The *Journal of Cotton Science* 2:110-116 (1998)
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ARTHROPOD MANAGEMENT

Efficacy of Ultra Low Volume and High Volume Applications of Fipronil Against the Boll Weevil

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INTERPRETIVE SUMMARY

Fipronil belongs to a new class of insecticides called *pyrazoles*. These tests indicate fipronil was effective at both low and high volume sprays against boll weevils. Laboratory bioassays substantiate fipronil's toxicity to this insect. Laboratory bioassays of fipronil using field-collected strains of boll weevil from both the Lower Rio Grande Valley of Texas and from Louisiana showed equality in toxicity. This bioassay did not reveal any differences in susceptibility of the laboratory strain tested as a reference in both Texas and Louisiana. These strains showed little variation in their response to fipronil and little potential for the development of resistance to this chemical. High volume sprays of fipronil were effective at reducing percentage damaged squares and bolls. Ultra low volume sprays of malathion and fipronil were equally toxic to the boll weevil. Residues of fipronil and its metabolites found on leaf surfaces during these tests were directly related to boll weevil mortality. Greater deposition on the undersides of leaves occurred when fipronil was applied at the highest volume.

ABSTRACT

Topical bioassays of fipronil against a field-collected and laboratory reference strain of boll weevil from the Lower Rio Grande Valley showed equal LD_{50} values. The LD_{50}'s of field-collected strains from Louisiana and Lower Rio Grande Valley were equal. In Texas in 1993 (Test 1), 15 applications of fipronil applied at 0.028, 0.056, and 0.084 kg(a.i.)/ha were equally effective at reducing percentage damaged squares and bolls below the untreated check. In Texas in 1994 (Test 2), 14 applications of 0.056 kg(a.i.)/ha effectively reduced damaged fruit below that of the untreated check. Ultra low volume ground application tests in Mississippi in 1995 (Test 3) showed no differences in toxicity of two rates (1.02 and 1.36 kg(a.i.)/ha) of malathion and fipronil at 0.056 kg(a.i.)/ha. Fipronil applied ultra low volume by aircraft at rates of 0.043 and 0.056 kg(a.i.)/ha (Test 4) were equally effective against boll weevils in bioassays of treated leaves. In a subsequent test (Test 5), 0.028 and 0.043 kg(a.i.)/ha rates of fipronil were equal in toxicity to boll weevils. Residues of fipronil + four metabolites on leaf surfaces determined during aerial tests were directly related to boll weevil mortality. Fipronil is highly effective against boll weevil in either low volume sprays in oil or high volume sprays in water.

Fipronil is an experimental pyrazole insecticide that is being tested on cotton. It was discovered in 1987 by Rhone Poulenc scientists and has been shown to be effective against the boll weevil *(Anthonomus grandis grandis* Boheman) as a foliar spray (Colliot et al.,1992). Efficacy has also been shown against other cotton insect pests such as thrips (*Frankliniella* spp.) and tarnished plant bugs [*Lygus lineolaris* (Palisot de Beauvois)] (Burris et al.,1994).

Animal and Plant Health Inspection Service personnel involved with the eradication program against the boll weevil are currently evaluating the compound against the boll weevil. Fipronil's low use rate, 0.056 kg(a.i.)/ha, against the boll weevil (Shaw and Yang,1996) combined with its effectiveness against plant bugs (Shaw et al.,1997) make it a potential candidate for registration for use on cotton and as an alternative insecticide for controlling the boll weevil/plant bug complex and/or for inclusion in the boll weevil eradication program.

In this research, the deposition and efficacy of fipronil applied in ultra low volume and high

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volume sprays was evaluated in the field in Mississippi and Texas at different rates and volumes by aircraft and ground sprayer.

MATERIALS AND METHODS

Technical fipronil (Regent) and metabolites I, II, III, and IV were obtained from Rhone Poulenc, Inc., 2 T. W. Alexander Dr, Research Triangle, NC. Fipronil, formulated as a 300 g/L emulsifiable concentrate, was also obtained from Rhone Poulenc. Malathion (Fyfanon), as 95% technical, was obtained from Cheminova, Inc., P. O. Box 9 DK-7620, Lemvig, Denmark. Reagent grade chemicals used in analytical analysis were obtained from commercial sources.

Laboratory

Technical fipronil was diluted in acetone and topically applied to the dorsum of the boll weevil thorax (Wolfenbarger et al.,1986) at 0.5, 0.1, 0.025, 0.0125, and 0.00625 µg fipronil/µL acetone/boll weevil. Mortalities were indicated after 48 h when boll weevils continue to lay on their side despite probing of proboscis. All reference boll weevils used in bioassays in Texas were obtained from USDA's Gast Rearing Laboratory at Mississippi State, MS. Field-collected boll weevils were reared from infested squares collected from untreated cotton near Weslaco, TX, in 1993. The lethal dose to kill 50% of the population (LD_{50}), slope ± standard error, and 95% confidence interval were determined with PROC PROBIT (SAS Institute, 1990). After 48 h, mortalities of untreated weevils were corrected for 8% natural mortality.

Tests 1 and 2

A high clearance John Deere sprayer was used to apply fipronil in 1993 and 1994 in cotton near Weslaco, TX. A 93 L/ha volume was applied through five flat-fan 65002 nozzles (Spraying Systems, Inc., Wheaton, IL)/row with pressure set at 310 kPa and speed at 6 km/h. Treated and untreated plots were eight rows wide (1 m apart) and 18 m long with five replicates. Experiments were arranged in a randomized complete block design. Plots were separated by 8 m with sweet sorghum [*Sorghum bicolor* (L.) Moench] (2–3 m tall) between replicates and four rows between plots. In Test 1, plots were sprayed at 0.028, 0.056, and 0.084 kg(a.i.)/ha on 1, 4, 10, 16, 18, 24, 26, 29 June and 2, 6, 9, 13, 16, 20, 23 July 1993. In Test 2, plots were sprayed at 0.056 kg(a.i.)/ha on 19, 20, 24, 27 May; 2, 7, 9, 10, 28 June; and 1, 5, 8, 12, 19, 21 July 1994. In Test 1, plots were sampled 13 times 1 to 3 days following each of the sprays. In Test 2, plots were sampled 26 times 1 to 3 days following each of the sprays. Plots were sampled for squares damaged by feeding and oviposition of the boll weevil and undamaged squares on the whole plant. Three to five whole plants that contained 3 to 15 squares and small bolls/plant were sampled in each plot in each replicate. Results are mean percentage damaged for the season. Significant differences between treated and untreated plots were determined by analysis of variance for boll weevils and differences between means were determined by least significant difference (LSD) (SAS Institute,1990).

Test 3

Sprays were applied by ground to cotton (DPL 50) on 1 July 1995 at Stoneville, MS, to compare toxicity of fipronil at 0.056 kg(a.i.)/ha, and malathion at 1.02 and 1.36 kg (a.i.)/ha.

Both insecticides were applied at a 1.17 L/ha volume using an ultra low volume air-assisted ground sprayer (Barrentine and McWhorter, 1988; Hanks and McWhorter, 1991; Mulrooney et al., 1997). A John Deere 600 high cycle equipped with a dual-boom spraying system consisting of both a conventional and an air-assisted ultra-low-volume spraying system was used in the ground tests. Conventional applications (93.5 L/ha) were at 10 km/h using TeeJet 9501 nozzles (Spraying Systems, Wheaton, IL) at a pressure of 317 kPa. Ultra-low-volume (1.17 L/ha) treatments were applied at 7 km/h with Bete T-Mizer nozzles (Bete Fog Nozzle, Greenfield, MA) by using an air pressure of 35 kPa to atomize the liquid into spray droplets. Test plots of cotton were four rows (1 m apart, running east and west) by 58.5 m arranged in a randomized complete block design with four replications. A four-row buffer was provided between each plot.

Leaves from the fourth node down from the terminal were collected at 0, 24, 48, and 72 hours after treatment at Stoneville, MS. Five leaves per

replicate were collected, and placed in zip-lock bags on ice packs in a cooler and transported to the lab. Each leaf was placed in a petri dish (9 cm) containing one 4-d-old adult boll weevil. Mortality was recorded at 24 and 48 h.

At Stoneville, MS, at 24, 48, and 72 hours after treatment, individual plants 0.5 to 1.0 m in height were caged with Fibre-air Plant Sleeves (20.3 by 48.3 by 55.9 cm, Kleen Test Products, Brown Deer, WI). Five 4-d-old adult boll weevils were placed in each cage. There were 10 cages per replicate. Twenty-four hours after placing five weevils in each cage, the caged plant was cut at the soil line and brought to the laboratory where mortality was recorded. Any surviving weevils were placed in petri dishes for an additional 48 h. Mortality was recorded at 24, 48, and 72 h after placing weevils in cages in the field.

Tests 4 and 5

Sprays were applied by aircraft on 22 Aug. and 5 Sept. 1995 at Stoneville, MS. In these tests comparisons of boll weevil mortality and residues of fipronil from treated leaves were made. In Test 4, 0.056 kg (a.i.)/ha of fipronil in a 1.17 L/ha volume of cottonseed oil and 0.043 kg (a.i.)/ha in a 0.88 L/ha volume were compared. The 1.17 L/ha volume application was made using an Air Tractor 402 aircraft equipped with 18, 8002 flat-fan nozzles (Spraying Systems, Wheaton, IL) at 262 kPa, and an air speed of 225 km/h. The 0.88 L/ha volume application was made using the same settings on the aircraft, except that only 13 nozzles were turned on during this application. Application was made parallel to the rows (north-south) in plots 27.4 by 195.1 m during the late afternoon. There were four replicates of each treatment, with a replicate consisting of one pass of the aircraft.

Leaves were collected at 12, 36, and 60 hours after treatment for bioassay and residues. Twenty leaves per treatment were bioassayed in petri dishes using five boll weevils per leaf. Fipronil residues from both the upper and lower surfaces of leaves were obtained at each of the sampling times by washing five leaves per replicate with ethanol using dual-side leaf washers (Carlton, 1992). Residues were analyzed using HPLC as described below. Boll weevils were caged on plants in the field at 36 and 60 hours after treatment. There were five cages per replicate, with five weevils in each cage. The weevils remained in the cages for 24 hours. Mortality readings were taken at 24 and 48 h after removing the weevils from cages.

Test 5 was applied on 5 Sept.1995 at Stoneville, MS. Fipronil at rates of 0.043 and 0.028 kg (a.i.)/ha was applied in volumes of 0.88 and 0.58 L/ha of cottonseed oil. Leaves were collected at 0, 24, 48, and 72 hours after treatment for bioassay and residues with methods described above. Caged-plant bioassays were also conducted at 24 and 48 hours after treatment.

Residue Analytical Procedure for Fipronil and Metabolites

HPLC analysis was with a Waters Millenium 2010 Chromatographic Manager (Millipore Corp, Waters Chromatographic Division, Milford, MA) consisting of Waters 486 variable wavelength detector, Waters 600 solvent pump, Waters 715 Sample Processor and the Millenium 2010 Chromatographic Data Processor. For the separation of fipronil and its degradation products, a reverse phase column and methanol/water mobile phase were used. A 25 cm by 4.6 mm i.d. Adsorbosphere column (Alltech Associates, Deerfield, IL), methanol/water (70:30) mobile phase at a flow rate of 1.0 mL min^{-1}, a 20 µL injection volume and detector wavelength of 280 n*M* were used to achieve optimum analysis. The mobile phase and standard solutions were prepared from HPLC grade solvents filtered through 0.45 µ solvent filter (Millipore Corp., Milford, MA). Elutions times were, fipronil, 7.50 min; I, 6.88 min; II, 9.35 min; III, 7.80 min; and IV, 4.50 min. Detection limits were found to be in the range of 50 ng/L. The linearity was verified with standard solutions in the range of 50 ng/L to 50 µg/L. Analytical grade fipronil and metabolites, (I) 5-amino-3-cyano-1-(2, 6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole, (II) 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethyl-sulphonylpyrazole, (III) 5-amino-1-(2, 6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylthiopyrazole, and (IV) 5-amino-3-carbamoyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulphonylpyrazole were provided by

Rhone-Poulenc AG Company (Research Triangle Park, NC). All the solvents were of HPLC grade.

Data Analysis—Mortality Data

A randomized complete block design was used with four replicates in both the ground and aerial application tests. Data were analyzed by analysis of variance using PROC GLM (SAS Institute, 1990). Means were separated by least significant difference where appropriate.

Data Analysis—Residue Data

A randomized block design with four replicates was used. Residues, expressed as ng/cm^2 of leaf sampled, were removed from leaves at various times during the test period; therefore, the effect of time was a repeated measure. Residue data were initially analyzed using analysis of variance to choose a model that best accounted for the repeated measurements in the time effect. The model chosen was a split plot with the main unit, treatment, having a randomized complete block design and the subunit, time, being stripped across main units. In a subsequent analysis the levels of time were treated as a trend by a linear equation, residue = intercept + slope x (time). Data (ng/cm^2) were not transformed.

RESULTS AND DISCUSSION

Laboratory

The LD_{50}'s of fipronil to reference strain of boll weevil in this study (Table 1) and by Burris et al. (1994) were statistically equal; 95% confidence intervals overlapped. The LD_{50} for our field-collected strain from the lower Rio Grande Valley was significantly greater than LD_{50}'s of several field-collected strains but statistically equal to others from Louisiana (Burris et al., 1994). Results indicate that these populations in Louisiana and Lower Rio Grande Valley show variation in susceptibility. Of interest was the result that the slope of the reference strain was flatter and standard error of this slope was greater than shown for field-collected strains in both states. More factors were responsible for toxicity of fipronil to the reference strain than the field-collected strain.

Table 1. Toxicity to boll weevil (as ng/weevil after 48 h) of fipronil by topical application. (Weslaco, TX, 1993.)

Strain	N	Slope ± SE	LD_{50}	95% CI
Reference	290	0.9 ± 0.4	0.07	0.0039 – 0.21
Field	178	1.82 ± 0.2	0.036	0.025 – 0.05

Table 2. Efficacy of fipronil in tests against boll weevils. (Weslaco,TX, 1993.)

	Percent damage	
Rate (kg a.i./ha)	Squares	Bolls
0.028	6 a	8 a
0.056	5 a	7 a
0.084	4 a	10 a
Check	18 b	14 b
LSD	11	3

Tests 1 and 2

Fipronil was applied as season long sprays (15 applications) in 93 L/ha at 0.028, 0.056, and 0.084 kg (a.i.)/ha and seasonal mean of percentage damaged squares and bolls were significantly less than the untreated check (Table 2). All three rates were equally effective. In Test 2, we made 14 applications at 0.056 kg (a.i.)/ha in the same volume and again treated plots had significantly less damage than untreated plots. Seasonal damage was much less in 1993 (Test 1) than in 1994 (Test 2). In 1994 at Weslaco, cotton treated with 0.056 kg(a.i.)/ha of fipronil showed a seasonal mean of 23% damaged squares (data not shown in table). Untreated plots had a seasonal mean of 37% damage. Difference between the treated and untreated was significant as least significant difference was 10% damaged squares.

Test 3

Ultra low volume ground application tests compared two rates of ultra low volume malathion, 1.02 kg (a.i.)/ha and 1.36 kg(a.i.)/ha, with fipronil at 0.056 kg (a.i.)/ha. The percent mortality (48 h) of boll weevils placed on treated leaves in the petri dish bioassay did not differ between treatments until 3 d after treatment (Table 3). On this date, the 1.36 kg (a.i.)/ha rate of ultra low volume malathion had significantly ($F = 22.19$; df = 3, 12; $P = 0.0001$) higher mortality (95%) than fipronil (60%) and ultra low volume malathion at 1.02 kg (a.i.)/ha (42%) (Table 4). In a bioassay of caged boll

Table 3. Percentage mortality (48 h) of boll weevils on leaves treated with ultra low volume insecticides in petri dish and caged plant bioassays in ground tests. (Stoneville, MS 1995.)

	Days after treatment				
Insecticide (kg a.i./ha)	0	1	2	3	0
		Petri dish			Caged plant
Malathion, 1.36	100 ± 0	100 ± 0	100 ± 0	95 ± 5	92 ± 4
Malathion, 1.02	100 ± 0	95 ± 5	100 ± 0	42 ± 10	82 ± 6
Fipronil, 0.056	100 ± 0	85 ± 10	95 ± 5	60 ± 12	92 ± 4
Control	5 ± 5	5 ± 5	10 ± 10	0 ± 0	6 ± 3
LSD	8	18	17	26	16

Table 4. Percentage mortality (48 h) of boll weevils in petri dish and caged plant bioassays of cotton treated with ULV application by aircraft. (Stoneville, MS, 1995.)

Quantity applied	Volume applied	Hours after treatment				
(kg a.i./ha)	L/ha	12	36	60	36	60
			Petri dish bioassay		Caged Plant Bioassay	
0.056	1.17	100 ± 0	100 ± 0	60 ± 14	98 ± 2	92 ± 3
0.043	0.88	100 ± 0	100 ± 0	95 ± 5	96 ± 2	84 ± 2
LSD		NS	NS	NS	NS	NS

Table 5. Residues (ng/cm^2) of fipronil + metabolites removed from upper and lower leaf surfaces. (Stoneville, MS 1995.)

Quantity applied	Volume applied	Hours after treatment		
(kg a.i./ha)	L/ha	12	36	6
			Upper surface	
0.056	1.17	365 ± 45	161 ± 44	140 ± 33
Predicted values		335	163	116
0.043	0.88	178 ± 53	119 ± 25	62 ± 12
Predicted values		303	132	94
			Lower surface	
0.056	1.17	142 ± 48	44 ± 9	19 ± 7
Predicted values		125	34	18
0.043	0.88	52 ± 17	76 ± 24	35 ± 11
Predicted values		51	43	40

Table 6. Equations of predicted trends [residue = intercept + slope(time)] of residues (ng/cm) of fipronil + metabolites on upper and lower leaf surfaces over time.

	Upper surface		Lower surface	
Insecticide	Intercept	Slope	Intercept	Slope
0.056 kg a.i./ha in 0.88 L/ha volume	7.58	-0.69	8.01	-1.24
0.043 kg a.i./ha in 0.58 L/ha volume	7.65	-0.76	4.36	-0.16

weevils, there were no differences in mortality between fipronil and malathion when boll weevils were caged on plants immediately after application. Mortalities in this bioassay in the field were 92% for 1.36 kg (a.i.)/ha malathion and 0.056 kg (a.i.)/ha fipronil, and 82% for 1.02 kg (a.i.)/ha malathion.

Test 4

This test compared 0.056 kg (a.i.)/ha of fipronil applied by aircraft at a 1.17 L/ha spray rate to a 0.043 kg (a.i.)/ha rate applied at 0.88 L/ha. There were no differences in boll weevil mortality in either the leaf or the caged plant bioassay (Table 4). Mortality in the leaf bioassay was 100% until 60 hours after treatment when it dropped to 60 and 95% for the 0.056 kg (a.i.)/ha and 0.043 kg (a.i.)/ha rates, respectively. However, these differences were not significant.

Fipronil residues removed from upper and lower leaf surfaces and equations of trends of residue over time are given in Tables 5 and 6. Slope comparisons of trends showed no difference between insecticide rates in fipronil residues on the upper (F = 0.12, $P > F$ = 0.74) leaf surface. However, significantly higher (F = 7.45, $P > F$ =

Table 7. Percentage mortality (48 h) of boll weevils in petri dish and caged plant bioassays of cotton treated with ULV application of fipronil by aircraft. (Stoneville, MS, 1995.)

Quantity applied (kg a.i./ha)	Volume applied (L/ha)	Hours after treatment					
		0	24	48	72	24	48
		Petri dish bioassay				Caged plant bioassay	
0.043	0.88	100 ± 0	85 ± 10	75 ± 10	40 ± 8	68 ± 2	63 ± 3
0.028	0.58	95 ± 5	85 ± 10	55 ± 5	55 ± 10	63 ± 6	50 ± 4
LSD		NS	NS	NS	NS	4.8	9.2

Table 8. Residues (ng/cm^2) of fipronil + metabolites removed from upper and lower leaf surfaces. (Stoneville, MS, 1995.)

Quantity applied (kg a.i./ha)	Volume applied (L/ha)	Hours after treatment			
		0	24	48	72
			Upper surface		
0.043	0.88	195 ±38	88 ±4	34 ±5	46 ±5
Predicted values		214	62	49	41
0.028	0.58	162 ±22	78 ±8	30 ±2	50 ±8
Predicted values		163	57	46	40
			Lower surface		
0.043	0.88	30 ± 4	31 ± 9	17 ± 6	20 ± 3
Predicted values		31	20	19	18
0.028	0.58	66 ± 20	11 ± 2	11 ± 2	19 ± 7
Predicted values		53	13	10	8

Table 9. Equations of predicted trends [residue = intercept + slope(time)] of residues (ng/cm) of fipronil + metabolites on upper and lower leaf surfaces over time.

	Upper surface		Lower surface	
Insecticide	Intercept	Slope	Intercept	Slope
0.043 kg (a.i.)/ha in 0.88 L/ha volume	5.36	-0.33	3.42	-0.13
0.028 kg (a.i.)/ha in 0.58 L/ha volume	5.09	-0.33	3.97	-0.43

0.013) amounts of fipronil were recovered from the lower leaf surface in the 0.056 kg (a.i.)/ha rate. This is possibly due to the fact that the 0.056 kg(a.i.)/ha rate was applied at a higher spray volume than the 0.043 kg(a.i.)/ha rate. The higher volume may have resulted in greater deposition on the underside of the leaf as turbulents from the airplane caused the lower leaf surface to be turned upward as spray particles entered the plant canopy.

Test 5

When fipronil was applied by airplane, there were no significant differences between boll weevil mortality on cotton leaves treated with 0.042 and 0.028 kg (a.i.)/ha rates by the petri dish bioassay (Table 7). The 0.043 kg (a.i.)/ha rate was slightly more toxic than the 0.028 kg (a.i.)/ha rate in a caged plant bioassay at both 24 and 48 hours after treatment.

Fipronil residues, as ng/cm^2, removed from upper and lower leaf surfaces and equations of trends of residue over time from each treatment are given in Tables 8 and 9. Using *F* tests of homogeneity, slopes showed no significant differences between treatments in the amount of fipronil on the upper ($F = 1.54$, $P > F = 0.24$) or the lower ($F = 3.63$, $P > F = 0.068$) leaf surface.

This research demonstrates fipronil's high toxicity to boll weevils at all volumes tested. Therefore, fipronil's low-use rate and toxicity to plant bugs (Burris et al., 1994) offer maximum flexibility for use in cotton pest management. The favorable comparison of fipronil and malathion indicated fipronil could be an alternative insecticide for boll weevil control, and that further testing should be conducted to investigate fipronil's potential in the Boll Weevil Eradication Programs.

ACKNOWLEDGMENT

Debra Gary and Ben Naron (Application and Production Technology Research Unit) assisted in this research. Debbie Boykin provided guidance in the analysis of data.

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