to effect both the behavior of organisms and the chemical and physical characteristics of LLISs. Therefore, testing these LLISs under field conditions would help to examine the interaction of LLISs with tested organisms as well as abiotic factors.

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