GROWTH INHIBITION OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) LARVAE REARED ON LEAF DIETS OF NON-HOST PLANTS

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ABSTRACT

Chemists and natural plant product scientists have shown an interest in compounds from exotic plants that are considered non-hosts for the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), but may serve as sources of materials that reduce feeding and growth of herbivorous insects. We report here results of laboratory bioassays with neonate and fifth instar fall armyworm fed on a standard diet alone and on an amended diet with celufil or leaves from dogwood, *Cornus florida* L., hydrangea, *Hydrangea macrophylla* (Thunb.) Seringe, black cherry, *Prunus serotina* Ehrh., or Bradford pear, *Pyrus calleryna* Decne. The neonates had reduced growth and frass production when fed diets containing leaves of dogwood, hydrangea, black cherry and Bradford pear while fifth-instars had reduced consumption and weight gain when fed the hydrangea leaf-diet. These results suggest a toxic component in the leaves of hydrangea that reduces the development of the fall armyworm neonates and fifth instars. The results also indicate that the leaves of dogwood, black cherry and Bradford pear have growth inhibitors present in their leaves that adversely affect growth of neonate fall armyworm. Because fifth instars performed similarly to controls when fed dogwood, cherry or pear leaf-diets, older larvae may be able to overcome the plants natural defenses better than neonates.

Key Words: *Spodoptera frugiperda*, antibiosis, deterrent, growth inhibition, protein

RESUMEN

Los químicos y los estudiosos de los productos de plantas naturales han mostrado interés en complejos derivados de plantas exóticas no consideradas como hospedantes de *Spodoptera frugiperda* (J. E. Smith) pero que pueden servir como fuente de materiales que reducen la capacidad de alimentarse y el crecimiento en los insectos herbívoros. Reportamos aquí los resutados de bioensayos de laboratorio con *S. frugiperda* neonatas y del quinto instar alimentadas en una dieta estándar solamente y con una dieta con hojas de *Cornus florida* L., *Hydrangea macrophyla* (Thunb.) Seringe, *Prunus serotina* Ehrh. o *Pyrus calleryna* Decne. Los neonatos mostraron reducción del crecimineto y de la producción de excretas cuando fueron alimentados con dietas conteniendo hojas de *C. florida* L., *H. macrophylla*, *P. serotina* y *P. calleryna*, mientras que el quinto instar tuvo reducción del consumo de alimento y de la ganancia de peso cuando fue alimentado con una dieta de *H. macrophylla*. Estos resultados suguieren que un componente tóxico en las hojas de *H. macrophylla* reduce el desarrollo de los neonatos y quintos instares de *S. frugiperda*. Los resultados además indican que las hojas de *C. florida*, *P. serotina* y *P. calleryna* tienen inhibidores de creciemiento presentes en sus hojas que afectan adversamente el crecimiento de los neonatos. Debido a que los quintos instares se comportaron como los testigos cuando se alimentaron con *C. florida*, *P. serotina* o *P. calleryna*, las larvas más viejas parecen ser capaces de vencer las defensas naturales de las plantas mejor que los neonatos.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), continues to be an important economic pest as was emphasized by participants in the 1996 Armyworm Symposium at the annual meeting of the Southeastern Branch of the Entomological Society of America. One of the important controls for the fall armyworm has been plant resistance. This has led to questions such as: (1) Why don't larvae of the fall armyworm feed on all plant species and (2) could fall armyworm larvae feed on a non-host plant in no-choice situations? Doskotch et al. (1977) stated that it is evident from field observations of the Gypsy moth, *Lymantria dispar* (L.), that phytophagous insects utilize very few plant species as hosts, although many plant species may be acceptable to the Gypsy moth species. Bernays (1983) suggested that the use of natural feeding deterrents is an intuitively satisfying but logistically questionable tactic in crop pest management. Possibly, in the near future, chemicals from non-host plants will be exploited as new environmentally safe pesticides that can be used in biotechnology to render domestic crop plants to a non-host status.

Warthen et al. (1982) and Wiseman et al. (1986) searched a number of non-host plants and found several antifeedant responses by larvae of the fall armyworm. Both research groups used one non-host plant in common, the dogwood tree, *Cornus florida*

L. Warthen et al (1982) evaluated twigs while Wiseman et al. (1986) evaluated leaves for response to larvae of the fall armyworm. Wiseman et al. (1986) also found non-host plants, such as Jalapeno peppers and banana leaves (unpublished) on which the larvae of the fall armyworm performed well when plant materials were mixed in diets.

The dogwood is a small forest tree, common in the Southeastern United States. Weiner (1982) reported that the Delaware Indians boiled the bark of the dogwood in water and used the solution to reduce fever. Hostettmann et al. (1978) found a molluscicidal saponin in the bark of the dogwood and that *Biomphalaria glabratus* snails were killed within 24 h by a 6 to 12-ppm solution of the saponins. Miller (1978) found that homogenized leaf solutions of dogwood applied to the soil were toxic to the root lesion nematode, *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa. Villani & Gould (1985) found that crude plant extracts of dogwood leaves did not deter feeding by the wireworm, *Melanotus communis* Gyll. Warthen et al. (1982) showed that 1.5 g of dogwood twigs in 100 g of diet resulted in about 30% mortality of fall armyworm larvae. However, when Wiseman et al. (1986) used 50 g of fresh dogwood leaves in 400 ml of diet, 8-day-old fall armyworm larvae weighed an average of only 0.4 mg. In addition, none of the larvae fed the dogwood leaf diet pupated.

Chemists and natural product scientists have shown an interest in some of the exotic plant materials that were reported by Wiseman et al. (1986). Other plant species whose leaves have compounds that also have possibilities as feeding deterrents against larvae of the fall armyworm are: hydrangea, *Hydrangea macrophylla* (Thunb.) Seringe, black cherry, *Prunus serotina* Ehrh., and Bradford pear, *Pyrus calleryna* Decne. Therefore, we report results of feeding tests with leaves of these plant species incorporated in artificial diets to determine their effects on fall armyworm larval performance.

MATERIALS AND METHODS

Fall armyworm larvae were obtained from a culture maintained on a modified pinto-bean diet (Burton & Perkins 1989) at the Insect Biology and Population Management Research Laboratory, Tifton, GA.

Four experiments each were conducted in a randomized complete-block design with 30 replications and 1 cup per replicate. An extra set of treatments of 15 cups each was set up to measure the decrease in moisture of each diet during the test period. Controls for each experiment were regular pinto bean diet (Burton & Perkins 1989) and pinto bean diet diluted by combining 3 ml of prepared diet with 2 ml of water and mixed at a rate of 50 mg of celufil per ml of dilute diet. Celufil, a fine mesh cellulose filler with minimum nutritive value, binds with laboratory diets and does not readily absorb water. Diets prepared with celufil as an inert material for the controls remain fluid and are easily dispensed. For each experiment and leaf material diet, the pinto bean diet was diluted by combining 3 ml of prepared diet with 2 ml water and 50 mg of ground (1 mm screen) oven-dried leaves per ml dilute diet. All experiments were held in a controlled environmental room maintained at $28 \pm 2°C$ and $65 \pm 2\%$ RH with a photoperiod of 14:10 (L:D) h.

Experiment 1

Treatments consisted of dogwood and hydrangea leaves mixed in dilute pinto bean diet, regular pinto bean diet, and a dilute bean diet plus 50 mg celufil per ml dilute diet. The diet treatments were dispensed about 10 ml per cup into 30-ml plastic diet cups. The diets were allowed to cool for about 2 h, after which 1 neonate fall armyworm was introduced into each cup and the cup was capped. Weights of larvae and cumulative frass produced (weighed immediately after collection from each cup) were recorded at 9 d.

Experiment 2

Treatments consisted of 50 mg each of oven-dried cherry and pear leaves mixed per ml dilute pinto bean diet, regular pinto bean diet, and a dilute pinto bean diet plus 50 mg celufil per ml dilute diet. The diet treatments were dispensed into 30-ml plastic diet cups of about 10 ml per cup. The diets were allowed to cool for about 2 h, after which 1 neonate fall armyworm was introduced into each cup and the cup was capped. Weights of larvae and cumulative frass produced (weighed immediately after collection from each cup) were recorded at 8 d; the same larvae, frass accumulation and diet consumption were weighed again 24 h later (9 d).

Experiments 3 and 4

Treatments consisted of 50 mg each of oven-dried dogwood or hydrangea leaves per ml dilute pinto bean diet (Experiment 3) and cherry or pear leaves (Experiment 4). Regular pinto bean diet and a dilute pinto bean diet plus 50 mg celufil per ml dilute diet served as controls. Treatments of diets and procedures were as described above except that fifth instar fall armyworm were used. Larvae were reared to the fifth instar on the regular pinto bean diet. Initial weights of diets and larvae were recorded as the larvae were placed on the diets. Weight of diets, larvae, and fresh frass was recorded after 24 h. Weight of dry frass and diets was recorded for each after they were dried in an oven at about 50°C for 7 d. The percentage of nitrogen in the dry diets and frass from the latter 2 experiments was determined by an FP-228 nitrogen determinator (Model 601-700, Leco, St. Joseph, MI). Protein content was estimated by the formula 6.25 X % nitrogen (Helrich 1990).

Data were analyzed by PROC GLM analysis (SAS Institute 1989) and significantly different means were separated by least significant difference ($P \leq 0.05$) (SAS Institute 1989).

Results and Discussion

Experiment 1

Weight of fall armyworm larvae (Table 1) after having fed on dogwood- and hydrangea-leaf diets for 9 d was significantly ($P \leq 0.05$) different from the weight of larvae that were fed the regular pinto bean diet or diluted diet plus celufil. In fact, none of the larvae fed hydrangea-leaf diet were alive at 9 d. The dilute diet with celufil is capable of supporting larval growth and development equivalent to the regular pinto bean diet. Therefore, addition of leaf materials did not simply dilute the diet and cause a reduction in growth, i. e. the dogwood-leaf diet. But, since the larvae died on the hydrangea-leaf diet, the presence of a toxic factor was indicated. Frass production during the 9 d feeding was significantly less for larvae that fed on dogwood- and hydrangea-leaf diets than for larvae that fed on the two control diets.

Experiment 2

Larvae weighed significantly ($P \leq 0.05$) less at 8 and 9 d when fed cherry- or pear-leaf diets than when they were fed on the control diets (Table 2). Frass production for larvae

TABLE 1. WEIGHT OF FALL ARMYWORM LARVAE AND FRASS FROM NEONATES REARED ON DIETS CONTAINING DOGWOOD OR HYDRANGEA LEAVES

	Weight (mg) ± SEM at 9 Days	
Food Source	Larvae[a]	Frass[1]
Reg. bean diet	208.8 ± 8.3a	192.3 ± 12.8b
Diet + celufil	193.0 ± 8.1a	347.1 ± 12.3a
Diet + dogwood	11.6 ± 8.3b	11.6 ± 12.5c
Diet + hydrangea	0.0 ± 8.1b	0.0 ± 12.3c

[1]Means within a column followed by the same letter are not significantly different ($P \leq 0.05$ LSD; SAS Institute 1989). SEM = standard error of mean was based on pooled error mean square in the analysis.

that were fed these diets was significantly ($P \leq 0.05$) less at both 8 and 9 d than that for larvae fed the control diets. Both consumption and weight gain during the 24-h feeding period between days 8 and 9 were significantly ($P \leq 0.05$) less for the larvae fed the black cherry- and Bradford pear-leaf diets than for larvae that fed on the control diets.

Experiments 3 and 4

The initial weights of the fifth instars for the various treatments were not significantly different before they were transferred to the test diets (Tables 3 and 4). However, after 24-h, larvae fed the hydrangea-leaf diet weighed significantly ($P \leq 0.05$) less than larvae that were fed the dogwood-leaf diet or the control diets. The mean weight of larvae that were fed the dogwood diet was not different from the weight of larvae fed the control diets. Larvae fed the regular pinto bean diet gained significantly ($P \leq 0.05$) more weight during the 24-h feeding period than did the larvae fed the diet plus celufil or the dogwood-leaf diet. Weight gain during the 24-h feeding period was significantly ($P \leq 0.05$) less for larvae fed the hydrangea-leaf diet than for larvae fed the other three treatment diets. Larvae consumed more diet and excreted more frass when fed the diet plus celufil and dogwood-leaf diet than for larvae fed the regular pinto bean diet. Consumption and weight of frass were significantly less for the larvae that fed on the hydrangea-leaf diet than for any of the other three treatments. Percentage nitrogen of the diet or frass as an indication of the amount of protein in the diets or frass were also significantly ($P \leq 0.05$) different among treatments. The regular diet had the highest percentage of nitrogen in the diet but an intermediate amount in the frass. The diet plus the celufil had the least nitrogen in the diet and the frass. The hydrangea-leaf diet had an intermediate percentage nitrogen in the diet but the greatest percentage nitrogen in the frass.

Larvae fed the regular pinto bean diet utilized more of the protein consumed than did those that were fed on the other diets. Larvae feeding on the diet plus the celufil consumed more diet than larvae fed the regular pinto bean diet. Nitrogen content was lower in the diet plus celufil and in frass from larvae fed the diet plus celufil, indicating that the larvae that were fed this diet had to consume more diet and excrete less nitrogen to obtain the necessary protein for the growth. However, since the larvae excreted more frass, they also excreted more nitrogen (5.04 versus 3.46 mg) than did larvae that were fed the regular pinto bean diet. Larvae that fed on the dogwood-leaf diet excreted more than twice as much frass as those fed the regular diet. Larvae fed the regular pinto bean diet and dogwood-leaf diet consumed almost equal amounts of pro-

TABLE 2. WEIGHT AND LEAF CONSUMPTION FOR FALL ARMYWORM LARVAE REARED ON DIETS CONTAINING BLACK CHERRY OR BRADFORD PEAR LEAVES[1]

	Weight (mg) ± SEM[2]					
	Larvae		Frass			
Treatment	8 Days	9 Days	8 Days	9 Days	Consumption[3] (mg)	Weight Gain in 24 hrs
Reg. Bean Diet Check	204 ± 6a	394 ± 14a	290 ± 13b	272 ± 18a	362 ± 31a	201 ± 13a
Bean Diet + Celufil Check	200 ± 6a	319 ± 14b	498 ± 13a	307 ± 19a	322 ± 32a	120 ± 13b
Black cherry leaves	1 ± 6b	4 ± 14c	35 ± 13c	18 ± 18b	134 ± 31b	1 ± 13c
Bradford pear leaves	4 ± 6b	1 ± 14c	0 ± 13d	0 ± 18b	0 ± 32c	0 ± 13c

[1]Means within a column followed by the same letter are not significantly different ($P \le 0.05$ LSD; SAS Institute 1989).
[2]SEM = standard error of mean was based on the pooled error mean square in the analysis.
[3]Diet consumption = (initial fresh weight of diet - final fresh weight of diet) × [(100 - % moisture loss)/100].

TABLE 3. WEIGHT, CONSUMPTION AND NITROGEN CONTENT OF DIET AND FRASS FOR FIFTH-INSTAR FALL ARMYWORM LARVAE REARED ON DIETS CONTAINING DOGWOOD OR HYDRANGEA LEAVES[1]

	Larval Weight (mg) ± SEM[2]				Percent Nitrogen ± SEM[2]	
Treatment	Initial Larval Wt	After 24 hrs Larval Wt	Consumption[c] (mg)	Frass (mg)	Diet	Frass
Reg. bean diet check	163 ± 5a	379 ± 10a	745 ± 41b	144 ± 18b	3.9 ± 0.01a	2.4 ± 0.1b
Bean diet + celufil check	158 ± 5a	351 ± 10b	859 ± 41a	315 ± 18a	2.9 ± 0.01c	1.6 ± 0.1c
Dogwood leaves	165 ± 5a	357 ± 10ab	880 ± 41a	319 ± 18a	3.3 ± 0.01b	2.2 ± 0.1b
Hydrangea leaves	161 ± 5a	191 ± 11c	425 ± 43c	79 ± 19c	3.3 ± 0.01b	2.8 ± 0.1a

[1]Means within a column followed by the same letter are not significantly different ($P \leq 0.05$ LSD; SAS Institute 1989).
[2]SEM = standard error of mean was based on the pooled error mean square in the analysis.
[3]Diet consumption = (initial fresh weight of diet - final fresh weight of diet) × [(100 - % moisture loss)/100].

TABLE 4. WEIGHT, CONSUMPTION AND NITROGEN CONTENT OF DIET AND FRASS FOR FIFTH-INSTAR FALL ARMYWORM LARVAE REARED ON DIETS CONTAINING BLACK CHERRY OR BRADFORD PEAR LEAVES[1]

	Larval Weight (mg) ± SEM[2]				Percent Nitrogen ± SEM[2]	
Treatment	Initial Larval Wt	After 24 hrs Larval Wt	Consumption[3] (mg)	Frass (mg)	Diet	Frass
Reg. bean diet check	144 ± 3a	345 ± 9a	760 ± 19b	259 ± 17c	4.0 ± 0.01a	2.0 ± 0.04a
Bean diet + celufil check	137 ± 3a	296 ± 9c	716 ± 19b	403 ± 17b	2.8 ± 0.01d	1.4 ± 0.04b
Black cherry leaves	145 ± 3a	324 ± 9ab	914 ± 19a	560 ± 17a	3.5 ± 0.01b	2.0 ± 0.04b
Bradford pear leaves	142 ± 3a	301 ± 9bc	731 ± 19b	378 ± 17b	3.4 ± 0.01c	2.1 ± 0.04a

[1]Means within a column followed by the same letter are not significantly different ($P \leq 0.05$ LSD; SAS Institute 1989).
[2]SEM = standard error of mean was based on the pooled error mean square in the analysis.
[3]Diet consumption = (initial fresh weight of diet - final fresh weight of diet) × [(100 - % moisture loss)/100].

tein (29 versus 28 mg), but excreted more than twice as much nitrogen (3.45 versus 7.02 mg). Fifth-instar fall armyworms fed the hydrangea-leaf diet consumed less than one-half the amount of diet consumed by larvae fed on the dogwood-leaf diet. And, the nitrogen consumption was about one-half as much. However, the nitrogen excreted by larvae fed the hydrangea-leaf diet was less than one-third as much as that excreted by larvae fed the dogwood-leaf diet and similar to the nitrogen excreted by larvae fed the regular pinto bean diet.

Weight of the larvae that fed on the pinto bean diet was significantly ($P \leq 0.05$) greater after 24-h than the weight of larvae fed the diet plus celufil and pear-leaf diet (Table 4). Larvae fed the cherry-leaf diet weighed significantly more than larvae that fed on the diet plus celufil diet. However, consumption was significantly ($P \leq 0.05$) greater for larvae fed the cherry-leaf diet than for larvae that fed on the other treatment diets. Frass production was also significantly ($P \leq 0.05$) greater for larvae fed the cherry-leaf diet than for the other treatments. Frass production was the least from larvae that fed on the regular diet. Percentage nitrogen in the pinto bean diet was significantly higher than the percentage nitrogen in the cherry, pear or celufil diets. Percentage nitrogen was lowest in the celufil diet as well as in the frass produced. The consumption of nitrogen was highest by larvae fed the cherry-leaf diet (32.0 mg) and least by larvae fed the celufil diet (20.0 mg). However, percentage nitrogen in the frass produced by larvae fed the diets of regular bean, cherry and Bradford pear-leaf were not significantly different. The amount of nitrogen in the frass varied considerably from 11.2 mg for larvae that fed on the cherry leaf diets to those that fed on the pear-leaf diet (7.94 mg), regular diet (5.18 mg) and celufil check (5.64 mg).

In summary, fall armyworm neonates did not perform well after feeding on the dogwood, hydrangea, black cherry and Bradford pear-leaf diets. Larvae may have exhibited compensatory feeding when offered the celufil and dogwood-leaf diets as their consumption increased over larvae that fed on the regular pinto bean diet. The results also indicated that the leaves of dogwood, black cherry and Bradford pear have growth inhibitors present in their leaves that adversely affect the growth of neonate fall armyworm. Fall armyworm fifth instars had reduced consumption and weight gain when fed a hydrangea-leaf diet. These results suggested either a severe growth inhibitor or a toxic component in the leaves of hydrangea that caused total mortality as neonates and reduced the performance of the fall armyworm fifth instars. Larvae that fed on the cherry- and pear-leaf diets excreted much higher amounts of nitrogen than larvae that fed on either control diets, indicating a poor assimilation of the protein in these diets. Lastly, these non-hosts offer good possibilities for the discovery of new chemicals that could be used in the management of the fall armyworm.

ACKNOWLEDGMENTS

We thank Johnny Skinner, Charles Mullis, and Robert Caldwell for their technical support.

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