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ADVANCEMENTS IN THE USE OF A LABORATORY BIOASSAY FOR BASIC HOST PLANT RESISTANCE STUDIES

B. R. WISEMAN AND R. E. LYNCH
Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, GA 31793

K. L. MIKOLAJCZAK
Northern Regional Research Center, USDA-ARS, Peoria, IL 61604
AND
R. C. GUELDNER
Russell Research Center, USDA-ARS-SAA, Athens, GA 31613

ABSTRACT

Modifications were made in laboratory bioassays for resistance of plants to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). A bioassay using a 6-mm diameter X 2-cm section of a plastic soda straw filled with a diet mixture proved sufficient to bioassay differences between resistant centipedegrass and susceptible bermudagrass. Chemical solvents used to dissolve plant fractions may in themselves be toxic to test insects. Ethyl alcohol required ca. 5 h to evaporate, when incorporated into an insect diet, before it was nontoxic to the fall armyworm. The bioassay was readily adaptable for use in the search for antibiotic factors from exotic plant species.

RESUMEN

Se hicieron modificaciones en el laboratorio de bioensayos de resistencia de plantas al gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). Un bioensayo usando una sección de 6 mm de diámetro X 2 cm de un plástico llenado con una dieta mezclada, fue suficiente para ver diferencias entre el resistente centipedegrass y el susceptible bermudagrass. Solventes químicos usados para disolver fracciones de plantas pudieran por si solos ser tóxicos a los insectos en las pruebas. El alcohol de etilo requirió aproximadamente 5 h para evaporarse cuando se incorporó a una dieta de insectos antes de no ser tóxico al gusano cogollero. El bioensayo fue adaptado prontamente para uso en la búsqueda de factores antibióticos en especies de plantas exóticas.

Bioassays are essential in the study of the chemical basis of host plant resistance (HPR) to insects. A laboratory bioassay using meridic diet, centipedegrass [*Eremochola*

ophiuroides (Munro) Hackel] as the resistant plant material and the fall armyworm (FAW) [*Spodoptera frugiperda* (J. E. Smith)] was first developed by Wiseman et al. (1984b). Modifications of this bioassay have been used to measure biological responses of FAW to various plant materials such as panicles of sorghum (Wiseman et al. 1984c), corn silks (Wilson et al. 1984), sorghum leaves (Wiseman et al. 1984a), corn leaves (Isenhour et al. 1985), *Tripsacum* leaves, and for initial screenings of germplasm (Wiseman et al. unpublished).

We report here some new uses and advances in techniques to bioassay plant materials for resistance against feeding by the FAW.

Materials and Methods

A series of related experiments was conducted to enhance the development of a laboratory bioassay for HPR studies. All experiments were maintained in a constant temperature room at 27 ± 2°C and 75 ± 5% RH.

Micro-technique

The laboratory FAW bioassay used in past tests required 250-300 ml of pinto bean diet in 100-150 ml of distilled water. A minimum of 10 g of resistant plant material, either fresh, freeze-dried, or air-dried, is added to the liquid diet and homogenized (Wiseman et al. 1984b). However, chemical fractionation of plant materials produces only small quantities of chemicals for assay. Thus, a microassay was needed to accommodate the smaller amount of material that would be added to the insect diet. This was accomplished by blending 20 ml of bean diet, 10 ml of distilled water, and 2 g of fresh grass leaves in a 37-ml mini-blender for ca. 3-5 min. The blended material was then aspirated into a plastic soda straw ca. 6 mm in diam and 20 cm in length and allowed to cool and solidify for ca. 2 h. The straw was dissected into 2 cm lengths (ca. 460 mg of diet mixture), sections of the straw were placed in 18.7-ml plastic diet cups, 1 neonate FAW was introduced, and the cup was capped with a polycoated lid to prevent rapid drying of the diet. The amount of diet initially mixed for this test yielded ca. 40 sections of straw.

Treatments consisted of centipedegrass, bermudagrass, and a diet check arranged as a randomized complete block with 18 replications and 2 cups/replicate. Larvae were weighed on the 4th and 7th days after infestation. Using only the amount of diet contained in 2 cm of soda straw, FAW larvae consumed all the diet by ca. 8 days. Therefore, the check diet was closely monitored and all larvae were weighed before the diet in any of the treatments was totally consumed. Standard statistical procedures were used, and Duncan's multiple range test was applied to separate means at $P = \leq 0.05$ (Duncan 1955).

Solvent test

Various solvents are used to extract fractions from plant materials for chemical isolation. One solvent, ethyl alcohol (ETOH), was evaluated to determine its effect on survival of FAW larvae.

Sixty ml of pinto bean diet was blended in a household blender with 37 ml of distilled water and 23 ml of 70% ETOH. Ca. 4 ml of each mixture was dispensed individually into each of thirty 18.7-ml plastic diet cups. Treatments were as follows: pinto bean diet check infested with larvae after allowing 2 h for diet solidification, ETOH check (0)

infested after allowing 2 h for diet solidification, pinto bean diet plus ETOH treatments allowed to solidify for 2 h and then air dried in a fume hood for l, 2, 3, 4, or 5 h before infesting with larvae. One neonate FAW larva was introduced into each cup, and all cups were capped with regular diet cup lids. The test was arranged as a randomized complete block design with 30 replications and 1 cup/replicate. Mortality and biological notes were recorded after 24 h, 3 days, and 7 days. Standard statistical procedures were used, and Waller-Duncan k-ratio t test was applied to separate means at $P = \leq 0.05$ (SAS 1982).

Combinations of Plant Materials

Combinations of resistant and susceptible plant materials were evaluated using the bioassay described by Wiseman et al. (1984b). The pinto bean diet (250 ml) was blended with 120 ml of distilled water and 10 g of freeze-dried plant materials. Eight diets of various combinations of plant materials were made and dispensed at ca. 7 ml/cup into thirty-six 30-ml plastic diet cups for each treatment. Treatments were: (1) bermudagrass; (2) corn leaves; (3) centipedegrass; (4) bermudagrass + centipedegrass (1:1 by weight); (5) corn leaves + centipedegrass; (6) corn silks; (7) corn silks + centipedegrass; and (8) an untreated check. One neonate FAW larva was introduced per cup and procedures were similar to those above. Weights of larvae were recorded at 8 days. The test was arranged as a randomized complete block with 18 replications with 2 cups/replicate. Standard statistical procedures were used and Waller-Duncan k-ratio t test was applied to separate means at $P = \leq 0.05$.

Antibiosis Factors in Exotic Plant Materials

The following test was conducted to evaluate possible effects of exotic plant materials on FAW growth and to encourage a search for antifeedants, toxic factors, etc., in natural plant products. Several plant species (Table 4) were harvested in 50-g samples. Each sample was blended as fresh material into slurry with 100 ml of water and then 300 ml of regular pinto bean diet was added. The diet mixtures were dispensed into thirty-six 30-ml cups. The diets were allowed to cool and solidify for ca. 2 h, then one neonate FAW was introduced for each cup and treatment. Cups were capped with paper lids. The test was arranged as a randomized complete block design with 18 replications and 2 cups/replicate. Weights of larvae were recorded at 8 days. In addition, days to pupation and weights of 1-day pupae were recorded. Standard statistical procedures were used for analysis and means were separated by Duncan's new multiple range test at $P = \leq 0.05$.

Evaluation of Natural Plant Chemicals

Natural plant chemicals from tea, i.e., caffeine, theophylline, and theobromine, were evaluated for FAW growth responses. These chemicals were incorporated into 300 ml of pinto bean diet with 100 ml of distilled water. Diets were dispensed into thirty-six 30-ml plastic cups for each treatment, allowed to cool and solidify for 2 h. One neonate larva was introduced and the cup was capped with a paper lid. The test was designed as a split plot with 18 replications and 2 cups/replicate. Whole plots were concentrations of natural products per diet of 0, 0.25, 0.50, 1.0, 2.0, and 4.0 g/400 ml. Sub-plots were caffeine, theophylline, and theobromine. Standard analysis (SAS 1982) of the data was

applied and Duncan's new multiple range test was used to separate mean differences at $P = \leq 0.05$.

Results and Discussion

Modifications of the bioassay developed by Wiseman et al. (1984b) were required because of the type of plant material, limited amounts of chemical fractions, or slight changes in the use of the bioassay for particular needs or programs.

Micro-techniques

Table 1 shows the mean weights of FAW larvae fed 4 and 7 days when the diets were placed in soda straw sections. The use of the 6-mm x 2-cm sections will not permit a longer observation period unless larvae remain small. The larvae fed the check diets consumed almost all the diet by 7 days. However, the use of a substandard diet such as the bean diet without yeast (Wiseman et al. 1984b) would possibly allow for a longer observation period because larvae would not consume as much diet and develop as rapidly as larvae on the regular bean diets. Significant differences were detected at 4 and 7 days between weights of larvae that fed on bermudagrass versus larvae that fed on centipedegrass diet mixtures. Thus, the microassay using the straw to hold the diet can be used where small amounts of plant materials or fractions are available.

Solvent test

The results of the ETOH test are presented in Table 2. ETOH incorporated into the pinto bean diet resulted in 100% mortality at 24 h for the 0- and the 1-h drying periods. Larval mortality declined with increased evaporation time for ETOH. It appears from these data that a solvent such as ETOH should be evaporated from the diet for at least 5 h before introducing larvae into test cups. This drying period was in addition to the 2 h normally required for diet cooling and solidification. Other solvents such as hexane, acetone, methanol, ether, etc., are also likely to produce larval mortality when incorporated into an insect diet unless they are adequately evaporated before insects are introduced. These solvents must be tested individually to determine evaporation time before valuable plant fractions are incorporated into insect diets.

TABLE 1. Weight of fall armyworm larvae at 4 and 7 days after feeding via plastic soda straws on resistant or susceptible grasses mixed in bean diet.

Treatment	Weight (mg) at[1]	
	4 days	7 days
Bermudagrass	4.8a	67.6a
Diet check	4.0b	64.5a
Centipedegrass	3.1c	49.1b

[1]Mean weights within each column followed by the same letter are not significantly different ($P \leq 0.05$, Duncan's multiple range test).

TABLE 2. PERCENT MORTALITY OF NEONATE FALL ARMYWORM LARVAE AFTER VARIOUS EVAPORATION TIMES WHEN 70% ETOH WAS MIXED IN THE PINTO BEAN DIET.

Evaporation time (h)[2]	Percent mortality[1]		
	24 h	3 days	7 days
0 ETOH check	100d	100d	100e
1	100d	100d	100e
2	67c	70c	80d
3	13b	10ab	33c
4	10ab	13b	17b
5	0a	0a	0a
0 Reg. check	0a	0a	0a

[1]Percentages within each column followed by the same letter are not significantly different ($P = \leq 0.05$, Waller-Duncan k-ratio t test).
[2]Evaporation time after initial 2 h for solidification period.

COMBINATIONS OF PLANT MATERIALS

Table 3 shows the weights of FAW larvae fed resistant or susceptible plant materials or combinations of the two incorporated into the pinto bean diet. The larvae fed the centipedegrass-diet mixture were significantly smaller than those fed the bermudagrass-diet mixture. When centipedegrass was mixed with other plant materials, the browning (Chang et al. 1985 and Wilson et al. 1984) that normally occurs on the top of the diet was enhanced. The larvae that fed on the centipedegrass mixed with other plant material were, in general, significantly smaller than those fed centipedegrass alone. Thus, it appears that other plant materials mixed with this resistant plant material may increase the detrimental effect on the larvae.

TABLE 3. WEIGHT OF FALL ARMYWORM LARVAE AFTER FEEDING ON RESISTANT, SUSCEPTIBLE, OR VARIOUS COMBINATIONS OF PLANT MATERIALS MIXED IN THE PINTO BEAN DIET.

Treatment[2]	Weight (mg) of larvae[1] at 8 days
Bermudagrass	151.4a
Corn leaves	126.6b
Centipedegrass	106.5c
Bermudagrass + centipedegrass	99.3c
Corn leaves + centipedegrass	77.4d
Corn silks	74.4d
Corn silks + centipedegrass	53.9e
Check	114.9bc

[1]Mean weights of larvae followed by the same letter are not significantly different ($P \leq 0.05$, Waller-Duncan k ratio t test). Corn leaves = Caca. X's 11-leaf stage; corn silks = Stowell's Evergreen 2-day-old silks.
[2]Combinations of plant material included 10 g of each material.

ANTIBIOSIS FACTORS IN EXOTIC PLANT MATERIALS

Several readily available plant materials show strong potential antifeedant activity against the FAW larvae (Table 4). Oleander leaves, commonly known to be highly toxic to humans, did not seriously affect FAW larval growth. Interestingly, however, Zapalote Chico silks, Texas purple sage leaves and dogwood leaves showed great potential for possible antibiosis factors. Pyracantha, Zapalote Chico, Texas purple sage, and dogwood diets also delayed FAW development more than 1 week. The development of FAW larvae fed dogwood leaf diets was greatly retarded at 8 days and all of the larvae failed to pupate.

EVALUATION OF PLANT CHEMICALS

Caffeine and several related methylxanthines have been evaluated against tobacco hornworm, *Manduca sexta* L., larvae for possible feeding inhibitors and for pesticidal activity (Nathanson 1984). Table 5 shows the antifeedant effects of caffeine, theophylline, and theobromine on the FAW when mixed in pinto bean diets. All three of the chemicals significantly reduced the weight of FAW larvae, but there were no significant differences between chemicals. However, concentration of chemicals did have a significant impact upon the growth of FAW larvae. There were no significant chemical X concentration interactions. As little as 0.06% of each chemical incorporated into the diet caused a detrimental effect on growth of FAW larvae by 8 days. A 1% concentration of the chemicals by diet weight essentially inhibited growth of FAW larvae. About 90% of the larvae on the highest caffeine treatment died at ca. 10 days, whereas ca. 45% of the larvae on the highest theophylline treatment were dead by ca. 10 days. None of the larvae on the highest theobromine treatment were dead at ca. 10 days. Also, at the 4-g (1%) concentration of all three chemicals, more than 88% of the larvae failed to pupate.

In summary, modifications of the FAW bioassay (Wiseman 1984b) can be readily adapted. The soda-straw technique with the 18.7 ml diet cup capped with a polycoated lid permits use in micro-procedures. When solvents are used, caution should be taken to dry or evaporate the solvent from the diet before larvae are introduced. The bioassay is also adaptable for use in searching for additional antibiosis factors in exotic plants.

TABLE 4. GROWTH RESPONSES OF NEONATE FALL ARMYWORM LARVAE FED EXOTIC PLANT MATERIALS MIXED IN THE PINTO BEAN DIET.

Plant materials	Wt. (mg) of larvae[1] at 8 days	Days to pupation	Wts. of pupae
Diet check	271.9a	13.4b	266.lab
SEG silks (*Zea mays*)	240.2b	14.1b	283.7a
Jalapeno pepper (*Capsicum* sp.)	214.1c	14.0b	260.2b
Oleander leaves (*Nerium oleander*)	94.4d	16.7c	256.9b
Cotton leaves (*Gossypium hirsutum*)	52.0e	18.9d	228.7c
Wild cotton leaves (*Gossypium* sp.)	47.9ef	19.5de	181.9d
Pyracantha berries (*Pyracantha* sp.)	26.5fg	20.7e	181.3d
Zapalote Chico silks (*Zea mays*)	9.1gh	25.1f	190.1d
Texas purple sage leaves (*Artemisia* sp.)	6.3gh	23.9f	217.0c
Dogwood leaves (*Cornus florida*)	0.4h	dead a	dead e

[1]Mean weights of larvae followed by the same letter are not significantly different ($P \leq 0.05$, Duncan's multiple range test).

TABLE 5. EFFECTS OF VARIOUS CONCENTRATIONS OF CAFFEINE, THEOPHYLLINE, OR THEOBROMINE IN PINTO BEAN DIETS ON FALL ARMYWORM DEVELOPMENT.

Conc (g)[2]	Mean: Wt. of larvae after feeding 8 days on[1] Caffeine	Theophylline	Theobromine	Overall	Days to pupation	Wt. of pupae
0	164.3	163.9	159.3	162.5a	13.8	258.8
0.25 (.06%)	157.9	153.5	152.2	154.5b	14.2	265.0
0.50 (.12%)	107.2	134.6	110.8	117.5c	15.0	255.9
1.0 (.25%)	34.6	28.8	36.4	33.3d	19.4	243.0
2.0 (.5%)	1.5	5.5	15.3	7.4e	25.7	204.2
4.0 (1%)	0.0	0.8	7.7	2.9e	+	+

[1]Mean weights of FAW larvae followed by the same letter are not significantly different ($P \leq 0.05$, Duncan's multiple range test).
[2]Diet included 300 ml pinto bean diet and 100 ml distilled water.
+ More than 88% died.

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