EFFECT OF DESTRUXINS FROM *METARHIZIUM ANISOPLIAE,* IVERMECTIN AND *BACILLUS THURINGIENSIS* DELTA-ENDOTOXINS ON ADULT SILVERLEAF WHITEFLY, *BEMISIA ARGENTIFOLII*

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Abstract

Using an assay system developed for the adult silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae), insects were exposed to a variety of potential toxins *per os.* Insects in this assay system were fed through a cellulose mixed-ester membrane on a diet of sucrose and the substance in question. Most of the substances tested in this manner were found nontoxic to this insect. Destruxins, extracted from the entomopathogenic fungus, *Metarhizium anisopliae,* and the natural insecticide/nematicide Ivermectin were among the few compounds found toxic to adult silverleaf whiteflies.

Introduction

Since about 1987, cotton fiber grown in the irrigated regions of the southwestern United States has been particularly subject to infestations of the sweetpotato whitefly, Bemisia tabaci, Genn. strain B, recently renamed the silverleaf whitefly, B. argentifolii, Bellows and Perring (Bellows et al., 1994). As it feeds, B. argentifolii can secrete more than 300 kg of phloem sap from the leaves of one hectare of a heavily infested cotton crop (Hendrix *et al.*, 1996). When this excreta, termed honeydew, lands on open bolls it causes the cotton fibers to become sticky and eventually moldy. Honeydew-contaminated cotton sticks to machinery in both cotton gins and textile mills, slowing or preventing its processing. Sticky cotton fiber also contains higher levels of leaf trash and dirt than clean fiber which can lead to health problems among textile mill workers (Ayars et al., 1986).

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Agents which much be ingested are difficult to assay against this insect because it feeds exclusively on plant phloem sap. We have designed and used a system to screen large numbers of potential intoxicants against this insect. We have been especially interested in agents such as systemic insecticides, microbial toxins, lectins or other agents which might be cloned into the cotton plant. Of the many substances screened, only a few proved toxic to adult silverleaf whiteflies over a 24 or 48 h exposure period. We report here on two promising intoxicants: destruxins, extracted from the entomopathogenic fungus, *Metarhizium anisopliae* and the natural insecticide/nematicide Ivermectin.

Materials and Methods

Conical 2 ml plastic microcentrifuge tubes with natural colored screw caps were used as assay devices. A whitefly aspirator device was devised by means of which whiteflies were aspirated directly into the screw top tubes for assay (Davidson *et al.*, 1995). Destruxins A, B, and E were extracted from culture filtrates of *Metarhizium anisopliae* strain Ma23 using methylene chloride, and subjected to chromatography on a silica column. The fraction eluted with acetone was evaporated to a powder. Destruxins from ARSEF23 were semipurified on 1:1 Dowex 50 and Dowex 1. The resulting dried powders were dissolved in 95% ethanol and diluted at least 100-fold in 27% sucrose containing yellow food dye.

The natural product insecticide/nematocide Ivermectin (Merck and Co., Rahway, New Jersey) was dissolved in 95% ethanol at 1 mg/ml, and diluted in 27% sucrose to concentrations of 1 and 10 μg/ml. For all *B. thuringiensis* toxins tested, except CytA, parasporal inclusions were purified on Renografin gradients and solubilized in 50 mM Na_2CO_3, 10 mM DTT, 5 mM EDTA, and 0.1 mM PMSF. Proteins were used either as solubilized (non-activated) or activated by trypsin treatment following dialysis against Tris-Cl, pH 8.5. Activation was necessary because this insect is thought to possess weak digestive proteases (A. C. Cohen, *pers. comm.*). CytA toxin was prepared by dissolving crystals of *B. thuringiensis* subsp. *israelensis* in 50 mM Na_2CO_3. CytA toxin was the gift of Dr. B. Knowles, Cambridge University. *B. thuringiensis* toxins were diluted in 20% sucrose for assay against whiteflies.

In the assays, 5 or 10 vials containing 5 insects each were utilized as controls. Whitefly mortality was scored after 24 and 48 h. Honeydew deposition on the sides and bottom of the vial was also scored on an arbitrary scale of 0 (no honeydew), 1 (single deposit) or 2 (more than one deposit). Vials of insects were incubated at 25°C and 50-55% RH. Control mortality under these conditions was around 10%, but rose to 94% when assays were conducted at 35°C and 30% RH. After 48 h, the vials were chilled and all whiteflies counted for a final assessment of mortality. Analysis of variance for a completely randomized design

was used to test for differences in honeydew scores and levels of mortality. Percent mortality was transformed by arcsine prior to analysis; however, untransformed values were presented in the results. Means were separated using LSD if a significant F-value ($P<0.05$) was indicated and they are presented with their standard errors (±SE).

Results and Discussion

B. thuringiensis toxins tested included CryIB (88 μg/ml, CryIC (59μg/ml), CryIE (152μg/ml)) , CryIA(a) (96 μg/ml), CryIA(b) 6.9 μg/ml), CryIA(c) (51 μg/ml), CytA (100 μg/ml), HNC (76 μg/ml), and HNE (91 μg/ml) all in the activated state and CryIA(c) (925 μg/ml) and CryIA(a) (140 μg/ml), solubilized only. For all these toxins no significant mortality or change in honeydew production by adult *B. argentifolii* was noticed. Nonetheless the wide range of targets recently discovered for *B. thuringiensis* toxins suggests that further screening may yet lead to discovery of one with activity against *B. argentifolii.*

Ivermectin, a member of a class of broad-spectrum antiparasitic antibiotics referred to as Avermectins, is an inhibitor of reproduction in such diverse insects as fire ants and mosquitoes. It exhibited a significant ability to kill adult silverleaf whiteflies when present in their diet at concentrations greater that 1 μg/ml (Table 1). Adult whitefly honeydew production was also strongly curtailed by this toxin, being completely eliminated at 10 μg/ml.

In addition to its ability to kill *B. argentifolii*, it may also inhibit its reproduction. The related compound, Abamectin, has been reported to kill both *B. tabaci* and the greenhouse whitefly (*Trialeurodes vaporariorum*) when applied to host plants (Price and Schuster, 1991; Zchori-Fein *et al.*, 1994).

After 48 h, destruxins from *M. anisopliae* strain Ma23 produced ca. 80% mortality and significantly reduced honeydew production at 3 μg/ml (Table 2). Destruxins from strain ARSEF23 produced 80% mortality and significantly reduced honeydew production at 100 μg/ml (data not shown). Destruxin E has been reported to kill and to repel aphids when applied as a spray to leaves or through the plant vascular system (Robert and Riba, 1989); however, the activity of destruxins to whiteflies has not been previously reported. In our bioassay system, the activity of destruxins from strain Ma23 was equal to that of the insecticides Imidocloprid (Davidson *et al.*, 1995) and Ivermectin (Table 1). These results suggest that destruxins are potentially useful agents against this cotton insect pest.

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Table 1. Mean percent mortality and honeydew production of *B. argentifolii* fed on Ivermectin added to 20% sucrose at 25°C, 50% relative humidity.[1]

Concentration of Ivermectin	% Mortality (S.E.) 24 h	% Mortality (S.E.) 48 h	Honeydew Score (S.E.) 24 h	Honeydew Score (S.E.) 48 h	n[3]
Control	7 (2.5)a	16 (5.0)a	0.4 (0.2)a	1.3 (0.2)a	4
1 μg/ml	22 (6.9)a	39 (15)a	0.7 (0.3)a	0.7 (0.3)	3
10 μg/ml	56 (9.8)b	100 (0)b	0.0 (0.0)b	0.0(0.0)b	3

[1]Means followed by the same letter are not significantly different at P<0.05
[2]Honeydew score = mean quantity per vial on an arbitrary scale of 0 to 2
[3]n = replicate treatments over time

Table 2. Mean percent mortality and honeydew production of *B. argentifolii* fed on destruxins extracted from *M. anisopliae* strain Ma23. Ethanol control contained ethanol at the same concentration as present in preparations containing 18 μg/ml destruxins.[1] Similar results were found with destruxins from *M. anisopliae* strain ARSEF23.

Destruxins,	% Mortality (S.E.)		Honeydew Score (S.E.)[2]		
Concentration	24 h	48 h	24 h	48 h	n[3]
Controls,					
Sucrose	0.6 (0.7)a	6.0 (2.3)a	0.8 (0.2)a	1.4 (0.3)a	3
Suc + Ethanol	7.0 (6.4)a	8.0 (7.8)a	1.0(0.0)a	1.3 (0.1)a	2
Destruxins,					
1.8 μg/ml	1.0 (0.6)a	14 (6.9)a	0.4 (0.2)ab	0.6 (0.2)b	3
3 μg/ml	33 (0.5)b	82 (0.7)b	0.2 (0.1)b	0.2 (0.1)bc	2
18 μg/ml	60 (15)b	92 (5.20)b	0.0 (0.0)b	0.0 (0.0)c	3

[1]Means followed by the same letter are not significantly different at $P<0.05$
[2]Honeydew score = mean quantity per vial on an arbitrary scale of 0 to 2
[3]n = replicate treatments over time