Novel Phenoxy Juvenile Hormone Analog (Pyriproxyfen) Suppresses Embryogenesis and Adult Emergence of Sweetpotato Whitefly (Homoptera: Aleyrodidae)

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ABSTRACT The juvenoid compound pyriproxyfen is a potent suppressor of embryogenesis and adult formation of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). Dipping of cotton seedlings infested with 0-1-d-old eggs in 0.1 mg (AI)/liter resulted in total suppression of egg hatch. Older eggs were affected to a lesser extent. Exposure of *B. tabaci* females for 48 h to cotton leaves treated with 5 mg (AI)/liter pyriproxyfen resulted in oviposition of infertile eggs. This effect persisted for an additional 2 d after transfer of the females to untreated leaves and continued, although to a lesser extent, for up to 8 d. Treatment of second instars with 0.04-5 mg (AI)/liter pyriproxyfen resulted in normal development until the pupal stage; however, adult emergence was totally suppressed. At similar concentrations, the suppression of emergence from treated third instars was 91-100%. Second instars exposed to 5 mg (AI)/liter pyriproxyfen excreted honeydew at a level similar to the control until the fourth instar (pupation), after which a strong reduction was observed. Inhibition of egg hatch on the lower surface of cotton leaves was observed when their upper surface was treated with 1-25 mg (AI)/liter, indicating a pronounced trans laminar effect. These findings indicate that pyriproxyfen is an efficient control agent for *B. tabaci;* the chemical has a strong translaminar effect and acts on all stages of this pest.

KEY WORDS *Bemisia tabaci,* pyriproxyfen, juvenile hormone analog

PYRIPROXYFEN, 2-[I-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine, is a potent juvenile hormone analog ORA) that acts on household pests such as houseflies, *Musca domestic a* L. (Itaya 1987), cockroaches, *Blattella germanica* (L.) (Kawada 1988, Koehler & Patterson 1991), and the tsetse fly, *Glossina* spp. (Langley 1990), on orchard pests such as the codling moth, *Cydia pomonella* (L.), and the oriental fruit moth, *Grapholita molesta* (Busch), and on the citrus scales *Aonidiella aurantii* (Meskell) and *Ceroplastes jloridensis* Comstock (Peleg 1988). Pyriproxyfen affects the hormonal balance in insects. In some cases, this JHA causes a strong suppression of embryogenesis, metamorphosis, and adult formation (Itaya 1987, Kawada 1988, Langley 1990).

Preliminary field trials done by the Israel Cotton Board during the past 2 yr indicated that pyriproxyfen exhibited high potency on the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), an important pest in cotton and vegetable crops. Whiteflies such as B. *tabaci* and *Trialeurodes vaporariorum* (Westwood) are among the most important economic cosmopolitan pests attacking cotton, vegetables, and ornamentals (Gerling 1990, Byrne & Bellows 1991). These homopteran insects damage crops by extracting large quantities of phloem sap which can result in >50% yield reduction (Lloyd 1922). The honeydew excreted by these pests serves as a medium for sooty mold fungi that discolors parts of the plants used for food and reduces the quality of cotton fiber used for textiles (Perkins 1987, Byrne & Bellows 1991). In some cases, B. *tabaci* transmit viruses and are considered limiting factors for growing agricultural crops (Muniyappa 1980). Our research was done to evaluate the biological activity of pyriproxyfen on the developing stages of B. *tabaci.*

Materials and Methods

Chemicals. Pyriproxyfen 10% emulsion concentrate (Tiger 10 EC) obtained from Agan Manufacturers, Ashdod, Israel, was used in all assays. The test concentrations were prepared by dilution with deionized water.

Rearing and Bioassays. Sweetpotato whiteflies were reared on cotton seedlings ('Acala' SJ2) under standard greenhouse conditions at 26 ± 2°C (Ishaaya et a1. 1988, Horowitz & Ishaaya 1992). The strain was collected from an Israeli cotton field in 1987 and since then has not been treated with pesticides. Cotton seedlings 20-25 em tall were dipped in various concentrations of

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pyriproxyfen or with deionized water (control). The plants were allowed to dry for 2 h. Twenty B. *tabaci* females confined in leaf cages (Ishaaya et al. 1988) were exposed to treated plants for 48 h and kept under controlled conditions of 26 ± 2°C, 60 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Adult mortality (after 48-h exposure), oviposition, and egg hatch were determined. In other tests cotton seedlings infested with eggs or larvae were treated with various concentrations of pyriproxyfen. Effects on egg hatch, pupation, or emergence were determined.

Transovarial Effect of Pyriproxyfen. Twenty females confined in leaf cages were exposed for 48 h to treated cotton seedlings. Effect on egg hatch was determined at various periods after the transfer of the females to untreated plants.

Translaminar Effect of Pyriproxyfen on Egg Viability. Leaves of cotton seedlings treated at their upper surface with various concentrations of pyriproxyfen were exposed at their lower surface to B. *tabaci* females. Aqueous solutions (50 IL) containing various concentrations of pyriproxyfen were spread uniformly on 7.6-cm^2 circles of upper leaf surface with a calibrated micropipetter. The leaves were allowed to dry for 2 h. Twenty females were then confined in leaf cages and were exposed to the lower surface of the leaf opposite the treated area for 48 h. The viability of the eggs oviposited during this period or during additional periods after the transfer of the females to untreated leaves was determined.

Vapor Phase Effect of Pyriproxyfen. Cotton seedlings were dipped in 5 and 25 mg (AI)/liter and allowed to dry for 2 h. Untreated leaves infested with 0-1-d-old B. *tabaci* eggs were kept at a distance of 2, 4, and 6 cm from treated leaves as described previously by De Cock et al. (1990). For control bioassays, infested leaves and untreated leaves were used. Egg hatch was then determined. Each treatment was done with five replicates of 50-100 eggs each.

Effect of Pyriproxyfen on Honeydew Secretion. Cotton seedlings infested with second-instar B. *tabaci* were treated with various concentrations of pyriproxyfen or with deionized water (control). Secretion of honeydew drops was then determined based in part on the method of Melamed-Madjar et al. (1983). A water-sensitive paper (Ciba-Geigy) was placed horizontally 3 mm beneath the infested leaves, and the number of honeydew drops secreted per individual during 30 min was determined at various periods during the larval and the pupal stage.

Statistical Analysis. All results were subjected to one-way analysis of variance (ANOVA), and means were separated by Scheffe's multiple range test (P = 0.05) (Day & Quinn 1989). Angular transformation for percentage egg hatch was done before the statistical analysis. POLO-PC (LeOra Software 1987) was used to estimate probit regression.

Table 1. Effect ofpyriproxyfen on 0-I-d-oldB. *tabaci* eggs

Concn, mg (AI)/liter	No. eggs	% Egg hatch
Untreated	251	73 ± 7a
0.02	222	21 ± 2b
0.10	345	2 ± 1c
0.50	355	2 ± 1c
2.50	249	0c

Values followed by the same letter do not differ significantly (P = 0.05; Scheffe's test [Day & Quinn 1989]). Angular transformation for percentage egg hatch was done before the statistical analysis. Data are averages (±SEM) of five or six replicates of 30-110 eggs each.

Results and Discussion

Ovicidal and Transovarial Activity of Pyriproxyfen. A strong ovicidal effect of pyriproxyfen was observed when cotton seedlings infested with 0-1-d-old eggs of B. *tabaci* were dipped in various concentrations of pyriproxyfen (Table 1). Egg hatch was suppressed 71 and 97% by concentrations of 0.02 and 0.1 mg (AI)/liter, respectively. Eggs 2-3 d old were less susceptible, and older ones were not appreciably affected by pyriproxyfen (Fig. 1). Thus, pyriproxyfen exerted its effect only on very young eggs. These results are similar to those obtained with eggs of *Spodoptera littoralis* (Boisduval) (Ascher & Eliyahu 1988). When B. *tabaci* females were exposed for 48 h to cotton plants treated with 0.5 mg (AI)/liter pyriproxyfen, adults were not affected, but eggs oviposited during this period were not viable (Fig. 2). Egg hatch continued to be totally suppressed for an additional 48 h after

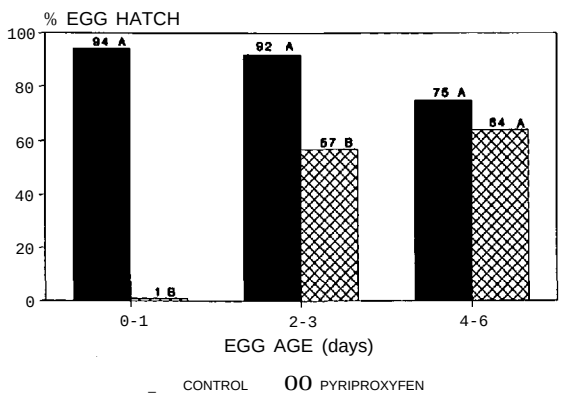

Fig. 1. Effect of pyriproxyfen on viability of eggs of different ages. Cotton seedlings infested with eggs of different ages were dipped in 0.5 mg (AI)/liter pyriproxyfen. Data are averages of five replicates of 150-350 eggs each. Within each period, figures followed by the same letter do not differ significantly (P = 0.05; Scheffe's test [Day & Quinn 1989]).

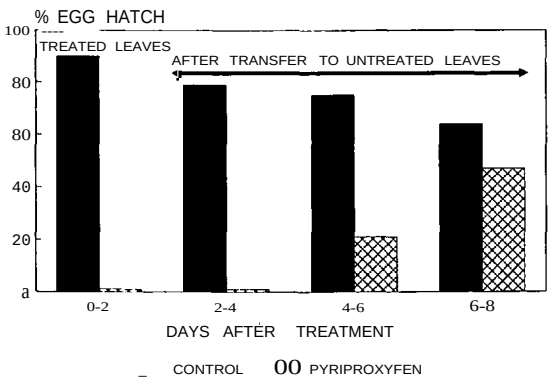

Fig. 2. Residual effect of pyriproxyfen through *B. tabaci* females (transovarial effect) on egg hatch. Five replicates of 20 females confined in leaf cages were exposed for 48 h to cotton seedlings treated with 5 mg (AI)/liter, then transferred for various additional periods to untreated plants. Viability of eggs obtained from treated females were Significantly different (P = 0.05; Scheffe's test [Day & Quinn 1989]) from the untreated females in all determinations.

the transfer of the females to untreated leaves' egg viability was reduced considerably for up t~ 6 d (Fig. 2). These results, i.e., suppression of egg viability for a relatively long period after a brief exposure of B. *tabaci* females to pyriproxyfen, indicate trans ovarial activity and are of practical importance. The egg mortality curve obtained after exposure of females to various concentrations of pyriproxyfen indicates that the LC_{50} and LC_{90} values were 0.026 and 0.049 mg (AI)/liter, respectively (Fig. 3).

Our results agree with the general concept that JHAs disrupt embryogenesis when treatments are done before blastokinesis; i.e., before the differentiation of the first instar (Staal 1975). Disruption of embryogenesis by JHAs has been

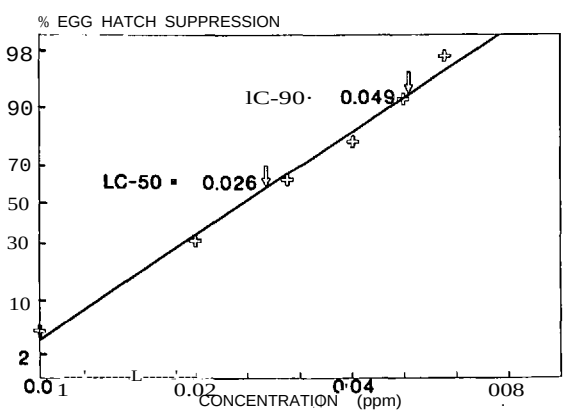

Fig. 3. Probit regressions of egg hatch when *B. tabaci* females were treated with various concentrations of pyriproxyfen. Number of eggs tested, 3,250; slope ± SE, 4.58 ± 0.19; LC_{50} (CL), 0.026 (0.024-0.027); LC_{90} (CL), 0.049 (0.046-0.053).

Table 2. Translaminar effect of pyriproxyfen on B. *tabaci* eggs

Concn, mg (AI)/liter	Oviposition on treated cotton leaves		Oviposition after transfer to untreated leaves	
	No. eggs	% Egg hatch	No. eggs	% Egg hatch
Untreated	533	84 ± 4a	841	92 ± 3a
1	355	1 ± Ib	583	88 ± 2a
5	774	Ob	339	6 ± Ib
25	501	Ob	245	7 ± Ib

Within columns, values followed by the same letter do not differ significantly (F = 0.05; Scheffe's test [Day & Quinn 1989]). Angular transformation for percentage egg hatch was done before the statistical analysis. Data are averages (±SEM) of five replicates of 20 females each. Leaves were treated on the upper surface, and females were introduced for 48 h to oviposit on the lower surface. They were then transferred to untreated leaves for an additional 48 h of oviposition. Egg viability of the two periods was detennined.

demonstrated in various groups of insects such as Lepidoptera (Riddiford 1972, 1974; Ascher & Eliyahu 1988), Coleoptera (Walker & Bowers 1970) and Homoptera (Nassar et al. 1973, Ascher & Eliyahu 1988). Pyriproxyfen was the most potent sterilizing agent among JHAs for tsetse flies when females were exposed to the compound (Langley 1990). Treatment of adults of the B. *germanica*, with pyriproxyfen resulted in suppression of ovary growth, immature oothecae, and nonviable eggs (Kawada 1988).

Translaminar Effect of Pyriproxyfen. We observed a strong translaminar effect of pyriproxyfen; treatment with relatively low concentrations (1,5, and 25 mg [AI]/liter) of pyriproxyfen on the upper surface of cotton leaves totally suppressed egg hatch of females present on the lower surface (Table 2). Pyriproxyfen affected egg viability for an additional period after the females were transferred to untreated leaves (Table 2). This effect may have important practical implications under field conditions for controlling sweetpotato whiteflies on the lower surface of the leaves. In usual field sprays, the compound does not reach, in sufficient quantities, the areas of the leaves where whitefly eggs and larvae are present.

No appreciable vapor phase toxicity of pyriproxyfen was observed in bioassays carried out according to a procedure described previously (De Cock et al. 1990). Egg fertility was not affected when cotton leaves infested with O-I-d-old eggs were placed at distances of 2 and 4 cm from leaves treated with 5 and 25 mg (AI)/liter pyriproxyfen.

Effect of Pyriproxyfen on the Larval Stage and on Honeydew Secretion. Pyriproxyfen had no direct effect on the larval stage (Table 3). Second and third instars treated with 0.04-1.00 mg (AI)/liter pyriproxyfen pupated at a level similar to that of the untreated control. However, total suppression of adult emergence occurred in bioassays with second instars and >90% suppression

Table 3. Effect of pyriproxyfen on pupation and emergence of *B. tabaci* treated at the second or third instal'll

Concn, mg (AI)/liter	Treatment of second instar			Treatment of third instar		
	n	% Pupation	% Emergence	*n*	% Pupation	% Emergence
Untreated	1,014	96 ± la	95 ± 2a	877	94 ± 2a	97 ± la
0.008	—	—	—	714	97 ± la	38 ± 2b
0.040	455	95 ± 2a	Ob	469	95 ± 2a	9 ± 2c
0.200	1,191	92 ± 2a	Ob	681	94 ± 2a	5 ± 2c
1.000	1,153	95 ± 2a	Ob	558	96 ± la	7 ± 3c
5.000	489	94 ± 2a	Ob	648	95 ± 2a	Oc

Within columns, values followed by the same letter do not differ significantly (P = 0.05; Scheffe's test [Day & Quinn 1989]). Angular transfomlation for percentage pupation and adult emergence was done before the statistical analysis. Data are averages (±SEM) of 5-10 replicates of 80-140 larvae each.

was observed in tests with third instars (Table 3). Honeydew secretion obtained from second instars treated with 5 mg (AI)/liter pyriproxyfen was not significantly affected during the first 3 d after application but was strongly affected after 7 d (Fig. 4). The reduction in honeydew secretion on days 7 and 10 probably resulted from pupal mortality (Table 3). These findings suggest an indirect effect of pyriproxyfen on the larval stage that results in a strong reduction of honeydew secretion during the pupal stage and almost complete suppression of adult formation. B. *tabaci* collected from cotton fields showed susceptibility to pyriproxyfen similar to that of the laboratory strain (unpublished results), indicating the possible use of this compound against strains resistant to conventional insecticides.

In other studies, pyriproxyfen was a potent inhibitor of mosquito developmental stages, acting as a JHA mimic, thereby suppressing adult formation. In general, mortality occurred at the pupal stage; at low concentrations, abnormal adults formed (Schaefer et aI. 1988). Dietary concentrations of 1 and 5 mg (AI)/liter pyriproxyfen resulted in >80% inhibition of eclosion of adult

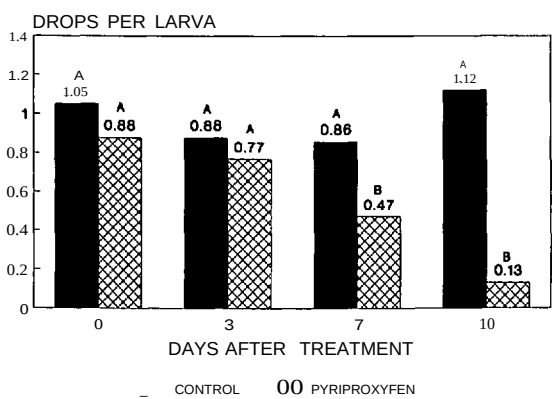

Fig. 4. Effect of pyriproxyfen on honeydew production. Second-instar *B. tabaci* were treated with 5 mg (AI)/liter pyriproxyfen. Number of honeydew drops per female was determined at various periods after start of assay. Data are averages of 10 replicates of 50-100 larvae each. Within each period, figures followed by the same letters do not differ significantly from each other (P = 0.05),

houseflies in manure of hens and pigs, respectively. In cattle, feeding ratios of 0.004 and 0.1 mg/kg body wt per d resulted in a similar degree of control for face and houseflies, respectively (Miller 1989). When applied topically to last-instar German cockroach females, pyriproxyfen suppressed adult emergence, caused morphological abnormalities, and prevented reproduction (Kawada et aI. 1989). Our findings indicate that pyriproxyfen affects all stages of B. *tabaci* (i.e., it suppresses egg hatch when adults or eggs are treated, and suppresses formation of adults when larvae are exposed to the compound). Pyriproxyfen is a selective insecticide, has relatively low mammalian toxicity (Peleg 1988, Yokoyama & Miller 1991), and could be considered a possible component in integrated pest management programs for controlling B. *tabaci* in cotton, vegetables, and ornamentals.

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