

**IMPACT OF Bt AND THIODICARB ALONE
AND IN COMBINATION ON TOBACCO
BUDWORM, MORTALITY AND EMERGENCE
OF THE PARASITOID**

MICROPLITIS CROCEIPES

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Abstract

Bacillus thuringiensis var. kurstaki (Bt) and thiodicarb (Larvin) were evaluated in laboratory and field tests to determine the effect on Heliothis virescens larvae, the tobacco budworm, and the parasitoid Microplitis croceipes. Laboratory trials were conducted using Bt rates of 0, 10, 50 and 250 ppm and Larvin rates of 0, 12.5, 25, 50, 100, and 200 ppm. Field tests were conducted using Bt and Larvin, independently and in combination, at rates recommended for resistance management in Arkansas. Tobacco budworm mortality was directly related to Bt and Larvin concentration. Bt only significantly impacted tobacco budworm mortality at the highest rate of exposure in the absence of parasitization. On day 6 and 16, parasitization increased host mortality at all Bt experimental rates. Larvin exhibited a linear relationship between host mortality and increasing insecticide concentration. However, parasitization did not significantly impact host mortality until day 16. Emergence of M. croceipes was inversely related to Bt and Larvin concentration.

Field tests indicate that neither Bt nor Larvin, alone or in combination, provide acceptable control of early third instar tobacco budworm in the absence of parasitization by M. croceipes. However, Larvin and Larvin/Bt mixes provided significantly greater tobacco budworm control than did Bt application alone. In addition, no significant advantage was determined for tank mixes as compared to Larvin application alone. Emergence of M. croceipes was significantly lower in Larvin and combination treatments as compared to Bt alone treatments. Overall, results indicate that Larvin is more effective than Bt against third instar tobacco budworms and more detrimental to M. croceipes emergence.

Introduction

Bacillus thuringiensis (Bt) and thiodicarb (Larvin) are effective insecticides against early instar Heliothis virescens (F.), the tobacco budworm. With the development of pyrethroid resistant tobacco budworm populations (Luttrell et al 1987, Plapp et al. 1990, Mullins et al. 1991, and Elzen 1992), these insecticides are important components of early

season control programs. While there are numerous non-insecticidal means of preventing or delaying resistance development (i.e. early crop maturity and adequate field scouting), insecticides are and will continue to be an important component of any pest management strategy. However, in order to fully exploit all means of tobacco budworm control it is essential to evaluate the impact of natural enemies and to assess the impact of insecticide use on these populations.

Microplitis croceipes (Hymenoptera: Braconidae) is one of the most common parasitoids encountered in Arkansas cotton fields. Field collections during 1993 and 1994 suggest that it is only second in abundance to Cotesia marginiventris in the Red River and Mississippi delta regions of Arkansas. Atwood et al. (In Press) determined increasing rate of tobacco budworm mortality and decreased parasitoid emergence for C. marginiventris relative to increasing concentrations of both Bt and Larvin in continuous exposure laboratory trials. Field tests indicate that neither Bt nor Larvin, alone or in combination, provide acceptable control of third instar tobacco budworm (Atwood et al. 1996) In addition, no significant impact was noted on the emergence of C. marginiventris. However, similar studies have not been conducted for M. croceipes. To this end, laboratory diet trials and tests with field treated cotton were conducted during 1995 and 1996 to evaluate the impact of Bt and Larvin on M. croceipes.

Materials and Methods

A laboratory colony of M. croceipes was established from field collected H. virescens larvae and from an established colony in Stoneville, MS. Heliothis virescens larvae, from a previously established laboratory colony, served as hosts for M. croceipes. Host larvae were reared on a standard semisynthetic diet (Burton 1969). Insecticides used in these tests were B. thuringiensis (Javelin WG, Sandoz Crop Protection) and thiodicarb (Larvin 3.2, Rhone-Poulenc Ag Company).

Laboratory Tests

Laboratory tests were conducted using Bt rates of 0, 10, 50 and 250 ppm and Larvin rates of 0, 12.5, 25, 50, 100 and 250 ppm to induce a wide range of mortality responses in H. virescens larvae. Each insecticidal rate was applied alone and in combination with parasitization, thus providing 8 Bt and 12 Larvin treatments. Bt tests were replicated 4 times and Larvin tests 7 times. All tests were conducted in an incubator at 27°C and a 14:10 L:D regimen.

Exposure of H. virescens larvae to Bt or Larvin was accomplished by mixing the insecticide with buffer and incorporating directly into the pinto bean diet. The insecticide mixtures were only added after diet had been cooled to 50°C to prevent breakdown of the material. The diet was again blended after the addition of insecticide to ensure consistent concentration throughout the diet. For each test, 10 late 2nd instar H. virescens larvae were placed

in wax-lined 266 ml parasitization cups (16 for Bt replicates and 24 for Larvin replicates) containing 20 ml of standard pinto bean diet. One female *M. croceipes* was introduced into half of the parasitization cups for a period of 14 hours. The remaining cups were not parasitized to evaluate insecticide effect alone.

Individual larvae from each treatment (parasitized and non-parasitized) were transferred to 30 ml souffle cups containing the experimental insecticide concentration. Larvae were held on each diet continually until larval death, larval pupation, or parasitoid emergence. Readings were initially obtained on day 2 following exposure to evaluate short term insecticide effect. Additional readings were recorded on days 6 and 16 to evaluate long term insecticide impact on both host and parasitoid development. Data analysis was conducted using Proc GLM ($P>0.05$) with comparisons made using LSD (SAS Institute, 1988).

Field Tests

Tests were conducted at the Agriculture Experiment Station Research Farm of the University of Arkansas in Fayetteville, AR (Washington Co.). Small plot tests were conducted as a randomized complete block design with six replicates. Plots consisted of a single row, 9.1 m in length, with 2-row buffers between sides of treatments and 3.05 m buffers between replicates. Spray dates were July 16, 17, 18, 19, 23, 24.

Insecticide concentrations used in these tests were those commonly recommended for tobacco budworm control in Arkansas. Bt rates were 0.56 and 1.12 kg product/ha (0.5 and 1 lb product/acre) and Larvin rate was 0.14 kg AI/ha (0.125 lb AI/acre). Each insecticide-rate was tested individually and in combination. A sixth treatment consisted of an untreated control. In addition, each treatment was evaluated alone and in combination with parasitization by *C. marginiventris* for a total of 12 experimental treatments.

Insecticide application was made using a CO₂ powered backpack sprayer at a pressure of 2.8 kg/cm². Treatments were applied in a volume of 94.6 liters/ha using 2 nozzles (TX-6) per row. All applications were made late afternoon.

One hour after treatment, 60 primary squares were picked from each treatment and placed in labeled plastic bags for transportation to the laboratory. Squares were placed individually in 30-ml plastic cups to which had been added a moistened filter paper disk. A single parasitized or non-parasitized tobacco budworm larva was then placed in each cup. For each replicate, 15 late second instar tobacco budworms were placed on semisynthetic diet in 36 wax-lined paper cups (266 ml) the previous day. Six larval cups were used for each treatment with half being provided with two female *M. croceipes* for parasitization. A total of 30 parasitized and 30 non-parasitized larvae were used for each treatment.

Tobacco budworm larvae were allowed to feed on the treated squares for 48 hours, after which time mortality readings were obtained and each larva transferred to an individual 30-ml plastic cup containing semisynthetic diet. Tobacco budworm mortality was also recorded on day 7 and 14 after initial exposure to the treated squares and emergence of *M. croceipes* was recorded on day 16. Data were analyzed using proc GLM (SAS 1988) with mean separation by LSD.

Results

Laboratory Tests-Tobacco Budworm Mortality

Mortality for tobacco budworm larvae exposed to Bt was observed to range from 1.7 to 5.0% for non-parasitized larvae and 3.3 to 26.7% for parasitized larvae two days following insecticidal exposure (Fig. 1). A significant two way interaction ($P<0.05$) was determined for Bt rate and parasitization. Mortality for larvae exposed to Larvin ranged from 1.0 to 37.1% for non-parasitized larvae and 4.8 to 55.2% for parasitized larvae after 2 days (Fig. 4). Both Larvin rate and parasitization were determined to significantly impact host mortality but did not interact.

Six days after exposure to Bt, tobacco budworm mortality was observed to range from 1.7 to 55.0% and for non-parasitized larvae and 8.3 to 80.0% for parasitized larvae (Fig 2). Both Bt rate and parasitization were determined to significantly affect host mortality. Mortality for Larvin exposed larvae on day six ranged from 6.7 to 93.3 % for non-parasitized larvae and 9.5 to 94.3% for parasitized larvae (Fig 5). Only Larvin rate was found to significantly affect mortality.

Sixteen days after initial Bt exposure, tobacco budworm mortality ranged from 6.7 to 95.0% for non-parasitized larvae and 78.3 to 95% for parasitized larvae (Fig. 3). Tobacco budworm mortality for larvae exposed to Larvin ranged from 10.5 to 97.1% for non-parasitized larvae and 93.3 to 100% for parasitized larvae (Fig. 6). A significant two-way interaction was noted between parasitization and Bt or Larvin rate.

Laboratory Tests-Microplitis croceipes Emergence

Emergence of *M. croceipes* from Bt and Larvin exposed tobacco budworm larvae on day 16 following initial exposure is presented in Figure 7. Parasitoid emergence from Bt exposed larvae ranged from 75.0% at 10 ppm to 1.7% at 250 ppm. Emergence of *M. croceipes* from Larvin exposed larvae ranged from 83.8% at 12.5 ppm to 2.9% at 200 ppm. Bt and Larvin concentration was determined to significantly effect parasitoid survival. For both Bt and Larvin, *M. croceipes* emergence was inversely related to insecticide concentration. In no instance was emergence observed to exceed 50% when Bt or Larvin concentration exceeded 50 or 12.5 ppm, respectively.

Field Tests-Tobacco Budworm Mortality

Mortality for tobacco budworm larvae was observed to range from 0.0 to 30.0% for non-parasitized larvae and 0.0 to 40.6% for parasitized larvae two days following insecticidal exposure (Fig. 8). Parasitization and larvin both significantly impacted early tobacco budworm mortality. Significantly greater tobacco budworm mortality was observed in both non-parasitized and parasitized larvae exposed to treatments which included Larvin as compared to experimental controls and Bt alone treatments. No significant difference in tobacco budworm mortality was noted in relation to Bt rate. Furthermore, mortality in Bt treatments did not significantly differ from the control treatments..

Seven days after exposure to insecticide, significantly greater mortality was observed for non-parasitized and parasitized tobacco budworm larvae exposed to Larvin or Larvin/Bt combinations (42.2 to 50.0% and 50.8 to 57.8%, respectively) (Fig. 9). Mortality in Bt and control treatments ranged from 0.0 to 25.6%.

Tobacco budworm mortality 16 days after insecticide exposure illustrates the impact of parasitization (Figure 10). Mortality for parasitized larvae ranged from 92.1 to 97.7% with mortality from insecticide exposure not differing significantly from the experimental control. In contrast, mortality for non-parasitized larvae was significantly less than that for parasitized larvae, ranging from 16.1 to 55.0%. Again, significantly lower tobacco budworm mortality was noted for non-parasitized larvae exposed to Bt alone as compared to larvae exposed to Larvin and Larvin/Bt mixes. Insecticide mixes were not significantly more effective than Larvin alone. In addition, Bt alone treatments did not significantly differ from the experimental control.

Field Tests -Microplitis croceipes Emergence

Parasitoid emergence from tobacco budworm larvae was observed to range from 36.1 to 87.6% (Fig. 11). Significantly lower M. croceipes emergence was noted from hosts exposed to Larvin or Larvin/Bt mixes (36.1 to 42.0%) as compared to control and Bt treatments (64.2 to 87.6%). No significant difference in parasitoid emergence was observed within Larvin and Larvin/Bt treatments or between the two Bt alone treatments. Emergence of M. croceipes from the Bt alone treatments did not significantly differ from the experimental control.

Discussion

Emergence of M. croceipes from Bt and Larvin exposed tobacco budworm larvae was dependent upon insecticide rate of application in laboratory trials. Similar findings were noted for C. marginiventris (Atwood et al. In Press). Results of laboratory investigations suggest that high rates of Bt or Larvin application, not unlike other pesticides, may reduce natural parasitoid populations.

It must be considered that the laboratory tests were conducted with continuous insecticide exposure. As shown by Dulmage (1978), Ali and Watson (1982) and Fast and Regniere (1984), lepidopterous larvae may recover from initial doses of Bt. Our field trials appear to confirm these findings with Bt having negligible impact on M. croceipes emergence. The lack of Bt impact on M. croceipes emergence has also been observed for C. marginiventris (Atwood et al. 1996). However, it must also be considered that the recommended rates for Bt application used during field trials are targeted at first and second instar larvae. As shown by laboratory tests, increased Bt concentration in field application may potentially prevent successful emergence of M. croceipes. Greater mortality may also have occurred in the current test with longer insecticide exposure. However, Ali and Young (1993) determined a decrease in Bt activity at identical application rates against first instar tobacco budworm in field tests, showing less than 23 and 31% of the initial activity after 3 days. Therefore, it is doubtful if longer exposure to Bt treated squares would have significantly increased larval mortality. In contrast to findings for C. marginiventris (Atwood et al. 1996), field trials do indicate a negative impact of Larvin on M. croceipes emergence at the recommended rate for field application.

Tobacco budworm mortality was directly related to Bt and Larvin concentration in laboratory tests. Furthermore, findings agree with the observations of Fusco (1980) and Weseloh and Andreadis (1982) indicating a synergism between Bt and parasitoids. Laboratory studies indicated that mortality in parasitized tobacco budworm larvae on day 6 approximates or exceeds that observed in non-parasitized larvae on day 16 for both Bt and Larvin. Similar results were noted for C. marginiventris on day 7 and 14 (Atwood et al. In Press). Overall, tests appear to indicate that Larvin has a greater impact on M. croceipes than does Bt.

Five conclusions can be drawn from this investigation. These are; 1) third instar tobacco budworm are susceptible to a Bt rate of 250 ppm and Larvin rate of 200 ppm, 2) Bt rates above 10 ppm and Larvin rates above 12.5 ppm significantly reduce emergence of M. croceipes, 3) neither Bt nor Larvin provide adequate control of early third instar tobacco budworm or at current recommended field rates, 4) Larvin and Larvin/Bt combinations provide significantly greater control as opposed to Bt applied alone, however, mixes are no more effective than Larvin alone and 5) tobacco budworm larvae parasitized prior to insecticide application may have the ability to survive and thereby maintain field populations.

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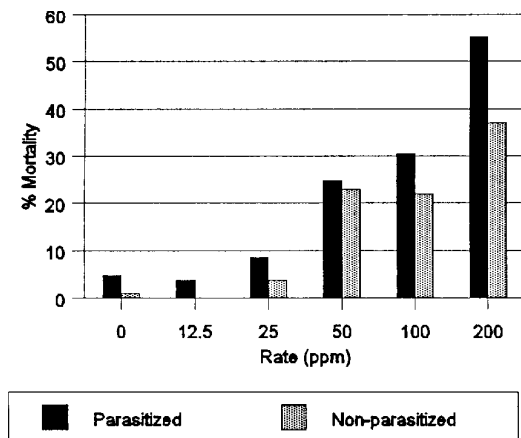


Figure 1. Effect of parasitization and different rates of Larvin on tobacco budworm mortality on day 2.

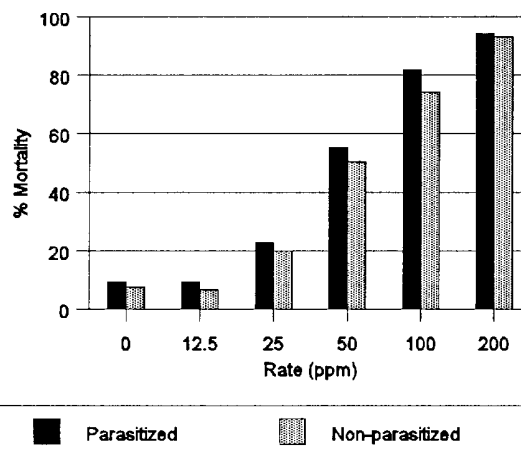


Figure 2. Effect of parasitization and different rates of Larvin on tobacco budworm mortality on day 6.

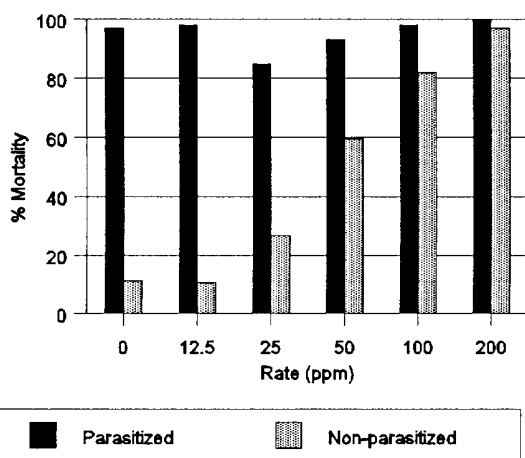


Figure 3. Effect of parasitization and different rates of Larvin on tobacco budworm mortality on day 16.

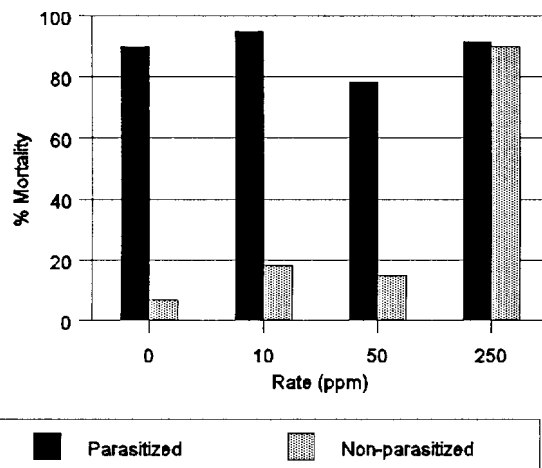


Figure 6. Effect of parasitization and different rates of Bt on tobacco budworm mortality on day 16.

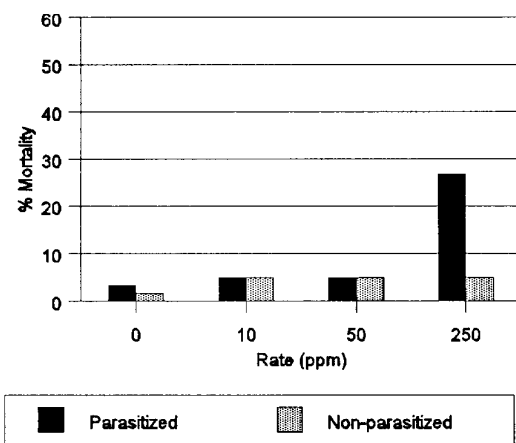


Figure 4. Effect of parasitization and different rates of Bt on tobacco budworm mortality on day 2.

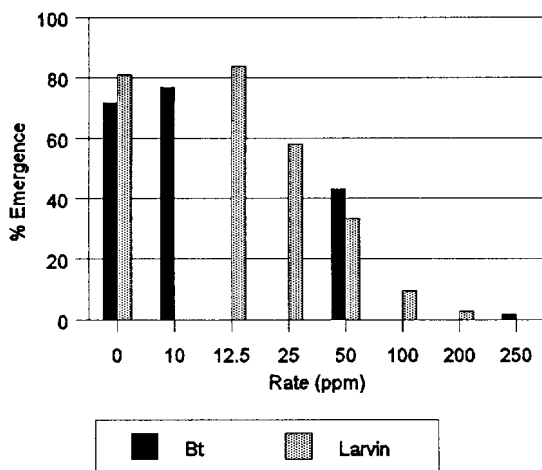


Figure 7. Effect of different rates of Larvin and Bt on emergence of *Microplitis croceipes* on day 16.

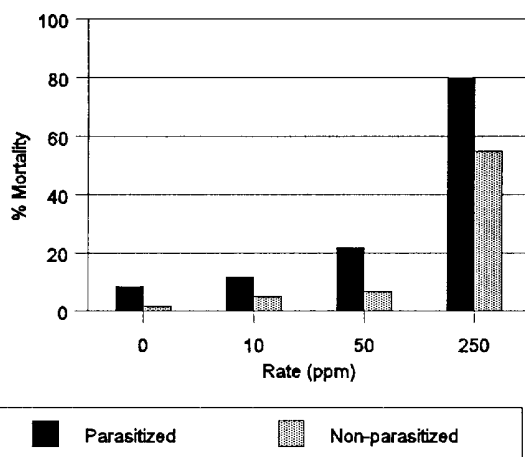


Figure 5. Effect of parasitization and different rates of Bt on tobacco budworm mortality on day 6.

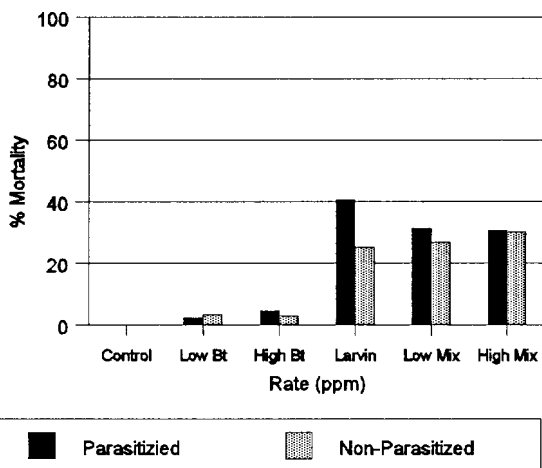


Figure 8. Effect of different field treatments on tobacco budworm mortality on day 2.

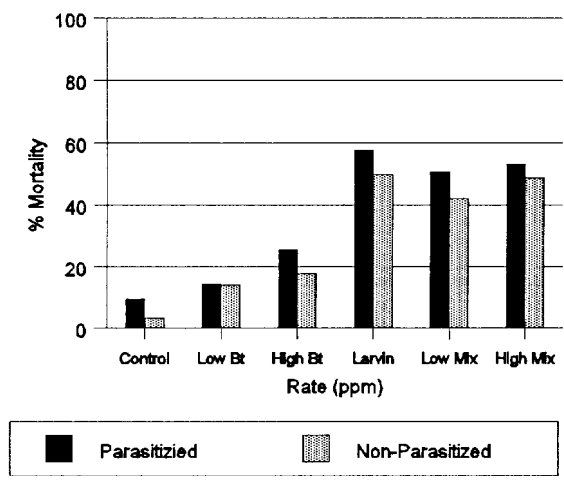


Figure 9. Effect of different field treatments on tobacco budworm mortality on day 7.

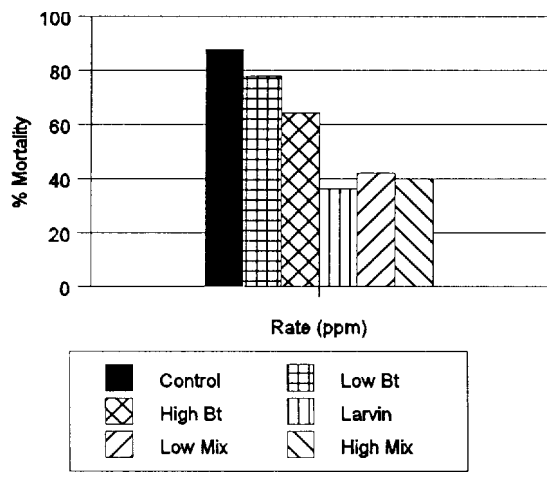


Figure 11. Effect of different field treatments on emergence of *Microplitis croceipes* on day 14.

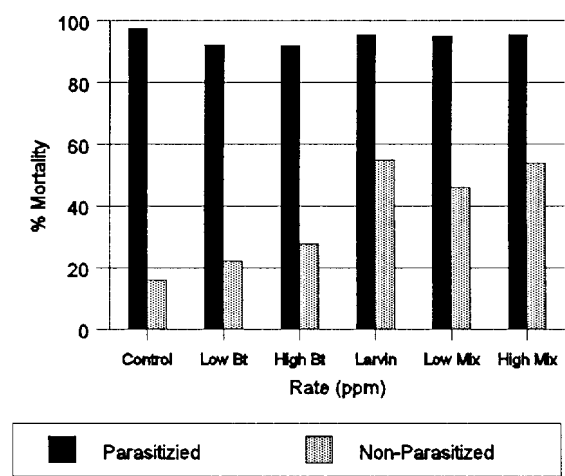


Figure 10. Effect of different field treatments on tobacco budworm mortality on day 14.