PERSISTENCE OF DIMILIN® (DIFLUBENZURON) ON COTTON FOLIAGE AS DETERMINED BY BEET ARMYWORM (SPODOPTERA EXIGUA) BIOASSAY

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Abstract

A laboratory beet armyworm bioassay was used to evaluate the persistence of DIMILIN® INSECT GROWTH REGULATOR (diflubenzuron) when applied at 0.062 lb ai/acre to foliage of either greenhouse- or field-grown cotton. In the first of four evaluations, plants were maintained in the greenhouse and leaves were bioassayed until 28 days after treatment (DAT). Larval survival was reduced by DIMILIN at all DAT's sampled. In the second evaluation, potted plants were maintained outside the greenhouse and leaf tissue was bioassayed until 30 DAT, during which there was a total accumulation of 2.7 inches of precipitation. Larval survival was reduced at all sampling dates by treatment with DIMILIN. In the third and fourth evaluations, field cotton was sampled for up to 34 days after application with DIMILIN during which up to 9.0 inches of rain was recorded. The bioassay again detected activity of DIMILIN throughout the exposure period. All evaluations indicated at least 4 weeks of persistence on cotton.

Introduction

Persistence on plant tissue of an insecticide which needs to be consumed for activity against foliar feeding larvae is advantageous as long as the insecticide expresses little activity on the beneficial insect populations. The insect growth regulator, diflubenzuron (DIMILIN), has been shown to be persistent on plant tissue (Bull and Ivie, 1978; Mansager et al., 1979) and to have little affect on beneficial cotton insects (e.g. Ruberson et al., 1993). It has been recommended against beet armyworms (BAW) by extension entomologists and county extension agents in a preemptive control approach. Repeat applications at approximately weekly intervals enhances coverage and allows newly expanded foliage to be treated. Subsequent control of BAW and reduced foliar cotton feeding using this approach has been documented (Lambert, 1992; Burris et al., 1994).

Several studies have been conducted to characterize the persistent activity expressed by DIMILIN on foliage. Bull and Ivie (1978) used ^{14}C-labeled DIMILIN 25W on leaves of field- and greenhouse-grown cotton. Approximately 90% of a field application remained associated with the leaf 14 days after it was applied. Following 3 inches of precipitation at 3 weeks, 30% of the initial application was still detected on and in the leaves. A greenhouse trial also indicated persistence on the foliage up to one month after treatment. The chemical was found predominantly on the surface of the leaf, there was little photodegradation, and losses to volatilization or weathering were minimal. Additionally, Mansager et al. (1979) found that absorption, metabolism or translocation of labeled DIMILIN 25W were minimal.

Schaefer and Dupras (1976,1979) also showed that the persistence of DIMILIN was not greatly affected by sunlight. Minimal loss of DIMILIN through volatilization is supported by a very low vapor pressure (Dobroski and Lambert, 1985). Persistence on cotton as related to rainfastness has been documented by McDonald and Weiland (1995). The authors noted that rainfastness was an attribute of the diflubenzuron active ingredient, irrespective of formulation. Rainfastness on plant foliage (McDonald and Weiland, 1995) would not have been unexpected since diflubenzuron has low water solubility (Dobroski and Lambert, 1985).

This study was undertaken to provide additional evidence of persistence of DIMILIN on cotton when applied at its lowest label rate and at typical volumes. More current formulations being used in the Cotton Belt, DIMILIN 2L and DIMILIN 2F, were evaluated. A significant feature of these investigations was the determination of the residual control effect of DIMILIN on BAW, a major pest on which field applications of DIMILIN on cotton are targeted. Thus, a BAW bioassay method was utilized to assess the potential residual activity of DIMILIN on cotton foliage for at least 28 days from both greenhouse and field plants.

Materials and Methods

During the first evaluation, Stoneville 825 cotton plants were individually raised in a greenhouse located in Bethany, CT in 4 inch azalea pots to the 4 true-leaf stage. Before treatment, all plants were stripped of foliage except for the 2 most recently expanded leaves. Application of DIMILIN 2F (0.062 lb ai/acre) was made in a trolley sprayer which was calibrated to deliver a volume of 20 gpa (2 passes of 10 gpa) at 22 psi from a flat fan 8002E nozzle, positioned 20 inches above soil level. Foliage was allowed to dry in the greenhouse prior to sampling.

A petri dish bioassay (McDonald and Weiland, 1995) was used for all evaluations. Either unsprayed or treated leaves were singly evaluated in the bioassay. Beet armyworms, received as eggs from the USDA-ARS Southern Field Crop Insect Management Laboratory, Stoneville, MS, were reared to the second/third instar stage on artificial tobacco budworm diet (BIOSERVE, Frenchtown, NJ). Five larvae were transferred onto a single excised cotton leaf resting on

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a moist filter paper in a 3.54-inch diameter petri dish for a single replicate, with a total of 8 replicates per treatment. Dishes were then placed in an incubator (80°F). This timing was designated as 0 days of bioassay (DOB). The number of survivors was determined daily for 5 days and reported for 1, 3 and 5 DOB. Survivors responded to gentle probing with a blunt dissecting needle. At 2 and 4 DOB, leaves in the bioassay were replaced according to treatment group with leaves from plants which had been reserved in the greenhouse. Additional moisture was also added to the filter paper at these times. The bioassay procedure was carried out on leaves at 0 (~1 hour), 14 and 28 DAT.

For the second evaluation Stoneville 825 cotton plants were raised in a greenhouse and sprayed on May 1 at a facility in Sanford, FL using procedures as described above. DIMILIN 2L (0.062 lb ai/acre) was used. After this first spraying all treated and untreated plants were permanently transferred outside the greenhouse. On May 15, a portion of the untreated plants was sprayed. On May 30 another group of untreated plants was sprayed. On both May 15 and May 30 three groups of leaves (untreated, treated on May 1, and treated the day of shipment [approximately 1 hour after treatment]) were shipped overnight in sealed plastic bags (ambient temperature) to Bethany, CT. Upon arrival, the BAW bioassay was initiated; extra leaves were stored in individual petri dishes with moist filter paper until used as replacement tissue at 2 and 4 DOB. Note that subsequent reference to leaves from Sanford as "1 hour after treatment", "13 DAT", etc., does not identify the one day needed for shipment.

For the third evaluation a uniform stand of Stoneville 825 cotton was grown under field conditions at Sanford, FL to the 6-leaf stage. At that time (September 5, 1995), 0.062 lb ai DIMILIN 2L/acre was applied to a portion of the stand at a volume of 20 gpa using a hand hydra gun. On September 18 or on October 2, other portions of untreated cotton were sprayed. On each of these latter dates, 5th and 6th true leaves from untreated plants, from plants treated on September 5, and from plants treated that day, were shipped to Bethany, CT for bioassay. Sixteen replicate leaf samples were bioassayed during this and the evaluation described below.

For the fourth evaluation, the same stand of Stoneville 825 cotton was at the 6- to 7-leaf stage. On September 12, 1995, 0.062 lb ai DIMILIN 2L/acre was applied to a previously untreated portion of the stand at a volume of 20 gpa using a hand hydra gun. On October 2 or on October 16 other portions of untreated cotton were sprayed. On each of these latter dates, 5th and 6th true leaves from untreated plants, from plants treated on September 12 and from plants treated that day, were shipped to Bethany, CT for bioassay.

Weather data were determined at the Central Florida Research and Education Center, Sanford, FL which was approximately 1 mile from the field/greenhouse site. Rainfall and maximum/minimum air temperatures for the months of May, September and October (1-16) of 1995 are shown in Table 1.

Data were analyzed using Duncan's Multiple Range test (Duncan, 1955) with a significance level of $\underline{P} = 0.05$.

Results and Discussion

The efficacy of DIMILIN against BAW when applied to 3rd and 4th true cotton leaves in a greenhouse is shown in Table 2. Results illustrate the number of larvae surviving at 1, 3 and 5 DOB on foliage which had been previously treated with DIMILIN at 1 hour, 14 days and 28 days prior to bioassay. Larval survival significantly decreased for all leaves with DIMILIN starting at 3 DOB. Although bioassays for each sampling were independently done, it appeared that survival on untreated 28-day foliage was lower than for both 1-hour and 14-day foliage. The assessment of leaf tissue for persistence of DIMILIN beyond 28 days was not attempted due to the onset of leaf senescence.

The persistence of DIMILIN on foliage sustained outside the greenhouse is illustrated in Table 3. Starting at 3 DOB cotton leaves sprayed with DIMILIN at both 1 hour and 15 days prior to bioassay expressed significant activity on BAW. Leaves treated with DIMILIN 30 days prior to bioassay expressed significant activity of DIMILIN on BAW starting at 5 DOB. A total of 2.21 inches of precipitation was recorded up to the 15-day harvest; another 0.53 inches fell between the 15- and 30-day harvests (Table 1). As in the greenhouse test above, check survival on the older foliage seemed to be reduced compared with that on the younger foliage. Senescence of the treated foliage did not permit assessment beyond 30 days for these early true leaves.

The persistence of DIMILIN on older cotton foliage (5th and 6th true leaves) of plants grown and treated in the field is shown in Tables 4 and 5. Activity of DIMILIN was noted 13 DAT, and then at 28 DAT, during the first field evaluation (Table 4). At 3 DOB for both samplings, leaves treated 1 hour prior to bioassay exhibited reduced BAW survivability more than either the 13- or 28-day samples. This indicates weathering had occurred. Nevertheless, both the 13- and 28-day samples reduced survival compared with that in the untreated. At 5 DOB treatments with DIMILIN were equivalent for this parameter. A total of 1.86 inches of precipitation was recorded by 13 DAT, as was a total of 4.20 inches by 28 DAT (Table 1).

The last field experiment tested activity through 34 days (Table 5). At both 3 and 5 DOB larval survivability decreased for samplings taken at either 20 or 34 days after treatment with DIMILIN, but with the 1-hour treatment showing more activity than the 34-day treatment. The

significant difference between treatment means for DIMILIN at 34 DAT occurred at both 3 and 5 DOB. This indicated that weathering took place through 34 days, yet enough DIMILIN remained to be effective against BAW. A total of 2.49 inches of precipitation fell through 20 DAT, while a total of 8.95 inches fell through 34 DAT (Table 1).

For all treatments with DIMILIN in the study, classic symptoms (Mulder and Gijswijt, 1973) of unsuccessful molting of BAW larvae due to this insect growth regulator were noted.

Based on these results using the lowest labeled rate, at least 4 weeks of persistence of DIMILIN can be expected whether the treated cotton is maintained in the greenhouse or in the field. Although the amount remaining on the leaves was not quantified in this study, Bull and Ivie (1978) found nearly 90% of the applied ^{14}C DIMILIN present on cotton foliage after a 2-week exposure under field conditions. This dropped to around 30% at 3 weeks after 3 inches of precipitation.

Across bioassays, significant activity of DIMILIN on BAW survivability may be determined as early as 1 DOB, but as late as 3 DOB. This is probably related to the time of application of DIMILIN and the timing of molting of the larvae. Fye and McAda (1972) determined there is approximately 1.9±0.5 days of development prior to molting for the second instar at 77°F (1.6±0.5 days for the third instar).

Earlier mortality of untreated BAW on older tissue is noteworthy. Signs of leaf senescence, such as development of chlorosis and necrotic spots, were seen in the latest sampled tissue (treated or untreated) of all experiments. Thus this tissue was not preferred by all BAW for consumption, after having hatched on and adapted to the artificial media. The use of foliage higher in the canopy, and thus more likely to express delayed senescence, should be attempted. This will potentially allow one to investigate greater than 34 days persistence of diflubenzuron on cotton tissue.

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Table 1. Air temperatures and rainfall amounts for the months of May, September, and October (1-16) in 1995 at Sanford, Florida as provided by the Central Florida Research and Education Center, Sanford, Florida.

	May			September			October		
	Temp(ºF)		Rain	Temp(ºF)		Rain	Temp(ºF)		Rain
Day	High	Low	(")	High	Low	(")	High	Low	(")
1	92	65	0.00	87	76	0.03	89	72	0.10
2	91	66	0.00	91	74	0.00	84	73	0.00
3	85	65	0.00	87	74	0.00	88	72	0.00
4	92	64	0.00	84	74	0.00	90	74	0.48
5	92	67	1.17	87	72	0.00	92	78	0.11
6	87	70	0.00	88	72	0.00	87	75	0.05
7	86	68	0.00	87	71	0.85	87	74	0.24
8	88	60	0.00	86	70	0.02	89	74	0.50
9	90	63	0.00	86	69	0.07	83	76	0.23
10	90	70	0.00	89	69	0.70	89	74	3.07
11	91	69	0.33	88	70	0.07	86	74	0.61
12	92	66	0.71	90	72	0.03	89	73	0.08
13	95	69	0.00	90	73	0.00	89	73	0.03
14	96	73	0.00	92	72	0.10	88	75	0.00
15	94	71	0.00	90	73	0.02	89	70	1.06
16	95	72	0.00	86	74	0.00	79	62	0.00
17	95	68	0.00	92	71	0.00			
18	96	71	0.00	92	74	0.00			
19	95	70	0.00	90	72	0.00			
20	90	70	0.34	90	69	0.00			
21	88	72	0.00	90	71	0.00			
22	87	70	0.00	89	71	0.14			
23	88	70	0.00	94	70	0.00			
24	87	69	0.19	90	69	0.79			
25	89	66	0.00	90	72	1.00			
26	92	65	0.00	93	74	0.15			
27	95	64	0.00	91	73	0.08			
28	92	67	0.00	86	73	0.00			
29	92	68	0.00	85	72	0.08			
30	95	69	0.00	89	73	0.00			
31	95	71	0.55						

Table 2. Beet armyworm survival on cotton leaves from the greenhouse through 5 days of bioassay (DOB) asaffected by DIMILIN 2F applied at 0 (1 hour), 14 and 28 days (DAT) prior to the initiation of the bioassay.

Treatment	Trt Appl	Rate in lb ai/ac	Mean # of Larvae Surviving 1 DOB	3 DOB	5 DOB
1st Sampling					
UNTREATED			4.6 a*	4.6 a	3.9 a
DIMILIN 2F	1 h	0.062	3.4 a	1.0 b	0.0 b
2nd Sampling					
UNTREATED			5.0 a	5.0 a	4.9 a
DIMILIN 2F	14 d	0.062	2.9 b	0.8 b	0.0 b
3rd Sampling					
UNTREATED			3.2 a	2.8 a	1.2 a
DIMILIN 2F	28 d	0.062	3.1 a	0.6 b	0.0 b

*Means followed by the same letter within a column for a given DAT, do not significantly differ (P = 0.05, Duncan's MRT).

Table 3. Beet armyworm survival on cotton leaves from the field during May of 1995 as affected by DIMILIN 2L applied at either 1 hr vs. 15 days or 1 hr vs. 30 days prior to the initiation of 5 days of bioassay (DOB).

Treatment	Trt* Appl	Rate in lb ai/ac	Mean # of Larvae Surviving 1 DOB	3 DOB	5 DOB
1st Sampling					
UNTREATED			4.9 a**	4.4 a	3.9 a
DIMILIN 2L	1 h	0.062	4.4 ab	2.1 b	0.1 b
DIMILIN 2L	15 d	0.062	3.9 b	2.0 b	0.5 b
2nd Sampling					
UNTREATED			3.4 a	1.4 a	1.1 a
DIMILIN 2L	1 h	0.062	3.3 a	0.3 b	0.0 b
DIMILIN 2L	30 d	0.062	3.8 a	1.7 a	0.4 b

*It took one day to initiate the bioassays due to shipment of leaf tissue from Sanford, FL to Bethany, CT.
**Means followed by the same letter within a column for a given sampling do not significantly differ (P = 0.05, Duncan's MRT).

Table 4. Beet armyworm survival on cotton leaves from the field in Sept/Oct 1995 as affected by DIMILIN 2L applied at either 1 hr and 13 days or 1 hr and 28 days prior to the initiation of 5 days of bioassay (DOB).

Treatment	Trt* Appl	Rate in lb ai/ac	Mean # of Larvae Surviving 1 DOB	3 DOB	5 DOB
1st Sampling					
UNTREATED			3.8 a**	3.1 a	2.6 a
DIMILIN 2L	1 h	0.062	3.5 a	0.6 c	0.0 b
DIMILIN 2L	13 d	0.062	3.8 a	1.4 b	0.1 b
2nd Sampling					
UNTREATED			4.1 a	2.8 a	1.8 a
DIMILIN 2L	1 h	0.062	3.3 a	0.3 c	0.0 b
DIMILIN 2L	28 d	0.062	3.3 a	1.2 b	0.1 b

*It took one day to initiate the bioassays due to shipment of leaf tissue from Sanford, FL to Bethany, CT.
**Means followed by the same letter within a column for a given sampling do not significantly differ (P = 0.05, Duncan's MRT).

Table 5. Beet armyworm survival on cotton leaves from the field in Sept/Oct 1995 as affected by DIMILIN 2L applied at either 1 hr and 20 days or 1 hr and 34 days prior to the initiation of 5 days of bioassay (DOB).

Treatment	Trt* Appl	Rate in lb ai/ac	Mean # of Larvae Surviving 1 DOB	3 DOB	5 DOB
1st Sampling					
UNTREATED			4.1 a**	2.8 a 1	.9 a
DIMILIN 2L	1 h	0.062	3.3 a	0.3 b	0.1 b
DIMILIN 2L	20 d	0.062	3.7 a	0.6 b	0.0 b
2nd Sampling					
UNTREATED			4.6 a	4.0 a	2.4 a
DIMILIN 2L	1 h	0.062	4.0 b	1.0 c	0.0 c
DIMILIN 2L	34 d	0.062	4.5 ab	3.1 b	1.3 b

*It took one day to initiate the bioassays due to shipment of leaf tissue from Sanford, FL to Bethany, CT.
**Means followed by the same letter within a column for a given sampling do not significantly differ (P = 0.05, Duncan's MRT).