REARING THE LESSER CORNSTALK BORER:[1] FUNGICIDES FOR CONTROL OF *ASPERGILLUS NIGER*[2]

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(Accepted for publication Oct. 26, 1984)

ABSTRACT

Several sanitary procedures were employed and the fungicides benomyl, captan, maneb-zinc, zinc ion-maneb complex, and triphenyltin hydroxide in autoclaved vermiculite were evaluated for control *Aspergillus niger* van Tieghem in rearing the lesser cornstalk borer, *Elasmopalpus lingnosellus* (Zeller). Sanitary procedures reduced the incidence of mold only slightly. Benomyl, at 0.25 g/100 g of vermiculite, had minimal detrimental effects on the biology of lesser cornstalk borer larvae and was highly effective in controlling *A. niger*. Triphenyltin hydroxide was highly toxic to lesser cornstalk borer larvae at all levels evaluated.

Key Words: Laboratory rearing, insect culture, mold control.

J. Entomol. Sci. 20(1): 26-33 (January 1985)

INTRODUCTION

The lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller, feeds on over 60 species of plants (Stone 1968), many of which are important cultivated crops. It often becomes an economic pest in the southeastern and southwestern U. S., especially during periods of drought. For example, during the 1980 drought in Georgia, the lesser cornstalk borer (LCB) caused a loss of ca. $37.8 million to peanut, soybean, and grain sorghum (Suber et al. 1982).

The ability to rear economically important insects such as the LCB is often essential for conducting research. Chalfant (1975) described a technique for rearing the LCB that utilized vermiculite placed on the surface of an artificial diet. In nature, LCB larvae construct silken tubes in the soil while feeding on plants; the vermiculite replaced sand in laboratory rearing and greatly enhanced survival. This technique has made it possible to conduct studies with this species on its sex pheromone (Lynch et al. 1984), damage to peanut pods in various stages of development (Lynch 1984), control with insecticides (Reed and Todd, unpublished data), and evaluation of soybean genotypes for resistance (Reed and Todd, unpublished data). Recently, however, *Aspergillus niger* van Tieghem has become an increasingly severe problem in the rearing of this insect, and often reduced

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larval survival > 75%. Funke (1983) reviewed the more common mold control agents used in insect rearing, and indicated that *A. niger* was the most important fungal contaminant and that it is probably the most difficult to control. We evaluated several sanitary procedures and five agricultural fungicides mixed with vermiculite for control of *A. niger* and report here their effects on the biology of the lesser cornstalk borer.

MATERIALS AND METHODS

The LCB colony at the Insect Biology and Population Management Research Laboratory was reared as described by Chalfant (1975) with the following modifications: (1) aureomycin was deleted from the pinto bean diet (Burton 1969); (2) neonate larvae were mixed with autoclaved, expanded vermiculite and dispensed into the rearing cups with a "Bazooka," a mechanical infestation device (Wiseman et al. 1980); and (3) larvae were reared at 26.7 ± 2°C, 70 ± 5% RH, and 14:10 photophase:scotophase. Several sanitary procedures were initially employed in an attempt to reduce the *A. niger* contamination in the LCB culture. These included covering the diet cups with a plastic bag as soon as the diet solidified, surface sterilization of the egg sheets by spraying them with 1% sodium hypochlorite when they were removed from the oviposition containers, surface sterilization of the rearing cups by spraying 1% sodium hypochlorite over the diet surface, and surface sterilization of the infestation station and hands of the person that infested the cups with 1% sodium hypochlorite. Data were recorded on percentage of the rearing cups contaminated with *A. niger*.

Captan fungicide was evaluated for control of *A. niger* at 0.125, 0.25, and 0.5 g of formulated captan per 100 g of vermiculite. One-hundred grams of sterilized vermiculite and the appropriate concentration of fungicide were placed in a 1-liter jar and rolled on a ball mixer for ca. 30 min. The experiment was conducted in a randomized complete block with 36 diet cups per treatment, each diet cup representing a replication. Each cup of diet was infested with 6 neonate LCB larvae with a camel's hair brush and ca. 1.0 ml of fungicide-vermiculite mixture was dispensed over the diet surface with a bazooka. Each cup was then contaminated by sprinkling spores of *A. niger* from an infested cup onto the vermiculite. After 15 days, data were recorded on *A. niger* contamination, larval survival, and larval weight. LCB larvae were extracted from 6 of the 36 cups per treatment by emptying the contents of a cup, i.e., diet, vermiculite, and larvae, into a beaker containing ca. 200 ml of water and then adding ca. 10 ml of 5.25 percent sodium hypochlorite to dissolve the silk. After ca. 5 min., the contents of the beaker were poured through a 40-mesh sieve and then sprayed with water to locate the LCB larvae. The larvae in the remaining 30 cups were allowed to pupate and emerge as adults. The cups were observed daily for adult emergence and data were recorded on the days to adult emergence and the number of adults that emerged. This experiment was conducted for four LCB generations and the data were combined in an analysis over generations.

Two additional tests with fungicide were conducted to control *A. niger* in LCB rearing cups. In the first, benomyl (Benlate, 50% AI), captan (50% AI), maneb-zinc (Manzate-D 80% AI), triphenyltin hydroxide (Duter, 47.5% AI), and zinc ion-maneb complex (Dithane M-45, 62% AI), were evaluated at 0.5 and 2.0 g of formulated material per 100 g of vermiculite. In the second, benomyl, maneb-zinc,

triphenyltin hydroxide, and zinc ion-maneb complex were evaluated at 0.125, 0.25, and 0.5 g of formulated material per 100 g of vermiculite for three generations of the LCB. All tests were designed, handled, and evaluated as described above, with one exception: *A. niger* contamination was recorded at 7, 10, and 15 days. Data for the multiple generations were combined as a split plot and all data were subjected to an analysis of variance. Significantly different means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Sanitary procedures to control fungal contamination of the LCB eggs and rearing media reduced *A. niger* contamination of the diet from ca. 80% prior to implementation to ca. 70% after implementation. Therefore, it was hypothesized that major contamination by *A. niger* was due to the moisture gradient between the autoclaved vermiculite and the diet surface rather than unsanitary handling procedures. Vermiculite placed on top of the diet in the rearing cups causes the diet to dehydrate more slowly than when no vermiculite is added. This hypothesis was supported by the fact that both the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and granulate cutworm, *Feltia subterranea* (Fabricius), are reared on the same diet, but without vermiculite, with little to no problems with *A. niger*.

The effect of captan on *A. niger* in LCB rearing cups is presented in Table 1. All captan treatments reduced *A. niger* contamination without influencing either larval survival or weight. However, adult emergence was delayed slightly and survival to adults was increased slightly by the use of captan.

The effects of selected fungicidal rates evaluated in Test 2 for control of *A. niger* in LCB rearing cups are presented in Table 2. Both rates of benomyl, maneb-zinc, and zinc ion-maneb complex provided significantly better ($P < 0.05$) control of *A. niger* on all evaluation dates than did the untreated check. Bell et al. (1981) reported that benomyl in combination with sorbic acid and folpet was the most effective of several fungicides tested for controlling *A. niger*. Gifawesen et al. (1975) and Bathon (1977) reported that benomyl was effective to ineffective for control of *A. niger* when incorporated into insect diets. Captan at 2 g/100 g vermiculite performed as well as the most effective fungicides after 7 days but was slightly less effective at 10 and 15 days. Captan also was effective for controlling *A. niger* in the test conducted by Bathon (1977). Triphenyltin hydroxide was ineffective in controlling *A. niger*.

The influence of these fungicides on LCB larval and adult biology is also presented in Table 2. Larval survival was significantly greater ($P < 0.05$) after 15 days with lower rates of benomyl and captan than with any other treatment. Larval survival for maneb-zinc and for the lower rate of zinc ion-maneb complex ranged from 47 to 55%. Triphenyltin hydroxide was extremely toxic to LCB larvae, and none survived. However, larval survival on the triphenyltin hydroxide treatment was not significantly lower than it was on the untreated check where less than 20% of the larvae survived. Triphenyltin hydroxide also was reported as the most toxic of several fungicide tested on three other lepidopterous insects (Livingston et al. 1978).

Several of the fungicides also significantly influenced larval weights, days to adult emergence, and the percentage of LCB adults that emerged (Table 2). Larvae from the low rate of benomyl and captan were significantly heavier ($P < 0.05$)

Table 1. Effects of captan mixed with vermiculite on control of *Aspergillus niger* and on survival and development of the lesser cornstalk borer.*

Treatment	Rate†	Diet with *A. niger* contamination (%)‡ after 15 days	Larval survival (%)‡ at 15 days	Larval wt. (mg) at 15 days	Days to adult emergence	Survival to adults (%)
Check	0	97.0 a	74.3 a	21.7 a	28.5 b	50.3 b
Captan	0.125	55.8 b	77.8 a	19.9 a	29.4 a	62.0 a
	0.25	58.4 b	72.2 a	23.0 a	29.3 a	59.4 a
	0.5	53.8 b	77.8 a	20.9 a	29.8 a	60.9 a

* Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.
† Grams of formulated fungicide per 100 g of vermiculite.
‡ Percentage data transformed to arcsin $\sqrt{\%}$ for analysis.

Table 2. Effects of fungicides mixed with vermiculite on control of *Aspergillus niger* and on the biology of the lesser cornstalk borer.*

Treatment	Rate† (g)	Diet with *A. niger* contamination (%)‡ after indicated day 7	10	15	Larval survival (%)‡ at 15 days	Larval wt. (mg) at 15 days	Days to adult emergence	Survival to adults (%)‡
Check	0	100 a	100 a	100 a	19.4 ed	10.2 cde	30.4 e	13.3 e
Benomyl	0.5	0 c	0 d	5.6 d	83.3 a	23.7 ab	30.2 e	65.6 a
	2.0	0 c	0 d	0 d	30.6 bcd	7.3 cde	34.9 d	30.0 cd
Captan	0.5	16.7 b	27.8 b	30.6 b	80.6 a	26.5 a	29.7 e	67.2 a
	2.0	5.6 c	11.1 c	19.4 c	25.0 cde	17.2 abc	37.0 c	22.8 d
Maneb-zinc	0.5	8.3 bc	8.3 cd	8.3 cd	55.6 b	14.1 bcd	29.7 e	67.2 a
	2.0	0 c	0 d	0 d	55.6 b	11.4 cd	37.0 c	22.8 d
Zinc ion-maneb	0.5	0 c	0 d	0 d	47.2 bc	9.7 cde	35.6 cd	35.6 c
	2.0	0 c	5.6 cd	5.6 d	22.2 cde	3.7 de	42.5 a	6.7 ef
Triphenyltin-hydroxide	0.5	91.7 a	97.2 a	100 a	0 e	0 e	–	0 f
	2.0	100 a	100 a	100 a	0 e	0 e	–	0 f

* Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.
† Grams of formulated fungicide per 100 g of vermiculite.
‡ Percentage data transformed to arcsin $\sqrt{\%}$ for analysis.

than larvae from most of the other treatments. None of the other treatments had a significant effect on the weight of LCB larvae. Days required for adult emergence did not differ significantly for the low rates of benomyl, captan, maneb-zinc treatments, and the check. However, the higher rate of these fungicides prolonged adult emergence significantly in comparison with the check. The percentage of larvae that survived to adults was significantly greater ($P < 0.05$) with the low rates of benomyl, captan, maneb-zinc than for all other treatments.

Table 3 presents data on the effect of lower rates of benomyl, maneb-zinc, and zinc ion-maneb complex evaluated in Test 3 for control of *A. niger* and on the biology of the LCB. All rates of benomyl provided significantly better ($P < 0.05$) control of *A. niger* than the untreated check. The three benomyl rates provided comparable mold control after 7 and 10 days, but by 15 days the lowest rate of benomyl had significantly more ($P < 0.05$) mold in the LCB rearing cups than did cups with the two higher rates of benomyl. Maneb-zinc provided better *A. niger* control after 7 days than did the untreated check, but only the higher rate of maneb-zinc provided better control than the untreated check after 10 and 15 days. Zinc ion-maneb complex produced conflicting results; all rates provided better control of mold after 7 and 10 days than did the untreated check, but the intermediate rate did not perform as well as the low or high rates after 15 days.

Survival of larvae was significantly higher ($P < 0.05$) in cups treated with all levels of benomyl, the intermediate level of maneb-zinc, and the low and intermediate levels of zinc ion-maneb comlex than it was in the untreated check. However, all fungicide treatments, except the low and intermediate levels of benomyl, signficantly reduced ($P < 0.05$) the weight of larvae in comparison with larvae in the untreated check. Similarly, all fungicide treatments, except for the low level of benomyl, significantly delayed ($P < 0.05$) adult emergence. The percentage of larvae that emerged as adults was significantly greater ($P < 0.05$) in the three benomyl treatments than in any other treatment. The zinc ion-maneb complex treatments resulted in adult emergence significantly lower than adult emergence for the benomyl treatments, but significantly higher than adult emergence for the untreated check. Survival to adults in cups treated with maneb-zinc was not significantly different from survival in the check.

The effects of these fungicides paralleled the effects of those reported by Singh and House (1970) for various antimicrobial agents on insects, i.e., several of the fungicides inhibited larval development and increased mortality in the larval or pupal stages. Singh and House (1970) also designated four categories for antimicrobials according to their effects on insect larvae. In reference to the LCB, benomyl would be categorized in the safe level, i.e., minimum deterimental effects, especially at lower rates; captan, maneb-zinc, and zinc ion-maneb complex would be categorized in the primary and secondary inhibitory levels, i.e., prolonged development, depending on their concentration; and triphenyltin hydroxide would be categorized in the toxic level.

In summary, benomyl at 0.25 g of formulated fungicide per 100 g of autoclaved vermiculite has been used to control *A. niger* for over six generations in rearing the LCB. This rate was selected due to its excellent control of *A. niger* with minimal effects on the biology of the lesser cornstalk borer. As to date, detrimental effects on the LCB or an increase in the incidence of *A. niger* that may indicate resistance have not been noted with continuous use of benomyl in rearing the LCB.

Table 3. Effects of fungicides mixed with vermiculite on control of *Aspergillus niger* and on the biology of the lesser cornstalk borer.*

Treatment	Rate† (g)	Diet with *A. niger* contamination (%)‡ after indicated day 7	10	15	Larval survival (%)‡ at 15 days	Larval wt. (mg) at 15 days	Days to adult emergence	Survival to adults (%)‡
Check	0	100 a	100 a	100 a	22.2 d	15.4 a	30.3 d	18.3 d
Benomyl	0.125	0 d	2.8 e	38.9 c	80.6 a	17.9 a	29.6 d	60.6 a
	0.25	0 d	2.8 e	2.8 e	80.6 a	14.9 a	32.0 c	69.4 a
	0.5	0 d	0 e	0 e	61.1 ab	8.9 bc	33.6 bc	63.3 a
Maneb-zinc	0.125	75.0 b	88.9 ab	94.4 a	44.4 bcd	9.9 b	32.1 c	16.1 d
	0.25	75.0 b	88.9 ab	88.9 a	58.3 ab	8.0 bc	32.3 c	18.9 d
	0.5	44.4 c	61.1 c	63.9 b	30.6 cd	4.2 c	34.6 b	21.1 cd
Zinc ion-maneb	0.125	36.1 c	44.4 d	50.0 bc	61.1 ab	8.0 bc	34.6 b	32.2 bc
	0.25	66.7 b	80.6 b	83.3 a	47.2 bcd	8.3 bc	32.1 c	34.4 b
	0.5	2.8 d	8.3 e	22.2 d	50.0 bc	6.6 bc	36.8 a	36.1 b

* Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.
† Grams of formulated fungicide per 100 g of vermiculite.
‡ Percentage data transformed to arcsin $\sqrt{\%}$ for analysis.

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