

Susceptibility of the adult eye gnat *Liohippelates collusor* (Diptera: Chloropidae) to neonicotinoids and spinosad insecticides

Yongxing Jiang[✉] and Mir S. Mulla

Department of Entomology, University of California, Riverside, CA 92521, U.S.A.

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ABSTRACT: Imidacloprid, thiamethoxam (first and second generation neonicotinoid insecticides), and spinosad (a naturally derived biorational insecticide) were evaluated in the laboratory against adult eye gnats, *Liohippelates collusor* (Townsend), using two modes of exposure. Ingestion experiments revealed that toxicity was the highest for thiamethoxam (Platinum™ soluble concentrate) followed by technical thiamethoxam, imidacloprid (Admire® 2 flowable concentrate), and spinosad (Success* soluble concentrate). When incorporated into 5% sucrose solutions, eye gnat mortality was significantly increased compared to the same concentrations of aqueous insecticide solutions. Contact toxicity experiments revealed that thiamethoxam formulation (Platinum) was the most toxic, followed by technical thiamethoxam, imidacloprid, and spinosad. Increasing the duration of exposure time from 15 min to 30 min significantly increased adult eye gnat mortality, but no further mortality occurred by extending exposure from 30 min to 60 min. Our results suggest that Platinum has good ingestion and contact activity against adult eye gnats. Admire and Success have appreciable ingestion toxicity but low contact activity against adult eye gnats. *Journal of Vector Ecology* 31 (1): 65-70. 2006.

Keyword Index: Susceptibility, imidacloprid, thiamethoxam, spinosad, ingestion toxicity, contact toxicity.

INTRODUCTION

Liohippelates eye gnats are readily attracted to man and animals, where they feed on mucous membranes and other parts of the body. Due to their persistent hovering and landing about the face, particularly the eyes, these pests cause a great deal of annoyance to their hosts (Mulla and Stains 1977). In addition to being highly pestiferous, they are known to be potential vectors of numerous infectious diseases such as mastitis in lactating cows (Sanders 1940), yaws (Kumm 1935), and bacterial conjunctivitis (Dawson 1960).

Liohippelates collusor (Townsend), the predominant species of eye gnats in many parts of the southwestern United States and northern Mexico, develops mainly in cultivated agricultural land with loose and friable soils that receive moisture from frequent irrigation or rains in warm weather. Normal agricultural practices such as tillage, irrigation, and disking under of cover crops and weeds provide ideal conditions for larval development. Currently, populations of these pests prevail in large numbers in some agricultural, residential, and recreational areas of the Coachella Valley and other valleys in southern California. Adult eye gnats, when not actively flying, rest on the ground and in or on lower portions of vegetation, weeds, and row crops. Due to their resting behavior, it is possible to control adult eye gnats in some habitats by applying residual insecticides to resting sites or use baits (attractants) containing toxicants. Therefore, evaluating new insecticides, especially those labeled for use in agricultural crops, is desirable, because insecticides used for crop pest control can impact eye gnats and reduce their numbers.

In recent years, two new classes of insecticides,

neonicotinoids, and spinosad (a natural product from soil actinomycete bacterium *Saccharopolyspora spinosa*) have become available for the control of a variety of insect pests in agriculture (Adan et al. 1996, Hu and Prokopy 1998, Liburd et al. 1999, Thompson et al. 1997). These insecticides possess a unique mode of action involving the nicotinic acetylcholine and GABA receptors (Salgado 1998). These insecticides also have low mammalian toxicity with low persistence in the environment (Thompson et al. 1997) as well as low toxicity to a number of predatory insects (Liu et al. 1999). Because it is very costly to develop and launch a new product for the control of public health insects, it is desirable to consider those control agents and products for use in public health that already have labels and established uses for insect control in agriculture. These two classes of insecticides meet these criteria. Moreover, these insecticides have not been evaluated against eye gnats.

Therefore, studies were initiated to determine the activity (both contact and ingestion) of several formulations of neonicotinoids and spinosad against adult eye gnats in the laboratory. The data gathered from the tests will provide valuable information that may form a basis for the potential use of these active ingredients for the control of eye gnat populations in the future.

MATERIALS AND METHODS

Eye gnat colony

Adult eye gnats from a laboratory colony established from wild populations collected in the Coachella Valley, CA in 1960 were maintained at the University of California, Riverside and used in the tests. To broaden the gene pool, the colony

Table 1. Ingestion toxicity of insecticides to adult eye gnats (*L. collusor*) fed on aqueous insecticide solutions without sucrose for 1-h and 24-h mortality.

Insecticide (Formulation AI)	Lethal conc. (mg AI/L)		Slope
	LC ₅₀ (95% CL)	LC ₉₀ (95% CL)	
Thiamethoxam			
Platinum (SC 21.6%)	29.9 (18.4-50.8)	362.5 (256.7-560.9)	1.2
thiamethoxam (Tech. Material 99%)	34.4 (15.1-67.5)	435.6 (270.3-890.1)	0.9
Imidacloprid			
Admire (FC 21.4%)	150.6 (135.7-437.6)	634.8 (415.5-896.7)	2.9
Spinosad			
Success (SC 22.8%)	213.7 (136.2-370.5)	698.7 (393.8-954.6)	2.5

was supplemented with wild eye gnats at various times. The colony was maintained according to the procedures of Mulla (1962).

Insecticides

Four formulations and products were tested in this study. Admire[®] 2, (21.4% AI), a flowable concentrate formulation of imidacloprid, was provided by Bayer Corporation, Kansas City, MO. Technical material of thiamethoxam (99% AI, Lot: 322-86A) was provided by Chem. Service, Inc., West Chester, PA. Platinum[™] (21.6% AI), a soluble concentrate (SC) of thiamethoxam, was provided by Syngenta Crop Protection, Inc., Greensboro, NC. Success* (22.8% AI), an SC formulation of spinosad, was provided by Dow Agrosciences LLC, Indianapolis, IN.

Ingestion activity of insecticides against adult eye gnats

One-h feeding test: Stock solutions (0.1%) and serial dilutions of all insecticides were prepared in distilled water. Four concentrations in the activity range of each insecticide were evaluated and distilled water was used as a control in each test. Each concentration and control was replicated four

times. For feeding trials 2.5 ml volumes of test solutions were pipetted into 15 ml glass vials and then plugged by a piece of dental cotton roll from which the eye gnats could imbibe the solution. Prior to plugging the glass vial, each cotton roll was moistened with the same concentration of prepared dilution. Each vial with the aqueous solution was inverted through a hole on the top of a plastic chamber, thereby providing a continuous supply of test solution to the eye gnats. The neck of each glass vial was wrapped with a piece of stretched parafilm to hold the vial firmly in the hole. Chambers used for this test were made from a 40-dram plastic vial vented with a screen window on the side and a hole on the top to access the glass vial. The plastic chamber was placed upside down so that its cap served as the base of the chamber (Figure 1). Adult gnats, in groups of 100-150 at a time, were anesthetized with carbon dioxide and 25 2- to 3-d-old mixed sexes were aspirated into each chamber. At the end of a 1 h exposure period, each glass vial with test solution was replaced by a new glass vial containing 5% sucrose solution (without insecticide) and plugged with a piece of dental cotton roll as a wick, providing a continuous supply of food for adult eye gnats during the 24 h holding period. Gnats were then held at

Table 2. Ingestion toxicity of insecticides to adult eye gnats (*L. collusor*) fed for 24 h on 5% sucrose aqueous solution, mortality read at the end of feeding.

Insecticide (Formulation AI)	Lethal conc. (mg AI/L)		Slope
	LC ₅₀ (95% CL)	LC ₉₀ (95% CL)	
Thiamethoxam			
Platinum (SC 21.6%)	0.4 (0.3-0.7)	1.0 (0.7-3.7)	3.3
thiamethoxam (Tech. Material 99%)	0.5 (0.3-0.9)	2.1 (1.1-22.3)	2.0
Imidacloprid			
Admire (FC 21.4%)	6.2 (5.4-7.1)	19.3 (15.9-24.9)	2.6
Spinosad			
Success (SC 22.8%)	13.1 (10.7-14.9)	20.0 (17.2-29.4)	5.0

Table 3. Contact toxicity of insecticides to adult eye gnats following 1-h exposure to insecticide deposits in flasks as assessed at 24-h mortality.

Insecticide (Formulation AI)	Lethal dosage (mg AI/cm ²)		Slope
	LD ₅₀ (95% CL)	LD ₉₀ (95% CL)	
Thiamethoxam			
Platinum (SC 21.6%)	0.002 (0.001-0.006)	0.085 (0.021-3.221)	0.82
thiamethoxam (Tech. Material 99%)	0.034 (0.009-0.126)	1.557 (0.956-25.321)	0.78
Imidacloprid			
Admire (FC 21.4%)	>0.08	-	-
Spinosad			
Success (SC 22.8%)	>0.08	-	-

28-30° C ambient temperature and a photoperiod of 14: 10 (L: D). Mortality was recorded 24 h post-exposure to the toxicants. The criterion of death was inability to move.

Effect of phagostimulant (sucrose): In order to compare the ingestion activity of insecticides, with or without the phagostimulant sucrose, adult eye gnats were exposed to the same concentration of insecticides as mentioned earlier in 5% sucrose and aqueous solution for 1 h and mortality was recorded at 24 h post-exposure. The insecticide concentration used in this test was the LC₅₀ level of each insecticide found in the above studies (Table 1) based on 1 h exposure. Each concentration was replicated four times, using a total of 100 eye gnats per concentration. A sucrose solution (5%) without insecticide was used as a control.

Prolonged feeding: To test the effects of a longer feeding period, adult eye gnats were fed on 5% sucrose-insecticide solution for 24 h, and mortality was assessed immediately after the end of the feeding period. The same methodology as the 1 h exposure test was used in this experiment. Sucrose solution (5%) without insecticide was used as a control.



Figure 1. Chambers used for eye gnat feeding on aqueous solutions of insecticides in water with or without 5% sucrose.

Contact activity of insecticides against adult eye gnats

Activity of insecticide deposits in flasks against adult eye gnats: The methodology used in this test closely followed that established by Georghiou and Mulla (1961). Stock solution (0.1%) of thiamethoxam (technical material) and serial dilutions were prepared in acetone; Stock solutions (0.1%) of Admire, Platinum, Success, and serial dilutions were prepared in distilled water on a weight/volume basis. One ml of appropriate strength solution was pipetted into 25 ml Erlenmeyer flasks and the solvent evaporated to dryness under a gentle stream of air for 60 min. Only the bottom of the flask was covered by the solutions leaving insecticide deposit after evaporation of the solvent. The treated area was calculated to measure 12.38 cm². Twenty-five adult eye gnats anesthetized with CO₂ were aspirated into each treated flask. After addition of the eye gnats, the mouth of each flask was covered with thin muslin cloth held in place by a rubber band and inverted through a hole in a wooden rack. The rack was set up in a box equipped with two overhead fluorescent lamps (Figure 2, Georghiou and Mulla 1961). In the flasks eye gnats, being negatively geotropic and positively heliotropic, move up in the flask crawling or resting on the area of insecticide deposits. At the end of a 1-h exposure period the gnats in each flask were anesthetized with CO₂ and transferred into a 75 ml glass squat jar with a 50-mesh screen porthole in the cap. A 15 ml glass vial filled with 5% sucrose solution was inverted through a hole in the screen and the glass vial was plugged with a piece of dental cotton roll, providing a continuous supply of food and moisture for eye gnats. Gnats in jars were then held in a room at 28-30° C ambient temperature with a photoperiod of 14: 10 (L: D). Gnat mortality was recorded at 24 h post-exposure. Four concentrations of each insecticide were evaluated and tests were repeated four times. Acetone was used as a control for the technical thiamethoxam test, while distilled water was used as a control for the other materials.

Exposure period and contact toxicity of insecticides: Results obtained from the above contact test using a 1-h exposure period indicated that Admire and Success had low contact toxicity to adult eye gnats, thus, only Platinum and technical thiamethoxam were tested in this study. Experimental

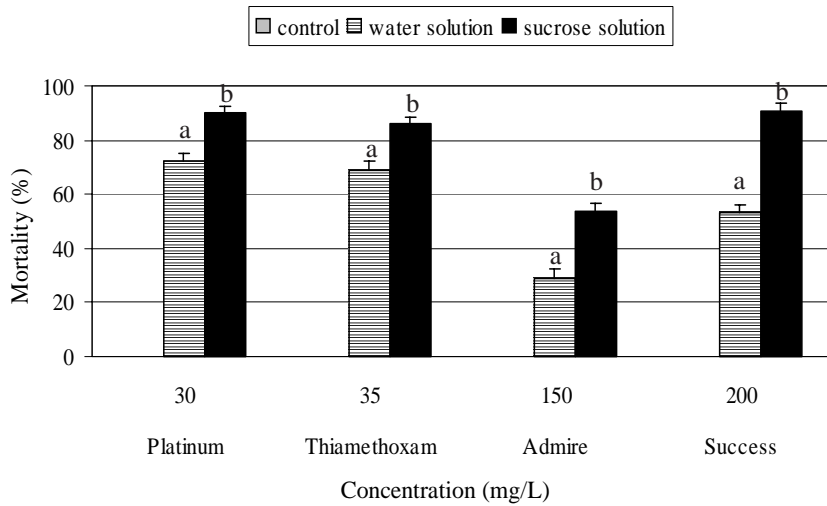


Figure 2. Mortality at 24 h post-exposure of adult eye gnats fed 1 h on water-insecticide or 5% sucrose-insecticide solution. Different letters represent significant differences at 0.05 level by Chi-square test.

and evaluation procedures were similar to those used in testing the activity of deposits of insecticide in flasks against eye gnats above. One ml of 0.1% Platinum and thiamethoxam solution was pipetted into the bottom of a 25-ml Erlenmeyer flask. Twenty-five (2- to 3-d-old mixed sexes) adult eye gnats per flask were exposed to deposits in inverted flasks and exposed for 15 min, 30 min, and 60 min at $28 \pm 1^\circ \text{C}$. At the end of each exposure period gnats in each flask were anesthetized again with CO_2 and transferred into a 75-ml squat jar. Sucrose solution (5%) was provided as food to eye gnats as in the previous test and held under the same conditions as mentioned above. Mortality was recorded at 24 h post-exposure.

Statistical analysis

For the dose-mortality response, data were subjected to probit regression analysis using POLO-PC (LeOra Software 1987) to obtain LC_{50} and LC_{90} , their 95% confidence limit, and slope values. The 95% confidence limit values were used to assess the significant differences between test materials. If the 95% confidence limits overlapped each other, it indicated

that there were no significant differences between the two tested materials. Data collected from 1 h exposure using insecticides in water and sucrose solution experiments were compared by chi-square test (Statistix 8[®] Analytical Software 2004), as well as the data collected from exposure periods of the contact activity experiment.

RESULTS AND DISCUSSION

Ingestion activity of insecticides against adult eye gnats

Mortality associated with the ingestion of aqueous solutions was greatest for thiamethoxam formulation (Platinum) followed by technical thiamethoxam, imidacloprid (Admire), and spinosad (Success) (Table 1). During the 1-h feeding period, no quick knockdown response (in 3-5 min) was observed. However, about 40-45% of eye gnats fed on high concentrations of thiamethoxam formulation (Platinum) and technical thiamethoxam became sluggish, resting at the bottom of the test chambers, either dying or sitting still at the end of the 1-h feeding period. In contrast, only 10-15% of eye gnats behaved similarly when fed on the same

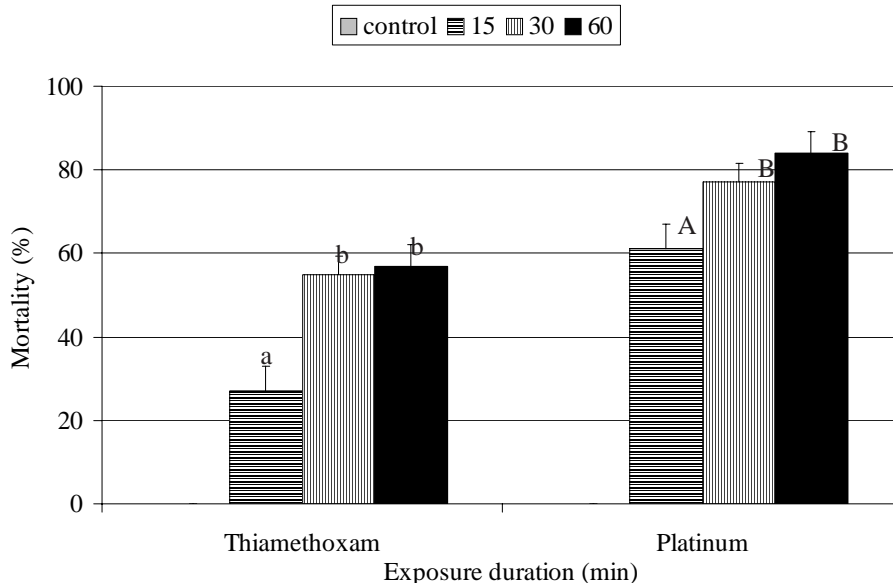


Figure 3. Contact activity of adult eye gnats exposed to thiamethoxam (technical and Platinum SC formulation) as deposits in flask for different periods. Different letters represent significant differences at 0.05 level by Chi-square test.

concentrations of imidacloprid (Admire) and spinosad (Success).

Offering the insecticides in sucrose solution yielded higher mortality in the eye gnats than the insecticides offered as water solutions (Figure 2). Mortality was significantly greater for eye gnats fed on 5% sucrose-insecticide solution than for eye gnats fed on aqueous insecticide solution alone for all the test materials ($P < 0.05$). Data presented here indicate that the addition of sucrose increased the intake and effectiveness of each insecticide in killing eye gnats. Feeding stimulants have been reported to effectively increase the efficacy of imidacloprid against *Rhagoletis pomonella* (Walsh) (Hu and Prokopy 1998), thiamethoxam against *Rhagoletis mendax* (Curran) (Ayyappath et al. 2000).

Eye gnats that fed at various concentrations of insecticides in 5% sucrose solutions continuously for 24 h showed a high level of toxicity as expected (Table 2). The level of toxicity of thiamethoxam formulation (Platinum), technical thiamethoxam, imidacloprid (Admire), and spinosad (Success) when fed upon 24 h was 75, 69, 25, and 16 times greater at LC_{50} , respectively, than that of 1 h feeding on aqueous insecticide solutions without sucrose. At the LC_{90} the toxicity was 362, 207, 33, and 35 times more, respectively, than that of 1 h feeding on aqueous insecticide solutions without sucrose. The addition of a phagostimulant, sucrose and extended feeding period are factors to contribute substantially the increase in activity of insecticides against eye gnats.

Contact activity of insecticides against adult eye gnats

The toxicity of insecticide deposits in flasks against adult eye gnats is presented in Table 3. Since the contact activity of imidacloprid (Admire) and spinosad (Success) was too low (Admire yielded 10% contact mortality and Success obtained lower than 20% mortality at the highest dosage of 0.08 mg AI/cm²), only the formulation of thiamethoxam (Platinum) and technical thiamethoxam LD_{50} and LD_{90} are presented (Table 3). Based on the nonoverlap of 95% CL, thiamethoxam formulation (Platinum) was significantly more toxic than technical thiamethoxam. Low level of knockdown of adult eye gnats (3-5%) was seen as early as within 15 min of exposure to the highest dosages (0.08 mg/cm²) of thiamethoxam formulation (Platinum) and technical thiamethoxam.

The relationship between exposure period and mortality was studied by exposing adult eye gnats to insecticide deposited flasks for 15, 30, and 60 min. Overall, mortality increased ($P < 0.05$) as duration of exposure increased (Figure 3). Greater mortality was observed when the exposure period increased from 15 to 30 or 60 min for both thiamethoxam formulation (Platinum) and thiamethoxam technical ($P < 0.05$), but there was no significant increase in mortality from 30 to 60 min. Relationship between exposure period and mortality was reported in the studies of thiamethoxam and imidacloprid against *Rhagoletis mendax* (Ayyappath et al. 2000). When adult *R. mendax* was exposed to thiamethoxam and imidacloprid-coated red spheres, *R. mendax* mortality increased as exposure duration increased from 10 to 60 s.

From these studies, it is clear that the insecticides

imidacloprid, thiamethoxam, and spinosad have a high level of toxicity to adult eye gnats upon ingestion of insecticide in sucrose solution. Thiamethoxam also showed appreciable contact activity against eye gnats. Therefore, the potential use of these insecticides for controlling adult eye gnats in the future could be through a combination of insecticide/bait/feeding stimulants or by applying insecticides to the resting sites where eye gnats prevail. Because adult eye gnats move around constantly, use of baits (attractants) along with phagostimulants and insecticides will be more appropriate for the control of these insects. The bait-insecticide mixture could be sprayed on spots or over a fraction of the eye gnat-infested habitats. However, the practical effectiveness of these compounds has yet to be determined under field conditions and with different application methods.

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