Effect of Beauveria bassiana on the Fecundity of Western Corn Rootworm, Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae)

Barbara S. Mulock¹ and Laurence D. Chandler²
Northern Grain Insects Research Laboratory, USDA-ARS, Brookings, South Dakota 57006

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INTRODUCTION

Western corn rootworm, Diabrotica virgifera virgifera LeConte, is an univoltine insect that is a major pest of corn, Zea mays L., throughout the corn-growing region of central and eastern North America (Levine and Oloumi-Sadeghi, 1991). In the spring of each year, larvae hatch from overwintering eggs and feed on establishing plant roots. Current management efforts are mainly directed toward the larvae. Chemical insecticides, primarily carbamates and organophosphates, are applied to the soil at planting to provide root protection (Metcalf, 1986). Alternatively, management can focus on the adults that begin to emerge from the soil in cornfields in late July. Through suppression of the adult population, the number of eggs deposited within a cornfield is reduced, as is the need to apply control measures the following year (Preuss et al., 1974). Recently, an increased interest in adult corn rootworm management has provided the opportunity to evaluate new control options (Lance and Sutter, 1992; Anonymous, 1999). The entomopathogenic fungus, Beauveria bassiana (Balsamo) Vuillemin, has received considerable attention as a microbial control agent and is currently registered for the management of several key agricultural pests (Anderson et al., 1988; Feng et al., 1988; Vandenberg et al., 1998; Brinkman and Fuller, 1999). In recent field-cage studies, Mulock and Chandler (2000) demonstrated a 50% reduction in adult populations of western corn rootworm following a single application of B. bassiana.

The ultimate aim of adult corn rootworm management is to reduce oviposition. Therefore, it is important to understand the effect of the pathogen on the reproductive capacity of the insect. The secondary effects of disease can be assessed at the level of individual fecundity, egg viability, and/or the reproductive potential of a cohort of insects. Wang and Knudsen (1993) found that B. bassiana treatment did not affect individual nymph reproduction in the Russian wheat aphid, Diuraphis noxia Kurdyumov, although total reproduction within the cohort was reduced due to differential mortality. Fargues et al. (1991) demonstrated a reduction in the total numbers of eggs laid by Colorado potato beetle, Leptinotarsa decemlineata (Say), surviving B. bassiana treatment at 22°C but not at 25°C. Furlong et al. (1997) reported that the diamondback moth, Plute-
Ilia xylostella (L.), infected with the fungal pathogen, Zoophthora radicans (Brefeld) Batko, laid fewer eggs prior to death than control females of the same age.

Oviposition in D. virgifera virgifera generally begins in early August, approximately 2 weeks after peak adult emergence, and extends through September (Hein and Tollefson, 1985). Branson and Johnson (1973) demonstrated that peak egg-laying occurs during the initial 10–15 days of the oviposition period and then gradually declines over time. Under optimal laboratory conditions a female may lay over 1000 eggs over an 11-week period, although under field conditions probably fewer eggs are laid (Hill, 1975). To be most effective, chemical-based insecticides are recommended when gravid females are observed in field surveys (Meinke, 1995). From our field and laboratory studies, we found that B. bassiana generally required 5–10 days between the initiation of an infection and death of the host in adult corn rootworm (Mulock and Chandler, 2000); therefore, the timing of the treatment is critical to effectively reduce oviposition.

The objective of this study was to evaluate the impact of B. bassiana on the reproductive potential of adult western corn rootworm. We examined the effect of B. bassiana on individual female fecundity and egg viability and estimated changes in the overall reproductive capacity within cohorts of beetles treated at increasing time intervals from adult emergence.

MATERIALS AND METHODS

Insects. Adult western corn rootworms were obtained from a laboratory colony at Northern Grain Insects Research Laboratory (NGIRL), USDA/ARS, Brookings, South Dakota. Insects were reared utilizing standard procedures described by Branson et al. (1975).

Pathogen. The B. bassiana isolate (BbPC-1) originated from infected D. virgifera virgifera adults collected from a single cornfield in Illinois and obtained from Mycotech Corp. (Butte, MT). The isolate was assessed for activity in laboratory-reared corn rootworm (LC50 3.4 × 10⁶ conidia/ml) and is currently maintained at NGIRL/USDA. Conidia were obtained by securing (two-sided adhesive tape) individual cadavers above Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY). Plates were incubated in darkness at 26°C for 10–14 days; dry conidia were harvested by scraping the media surface with a sterile inoculating loop. Conidia were suspended in a wetting agent, 0.04% Silwet L-77 (polyalkyleneoxide heptamethyltrisiloxane) (OSi Specialties, Inc., Greenwich, CT), and the concentration was measured using a hemocytometer. A sample of the suspension was streaked on SDAY plates and percentage germination estimated following 16–18 h of incubation. The suspension was adjusted to the treatment concentration, 5 × 10⁷ conidia/ml, by the addition of wetting agent. This concentration approximated the LC50 value previously obtained in laboratory bioassays conducted with adult female western corn rootworm.

Fecundity. Newly emerged adult western corn rootworms were sexed and 1:1 male-female pairs (80) were placed in individual plastic, screened, cages (8 cm high by 8.5 cm diameter) containing artificial diet and water agar (Branson et al., 1975). Pairs were divided into groups of 20 and at 5, 10, or 15 days postemergence, all females within a single group were removed from cages and treated with B. bassiana (5 × 10⁷ conidia/ml). The remaining group was used as a control. Initially, beetles were immobilized with CO2 and then submerged in the conidial suspension for 15 s. Treated beetles were transferred to dry filter paper where they regained activity before being returned to their original cages. Cages were held at 26 ± 2°C, 50–60% RH, and a photoperiod of 16:8 (L:D) h. Control beetles were treated with wetting agent only at 5 (7 females), 10 (7 females), or 15 days postemergence (6 females). Ages of female adults at treatment were chosen to approximate progressive stages of oviposition: previposition (5 days), egg development/maturation (10 days), and egg laying (15 days). Each treatment was replicated 4 times.

Oviposition dishes containing 3 ml of saturated, sieved (80-mesh) soil and covered with fluted aluminum foil as described by Branson et al. (1975) were placed in each cage at 10 days. Oviposition dishes, diet, and water agar were replaced at 7-day intervals over a 6-week period or until the death of the female. Oviposition dishes were sealed with parafilm to prevent moisture loss and stored at room temperature. Cages were checked daily and adult mortality recorded. Dead beetles were placed on moist filter paper in petri dishes. Cadavers presenting white mycelial growth within 48 h were microscopically examined for B. bassiana. Cages with females dying from causes other than B. bassiana during the course of the study were removed from the data set. At the end of the study (52 days), all living beetles were freeze-killed and placed on moist filter paper in petri dishes to check for B. bassiana.

After a minimum period of 2 weeks to allow for hardening of the chorion, oviposition dishes were washed through a 60-mesh sieve to remove soil, eggs were collected, and number of eggs per dish was recorded from three of the replicates. Mean cumulative numbers of eggs per female were compared among treatments for survivors and for cohorts using analysis of variance (GLM procedure, SAS Institute, 1985). Data were transformed (log + 1) before analysis when required to normalize variances. Where significant differences occurred, means were separated with least-significant difference (LSD).
Viability. Oviposition dishes, collected at weekly intervals from the 4th replicate in the above experiment, were maintained at 4°C for 120–140 days to simulate diapause conditions. Eggs were removed from individual dishes by washing soil through a 60-mesh sieve. Eggs were counted and a random subsample containing a maximum of 50 eggs/dish was removed and placed on moist filter paper inside a petri dish. Egg dishes were incubated at 27°C. After 10 days dishes were examined daily and percentage hatch was determined by counting the number of empty chorions. Within the cohorts treated with B. bassiana, only eggs deposited by beetles which where known to subsequently die due to the fungus were included within the data set. Percentages of egg hatch per week were angular-transformed and data analyzed using analysis of variance (GLM procedure, SAS Institute, 1985) to compare difference among treatments. Within the cohort of beetles treated at 5 days, there were insufficient beetles surviving fungal treatment to provide a data set for the final week.

**RESULTS**

B. bassiana-related mortality in mated female beetles treated at different ages was first observed 4–5 days following treatment with the majority of mortality occurring within the initial 15–20 days posttreatment for each cohort (Fig. 1). There was no significant difference in female mortality among cohorts treated with B. bassiana at different ages and stages of reproductive maturity ($F = 0.22$; $df = 2,11$; $P = 0.8088$). Final mortality rates ($± SE$) were 73 ($± 10.5$), 71 ($± 9.8$), and 79 ($± 4.1$)% for cohorts treated at 5, 10, and 15 days, respectively. No mortality due to B. bassiana was observed within control cohorts.

The mean number of eggs deposited by female survivors at the end of the study was lowest in beetles treated with B. bassiana at 10 days compared with either the control or the females treated at 5 and 15 days, although differences in egg numbers were statistically significant only during the initial 2 weeks of egg recovery (Table 1). By this time, surviving females treated at 10 days deposited an average of 30% fewer eggs than females treated at other ages or from within the control cohorts. By the end of the study, the difference between 10-day treatment and other groups had decreased to an average of 18% fewer eggs. Of those female survivors remaining at the end of the study, 35, 50, and 41% from 5-, 10-, and 15-day treatments, respectively, tested positive for the presence of B. bassiana when freeze-killed and placed on moist filter paper. Within this group, the mean number of eggs per surviving female ($± SE$) at the end of the study was 577 ($± 132.5$), 391 ($± 71.0$), and 449 ($± 65.1$) for beetles treated at 5, 10, and 15 days, respectively. There was no significant difference in the mean number of eggs deposited by female survivors at the end of the study from all replicates combined.

**TABLE 1**

<table>
<thead>
<tr>
<th>Adult age (days) of female at treatment ($n$)</th>
<th>Mean cumulative No. eggs per surviving female (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (20)</td>
<td>128.8 ($± 19.6$) $a$² 232.0 ($± 31.3$) $a$ 339.8 ($± 40.6$) $a$ 430.8 ($± 54.6$) $a$ 502.1 ($± 62.4$) $a$ 567.8 ($± 72.3$) $a$</td>
</tr>
<tr>
<td>10 (27)</td>
<td>80.9 ($± 13.1$) $b$ 153.5 ($± 18.0$) $b$ 249.1 ($± 26.2$) $a$ 346.9 ($± 36.5$) $a$ 382.3 ($± 40.6$) $a$ 440.3 ($± 50.1$) $a$</td>
</tr>
<tr>
<td>15 (27)</td>
<td>115.6 ($± 13.2$) $a$ 213.8 ($± 17.3$) $a$ 321.6 ($± 20.9$) $a$ 437.3 ($± 30.5$) $a$ 482.9 ($± 34.6$) $a$ 518.4 ($± 39.4$) $a$</td>
</tr>
<tr>
<td>Control (73)</td>
<td>130.5 ($± 9.1$) $a$ 215.2 ($± 12.4$) $a$ 326.3 ($± 16.2$) $a$ 416.6 ($± 21.7$) $a$ 475.2 ($± 24.6$) $a$ 532.7 ($± 28.3$) $a$</td>
</tr>
<tr>
<td>F value</td>
<td>2.26 2.89 1.57 1.17 1.36 1.07</td>
</tr>
<tr>
<td>df</td>
<td>3 3 3 3 3 3</td>
</tr>
<tr>
<td>$P &gt; F$</td>
<td>0.0430 0.0377 0.2013 0.3253 0.2584 0.3635</td>
</tr>
</tbody>
</table>

1 $n$, total number of female survivors at end of study from all replicates combined.
2 Numbers followed by different letters within the same column are significantly different $P < 0.05$ (LSD test).
between treatment schedules at any of the weeks tested ($P > 0.05$).

Egg production by cohort was significantly lower for all *B. bassiana* treatments compared with the control ($F = 34.63; \text{df} = 3, 304; P < 0.0001$) (Fig. 2). At the termination of the study, the mean number of eggs deposited per female ($\pm$SE) within control cohorts was $532 \pm 28.3$ compared with $170 \pm 29.7$, $225 \pm 24.1$, and $300 \pm 23.2$ eggs/female in cohorts treated with *B. bassiana* at 5, 10, and 15 days, respectively.

Egg viability, as estimated by percentage hatch, was initially $\approx 80\%$ for all treatments (Fig. 3). Hatch declined over time for all treatments to $\approx 50\%$ by 6 weeks. Percentage egg hatch per week significantly declined for eggs collected after 5 weeks for all treatments ($F = 19.97; \text{df} = 5, 97; P < 0.0001$). There was no significant difference in percentage hatch in eggs collected from any of the *B. bassiana* treatment schedules compared with the control ($F = 1.76; \text{df} = 3, 97; P = 0.161$).

**DISCUSSION**

Fecundity of *D. virgifera virgifera* within untreated cohorts was comparable to results obtained in previous laboratory studies (Branson and Johnson, 1973; Hill, 1975). Within treated cohorts, depending on when the insect was exposed to the fungus, *B. bassiana* reduced the number of eggs deposited by females. Females treated at 10 days produced $\approx 30\%$ fewer eggs during the initial 2 weeks of the oviposition period compared with other treatments, suggesting a possible link between sublethal infection and the reproductive capacity of the insect. The secondary effect of entomopathogens on host reproduction has been documented but primarily for insect viruses. Several studies have suggested that adult fecundity in lepidopteran species is reduced when the insects are infected with virus in later instars as opposed to early instar larvae (Young and Yearian, 1982; Perelle and Harper, 1986; Santiago-Alvarez and Osuna, 1988). Fewer conclusive studies are available concerning sublethal effects of fungal pathogens on host reproductive potential. Reduced fecundity in Colorado potato beetle surviving treatment with *B. bassiana* was shown to be temperature dependent by Fargues et al. (1991), whereas Wang and Knudsen (1993) found no difference in nymph production in surviving Russian wheat aphids. Noma and Strickler (2000) recently reported a reduction in the oviposition rate of lygus bugs treated with *B. bassiana* but only in insects that did not produce spores at the end of the experiment.

Entomopathogenic fungi affect the host insect through a combination of events including mechanical damage by hyphal growth, nutrient depletion, and the production of toxins (Hajek and St. Leger, 1994). Any of these processes at a sublethal level could negatively affect the reproductive system of the host. The preovipositional period for adult *D. virgifera virgifera* is estimated between 12 and 15 days (Hill, 1975). By 10 days, the first clutch of eggs is maturing within the ovaries of the female. A fungal infection initiated at this time and the resulting physiological stress could interfere with egg development. However, it is unclear why *B. bassiana* treatment of female adults at other ages did not produce a similar effect. One of the difficulties in data interpretation was the inability to confirm (or quantify) the presence of *B. bassiana* in all survivors.

A more striking difference in overall egg production was noted in the comparison between treated and nontreated cohorts. Due to *B. bassiana*-related mortality (74% averaged over all treatment schedules), treated cohorts produced between 44 and 68% fewer eggs than untreated cohorts by the end of the study period. The

![Fig. 2. Mean cumulative No. eggs per female *Diabrotica virgifera* treated with *Beauveria bassiana* at different adult ages. Different lower case letters indicate significant differences among treatments at Week 6 (LSD, $P < 0.05$).](image)

![Fig. 3. Percentage hatch of eggs ($\pm$SE) collected over time from *Diabrotica virgifera virgifera* females treated with *Beauveria bassiana* at different adult ages.](image)
earlier a treatment was applied following adult emergence, the fewer total number of eggs was deposited by the cohort despite similar mortality rates and disease incubation periods for all treatment schedules. Because a greater proportion of the total number of eggs is laid during the initial 10–15 days (Branson and Johnson, 1973), later treatment would not impact the female population until after the peak period of egg laying.

Branson and Sutter (1985) reported >80% hatch in eggs laid by females during the initial week of egg laying. They also found that egg viability declined with female age. Likewise, in findings similar to ours, Fargues et al. (1991) found B. bassiana treatment had no significant effect on egg viability in surviving female Colorado potato beetles. However, N’Doye (1976) observed a reduction in fertility of eggs laid by surviving Chilo suppressalis Walker when infected by B. bassiana as larvae, and Nnakumusana (1985) noted a reduction in egg viability of mosquitoes infected with the entomopathogenic fungus, Aspergillus parasiticus Speare.

As a tool for the management of adult D. virgifera virgifera and subsequent damage, our results suggest B. bassiana can significantly reduce the number of eggs deposited by a population depending on the timing of the application and the resulting beetle mortality. An application of B. bassiana within 5 days of peak female emergence causing approximately 75% mortality over a 15-day period could potentially reduce the number of eggs deposited by approximately 70% compared with no treatment. Because peak adult emergence in the field generally extends over a 7- to 10-day period near the end of July (Short and Hill, 1972), repeat applications may be advisable during this time. Treatments applied later in the season would reduce overall numbers of beetles, but they are less likely to impact oviposition. While this observation is applicable to all forms of adult control, it is particularly relevant to application of entomopathogens that require an incubation period between initial exposure and death of the host insect.

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REFERENCES


