Comparison of Five Food Sources for use in Bioassaying Soil Insecticides Against the Lesser Cornstalk Borer (Lepidoptera: Pyralidae)[1]

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ABSTRACT Five food sources were compared for use in bioassaying soil treated with granular insecticides against lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller), larvae. The least efficient food source was sorghum seedling grown from treated or untreated seed. Larval survival was variable when these seedlings were used with untreated soil; seedlings were also time-consuming to produce. Survival was acceptable when peanut and lima bean seedlings were used, but these seedlings were also time consuming to produce. Artificial diet plugs were the most efficient food source for the soil pesticide bioassay. Diet plugs were easy to produce and handle, and larvae survived well on plugs in untreated soil.

KEY WORDS Insecta, *Elasmopalpus lignosellus;* methods; bioassay.

The lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), is a major pest of peanuts in the southeastern United States (Smith and Barfield 1982). Soil insecticides are commonly used to manage this insect in peanuts. Efficacy of insecticides most widely used against the LCB was recently determined by soil bioassay using sorghum seedlings grown from untreated seed as the larval food source (Mack et al. 1989). Sorghum seedlings were an acceptable bioassay food source for use in these insecticide bioassays.

There are, however, some difficulties inherent in the use of sorghum seedlings as a food source for LCB larvae. The production of sorghum seedlings requires about 1 wk and seedlings may also be contaminated by saprophytic bacteria and fungi which cause high insect mortality. Insect survival rates can be improved by daily washing of the growing seedlings with an anti-bacterial solution and by using an aqueous fungicidal solution in which to grow seedlings. However, washings and fungicides may cause root pruning and slow plant growth. These chemicals may also be toxic to the LCB (Lynch and Reed 1985). This study determined if seedlings of other plants or artificial diet (Chalfant 1975) were more effective and less labor-intensive than sorghum seedlings for bioassaying soil insecticides against the LCB.

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Materials and Methods

Four kinds of plant seedlings and an artificial diet were evaluated as food sources for neonate (< 24 h old) LCB larvae to be used in soil insecticide evaluations. The seedlings were 'Topaz' sorghum, *Sorghum vulgare* Persoon, (untreated seed or seed treated with chlorpyrifos methyl), and untreated seeds of 'Henderson' bush lima bean, *Phaseolus limensis* Macfadyen, and 'Florunner' peanut, *Archis hypogaea* L. Suitability was determined as a combination of the effectiveness and perceived efficiency of each food source. Food source effectiveness was defined as a significant change in LCB survival compared with that of insects feeding on the standard, i.e., sorghum grown from untreated seed. Food source efficiency was defined as ease of handling and production of each food source, including the time involved with seedling maintenance and growth.

All seeds were surface-sterilized before use. Treated sorghum seeds were washed in acetone for 2 min to remove any external chemicals, rinsed with running water for 5 min, and then dried to evaporate the remaining acetone. All seeds were externally sterilized for 2 min in an aqueous solution containing 0.1% sodium hypochlorite in distilled water to reduce fungal and bacterial contamination. Sterilized seeds were rinsed in distilled water and then soaked for 30 min in a 0.25% solution of benomyl fungicide in distilled water. Seeds were germinated in clear plastic boxes lined with paper towels dampened with a water solution containing 0.025% benomyl. Boxes were held in a controlled environment chamber at 27 ± 2°C, 70% RH and 14:10 (L:D) photoperiod. Seedlings were washed daily with aqueous 0.1% sodium hypochlorite and additional aqueous benomyl solution was added as needed. Seedlings were grown, for about 1 wk, to the first true leaf stage for lima beans and peanuts, and until sorghum seedlings were 6 cm tall.

Diet plugs were stamped out of cubes of solidified artificial diet with a modified 3 cc plastic syringe. The syringe was modified by removing the tip to form an open cylinder. Each diet plug was approximately 9 mm diameter by 11 mm height with a weight of 78.2 ± 1.6 g ($\bar{X}$ ± SEM).

Insects were obtained from a laboratory colony maintained at Auburn University on artificial diet, with modifications as described by Lynch and Reed (1985). The neonate larvae eclosed and were held until use in a controlled environmental chamber at 27 ± 2.°C, 70% RH and 14:10 (L:D) photoperiod.

Soil samples were procured from a granular insecticide test conducted in 1988 on Dothan sand loamy soil (0.5% organic matter) at the Wiregrass Substation at Headland, Ala. Soil treatments consisted of chlorpyrifos 15G at 2.2 kg (AI)/ha applied in a 25- to 30-cm band over the row with a small-plot granular applicator. Chlorpyrifos was applied to selected plots at planting on 23 May, and to other plots at pegging (R2 plant growth stage, as defined by Boote 1982) on 12 Jul 1988. Some plots were left untreated on both dates. Soil samples were collected by using methods identical to those described in Mack et al. (1989). Samples were removed from each plot weekly for the duration of the field test. Soil was refrigerated at 0 ± 2°C then air dried to approximately 0% soil moisture prior to use. Soil samples collected on 23 May and 12 July were selected for use in this test, based on previously reported bioassay results (Mack et al. 1989). According to the previous results, < 20.0% survival could be expected in the treated soil sampled the day of treatment (i.e., 23 May or 12 Jul), 50 to 75% survival could be expected in the treated soil sampled 6 wk after treatment (i.e.; treated 23 May and sampled 12 Jul), and > 90% survival could be expected in the untreated soil sampled on either date.

The bioassay arena consisted of a 30-ml plastic creamer cup filled with about 1.0 cm of soil. Three neonate larvae were placed on the soil in each cup and provided with one of the food sources tested. Sorghum seedlings were added to the cups daily because they dried out rapidly, but other seedlings did not have to be replenished. When diet plugs were used, a 20-mesh nylon screen was placed on the soil surface to prevent the diet plug from contacting the soil surface. The screen prevented water in the diet from leaching additional pesticide out of the granules, and allowed larvae to travel freely from the diet to the soil. Cups were held in a controlled environmental chamber at 27 ± 2°C, 70% RH and 14:10 (L:D) photoperiod. Plant material, diet, and soil were examined for larvae at 72 h, and the number of surviving and dead larvae was recorded.

Five tests were conducted, with each test corresponding to a soil sample collected from the field on a specific date and series of plots (Table 1). A randomized complete block design with five treatments (food sources) and 6 to 8 replicates was used for each test. Analysis of variance and a Waller-Duncan k-ratio mean separation test (k = 100) were used for each test to compare larval survival from each food source (SAS Institute 1985).

Results

The five food sources varied in their suitability for use in soil insecticide screening. Survival in cups containing untreated soil and peanut seedlings or diet was > 80%, while survival in cups with sorghum seedlings was < 60% (Table 1). No difference in survival was detected between the untreated sorghum seedlings and any other food source in untreated soil sampled on 12 Jul. Larval survival in this case was > 60% in all treatments. Thus, survival in the two tests with untreated soil was variable when sorghum seedlings were used, and was uniformly good (> 60%) when peanut seedlings or artificial diet were used.

In treated soil, larval survival was lower in cups with artificial diet, lima bean, or peanut seedlings than in cups with untreated sorghum on 23 May (Table 1). Low survival was expected from this soil sample, since it was sampled immediately after treatment with chlorpyrifos. Survival was < 10% in cups containing lima bean seedlings, peanut seedlings, or artificial diet, and was > 25% in cups with untreated sorghum. Low survival was also expected for soil treated and sampled on 12 July and survival was < 20% for larvae in cups with any of the food sources. Survival in the two tests with treated soil was uniformly low when artificial diet or when lima bean or peanut seedlings were used. However, survival was variable when untreated sorghum was utilized.

An intermediate level of survival (50 to 75%) was expected for soil treated on 23 May and sampled on 12 Jul (Table 1). Larval survival was greater in cups with peanut and lima seedlings, or artificial diet than in cups with treated sorghum. Treated sorghum did not produce an intermediate level of survival, as expected.

Table 1. Effect of food source on mean percent survival of LCB larvae.

	SAMPLE DATE									
	23 May				12 July					
	untreated		treated †		untreated		treated ‡		treated †	
Food Source	N	% Surv.	N	% Surv.	N	% Surv.	N	% Surv.	N	% Surv.
Untreated Sorghum	8	54.2b*	8	29.2a	6	61.1a	6	16.7a	6	66.7ab
Treated Sorghum §	8	58.3b	8	16.7ab	6	61.1a	6	5.6a	6	38.9b
Peanut	8	95.8a	8	8.3b	6	88.9a	6	5.6a	6	88.9a
Artificial Diet	8	83.3a	8	4.2b	6	77.8a	6	0.0a	6	88.9a
Lima Bean	8	79.3ab	8	0.0b	6	88.9a	6	11.1a	6	77.8a

* Means in each column followed by the same letter are not significantly different according to Waller-Duncan k-ratio mean separation test (k = 100). N = number of replicates used for each treatment, with three larvae per replicate.

† Soil treated on 23 May with chlorpyrifos 15G at 2.2 kg (AI)/ha.

‡ Soil treated on 12 July with chlorpyrifos 15G at 2.2 kg (AI)/ha.

§ Seed treated with chlorpyrifos methyl.

Discussion

Methods for bioassay of soil pesticides have been reported for insects other than the LCB (Harris 1972, Sutter 1982 and Whitney 1967). The previous bioassay method that used untreated sorghum seedlings (Mack et al. 1989) is time-consuming. Larval survival in untreated soil was quite variable, and ranged from 53 to 95% (Mack et al. 1989). This wide range of survival was probably due to cannibalism and to difficulties associated with handling the sorghum seedlings. They must be grown for approximately 1 wk before use, which allows saprophytic fungi and bacteria to contaminate the seedlings. This contamination may contribute to reduced larval survival.

A bioassay method that is both efficient and effective is desirable to compare pesticide efficacies. The bioassay method should accurately compare treatment effects without confounding the results with unexplained mortality. Artificial diet was the most suitable of the five food sources tested and has long been utilized as an inoculation and food substrate for the bioassay of entomopathogenic viruses (Chauthani 1968). Artificial diet provided adequate larval survival in untreated soil and low survival in treated soil, making it every effective for evaluating insecticide effectiveness. Diet required little time to produce and could be used immediately, eliminating the 1-wk period needed for seed germination and seedling growth. Also, most of the living larvae could be found in silken tubes attached to the diet plug or to the screen on which the diet is placed, making it more efficient by decreasing the time spent searching for living larvae in the soil.

The two types of sorghum seedlings (i.e., treated and untreated seed) were the least suitable of the seedlings tested. They were prone to contamination by saprophytic organisms, required more handling time than the other seedlings, and additional seedlings had to be added to the cups to insure a continuous larval food source. Larval survival in the untreated soil was variable when sorghum seedlings were used as the food source, making them also a less effective food source.

Survival was good with peanut and lima bean seedlings, making them an effective food source for LCB larvae. However, neither seedling species was as efficient as artificial diet. Seedlings were prone to contamination by saprophytic bacteria and fungi during germination and growth. Infestations were reduced, however, if the seed coat was removed prior to or soon after germination. Also, some larvae burrowed into the endosperm, which limited larval contact with treated soil and could increase survival in treated soil. This would decrease the accuracy of a bioassay. However, larvae constructed soil-covered silken tubes that were often attached to these seedlings, which increased bioassay efficiency by decreasing time consumed searching for living larvae in the soil.

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