Effect of *Acremonium* Endophytes on Four Species of Billbug Found on New Jersey Turfgrasses

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Abstract. Laboratory studies were conducted to determine the effects of *Acremonium* endophytes on four species of billbug (Coleoptera:Curculionidae: *Sphenophorus parvulus* Gyllenhal, *S. venatus* (Say), *S. inaequalis* Say, and *S. minimus* Hart) found damaging cool-season turfgrasses in New Jersey. Billbug adults feeding on potted tall fescue (*Festuca arundinacea* Schreb.) plants infected with *Acremonium coenophialum* Morgan-Jones and Gams showed significantly greater mortality than billbugs feeding on endophyte-free tall fescue. Little difference was observed in amount of feeding. In petri dish preference tests, billbug adults were given a choice and, again, no significant difference was observed in the amount of feeding on endophyte-free versus endophyte-infected tall fescue tillers. In a third experiment, billbug adults were placed in petri dishes with either tall fescue with or without *A. coenophialum* or perennial ryegrass (*Lolium perenne* L.) with or without *Acremonium lolii* Latch, Christensen, and Samuels. Only small differences were seen in number of eggs laid and amount of feeding. Mortality of all four billbug species, however, was greater on both grasses when endophyte-infected.

The recent discovery of endophyte-enhanced turf performance has caused considerable excitement in the turfgrass industry. Reviews by Siegel et al. (1987), Pottinger et al. (1985), Funk et al. (1985), and others indicate that enhanced stress tolerance and insect resistance are associated with the presence of *Acremonium* endophytes. Since the initial findings of endophyte-enhanced resistance to Argentine stem weevil (*Listronotus bonariensis* Kuschel), a serious pasture pest in New Zealand (Prestidge et al., 1982), 11 additional insects have been reported to be affected by endophytes (Siegel et al., 1987).

Among these insects are the billbugs (*Sphenophorus* spp.), which are major turfgrass pests. Endophyte-enhanced billbug resistance was first observed in two perennial ryegrass cultivar evaluation trials at Adelphia, N.J.; grasses with high endophyte had little damage and few billbug larvae, while grasses free of endophyte were generally very susceptible (Ahmad et al., 1986). These field observations led to our interest in learning more about how *Acremonium* endophytes affect billbugs.

In designing the experiments conducted in this study, endophyte experiments with the closely related Argentine stem weevil (Coleoptera:Curculionidae) were taken into consideration. For example, in both choice and nonchoice petri dish trials, Argentine stem weevil adults exhibited a strong preference for leaf segments of endophyte-free ryegrass and tall fescue. On endophyte-infected potted plants, stem weevil adults fed less and deposited fewer eggs (Barker et al., 1983).

When we began collecting billbug adults for our experiments, we were surprised to find four species of billbugs causing injury to cool-season turfgrasses. Instead of just *S. parvulus* (bluegrass

billbug), as previous studies indicated, we also found *S. venatus*, *S. inaequalis*, and *S. minimus* in almost equal abundance (Johnson-Cicalese et al., 1989; Johnson-Cicalese, 1988). Our objectives in this study were to examine the effects of endophytes on these four billbug species and to determine if, in addition to endophyte-infected perennial ryegrass, tall fescue also shows enhanced resistance to billbugs.

Materials and Methods

Three separate experiments using billbug adults were conducted: 1) mortality and feeding on potted tall fescue plants with and without endophyte; 2) petri dish preference tests with endophyte-free and infected tall fescue tillers; and 3) a non-choice petri dish experiment measuring mortality, egg-laying, and feeding of billbugs on either tall fescue or perennial ryegrass tillers, with and without endophyte.

Billbug adults used in these experiments were obtained from six linear pitfall traps (similar to Lawrence, 1982) placed in turf plots at Adelphia and North Brunswick, N.J. For the first experiment, an unidentified mixture of billbug species was used because the presence of several species was not recognized at the time the test was initiated. However, the second and third experiments measured the effect of endophyte on each of the four billbug species.

The plant material used in these experiments consisted of three tall fescues selected from old turf plantings in Mississippi and Georgia and infected with the endophyte *Acremonium coenophialum* Morgan-Jones and Gains (clones TF2, TF3, and TF4), and two perennial ryegrass selections from Maryland infected with *Acremonium lolii* Latch, Christensen, and Samuels (clones PR1 and PR4). These five selections were divided into individual tillers and planted in 9 × 9-cm (0.7 liter) plastic pots. Half the plants from each selection were treated with fungicides to kill the endophyte, unsuccessfully at first with methyl[1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate (benomyl) in Feb. 1984 and then successfully with granular 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole (propiconazole) at 5.52 kg·ha⁻¹ (six times the label rate) in Mar. 1984. Thus, genetically identical plants, both infected and free of *Acremonium* spp., were available. Additionally, because the plants were subdivided and repotted several times and

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2 years passed before experiments were conducted, they were probably free of any fungicide residue.

Mortality and feeding of adult billbugs on whole plants was measured from 17 June to 15 July 1986. Ten to 12 billbug adults (mixed species) were placed on six pairs of tall fescue plants (clone TF2), with and without endophyte, growing in the greenhouse. The plants were potted in 9 × 9-cm plastic pots and, to prevent billbug escape, covered with a tubular clear acetate cage, 8.7 cm diameter × 25.4 cm high. Three feeding damage ratings were taken using a scale of 1 to 5, where 5 = no feeding and 1 = extensive feeding and severe leaf damage. Billbug mortality was recorded 11–15 July 1986. At the conclusion of the experiment, 60 of the 134 billbugs used were identified as: 28 *S. venatus*, 23 *S. minimus*, 5 *S. parvulus*, and 4 *S. inaequalis* (species confirmed by G.W. Wolfe, Rutgers Univ., New Brunswick, N.J.).

Petri dish preference tests were conducted from 26 Aug. to 9 Sept. 1986. Four tillers of clone TF4 were arranged alternately endophyte-free and infected, stapled to a piece of moistened filter paper and placed in a 10-cm-diameter petri dish. Twelve petri dishes with *S. venatus* and seven with *S. inaequalis* were run. At the time the tests were conducted, only few *S. minimus* and *S. parvulus* were available, so only three dishes each of these two species were run. Four or five adults were added to each dish, except that one dish of *S. parvulus* contained only three billbugs. Tests were conducted for 3 to 5 days, depending on the length of time the tillers stayed green. Feeding damage was visually estimated each day.

The third study, a nonchoice petri dish test, was conducted from 17 Apr. to 21 Sept. 1987. Eight billbugs, half of them males, were placed in a petri dish containing moist filter paper and either endophyte-free or infected tillers of either perennial ryegrass (PR1 and PR4) or tall fescue (TF2 and TF4). A replication of this experiment was initiated as soon as enough billbugs of a species were collected from pitfall traps. Therefore, the number of replications for each species and starting dates for each replication were: *S. inaequalis*—seven, 17 Apr.–14 May; *S. venatus*—six, 11 May–6 July; *S. parvulus*—four, 15 May–10 July; and *S. minimus*—three, 8 June–6 Aug. Dishes were held in the laboratory at 14:10 hr light:dark at a photosynthetic photon flux of 150 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Temperatures ranged from 21 to 27°C. Mortality, number of eggs oviposited, and feeding damage were recorded, and fresh filter paper and grass tillers were provided twice a week.

All data were subjected to analysis of variance and, when a significant F ratio occurred for a treatment effect, Duncan's multiple-range test or a least significant difference test was used for mean comparison (Ott, 1984). For mortality of adults on potted tall fescue, a χ^2 test (Ott, 1984) was conducted, comparing total number dead on endophyte-infected with total dead on endophyte-free plants.

Results and Discussion

When billbug adults (mixed species) were placed on potted tall fescue plants, only slightly more feeding occurred on endophyte-free plants (not significant at $P 0.05$). The average feeding score for infected plants was 3.4 and for endophyte-free plants, 2.8. The difference in billbug survival, however, was highly significant: 80% mortality on the six endophyte-infected plants vs. 42% mortality on the six endophyte-free plants ($\chi^2 = 11.6$, $P 0.01$). On the endophyte-infected plants, 54 were dead, five were alive, and the remaining eight were not found. On endophyte-free plants, 28 were dead, 31 alive, and eight

were not found. Those not found had either died and disintegrated or crawled out of the pots.

The petri dish preference tests indicated that adults of all four species did not have a strong preference for feeding on endophyte-free tall fescue tillers, even when given a choice. Feeding scores on endophyte-free tillers (ranging from 2.3 to 4.2, depending on billbug species) did not differ significantly from feeding scores on infected tillers (2.6 to 4.4). This result is similar to those obtained with potted tall fescue plants, but contradicts results obtained in other studies with the Argentine stem weevil (Barker et al., 1983).

In the experiment that examined the effects of both the tall fescue endophyte (*A. coenophialum*) and perennial ryegrass endophyte (*A. lolii*) on billbug survival, feeding, and ovipositing, few differences were found between tall fescue and perennial ryegrass; therefore, the data were pooled. In addition, there were few differences in how each of the four billbug species reacted to endophyte-infected tall fescue or perennial ryegrass.

In this nonchoice petri dish trial, important differences were observed in the survival of billbug adults (Fig. 1, top), similar to those found on potted tall fescue plants. It took significantly fewer days for all four billbug species to reach 90% mortality when confined on endophyte-infected grass tillers than on endophyte-free grass. Although the endophyte appeared to have the greatest effect on *S. parvulus*, with 90% mortality on endophyte-infected grass occurring in almost half the number of days as on endophyte-free grass, this difference was not statistically greater than the differences that occurred for the other billbug species.

The effect of endophytes on the amount of feeding by adult billbugs and number of eggs laid was less dramatic. *Sphenophorus inaequalis* and *S. venatus* fed significantly less on the endophyte-infected tillers, but for *S. parvulus* and *S. minimus*, there was essentially no difference (Fig. 1, center). This result is similar to those obtained in the potted plant and preference tests. *Sphenophorus venatus* is the only species that laid significantly more eggs in the endophyte-free dishes (Fig. 1, bottom). The diets fed to each billbug may have had a limited effect on number of eggs because most eggs were laid within a few days of being confined on a particular diet. However, Barker et al. (1983) reported that, after being confined for only 4 days on endophyte-infected plants, Argentine stem weevil females contained fewer eggs than those on endophyte-free plants.

The results of these three trials indicate that *Acremonium* endophytes in perennial ryegrass and tall fescue adversely affect the survival of adults of all four billbug species, but have little, if any, effect in deterring adults from egg-laying or feeding. In the long term, fewer eggs may be deposited on an endophyte-infected stand of turf because the females do not live as long. To the best of our knowledge, we believe this to be the first reported case of the tall fescue endophyte having an adverse effect on billbugs; whether this also occurs in the field, as it does with perennial ryegrass (Ahmad et al., 1986), remains to be confirmed. Ahmad et al. (1986) cited only the bluegrass billbug as the species present in their perennial ryegrass study, but we now have evidence that all four species of billbug are present in the same test area.

It would be extremely useful to determine how billbug larvae are affected by endophytes, as most turf damage results from larval feeding. Because of dramatic cases of endophyte-enhanced billbug resistance in the field (Ahmad et al., 1986), one would suspect that larvae are also adversely affected by endophyte, perhaps even more so than adults. Survival of Argentine

Fig. 1. Effect of endophyte on (top) mortality of billbug adults (LSD = 6.9), (center) amount of feeding by billbug adults (LSD = 0.2), and (bottom) number of eggs laid by billbug adults (LSD = 0.5) in a petri dish trial (* indicates significant difference between endophyte-infected and -free, data from tall fescue and perennial ryegrass were pooled; values are the average of at least three replications).

stem weevil larvae (Barker et al., 1984) and other insect larvae are adversely affected by endophyte-infected grasses. Unfortun-

nately, there are several problems in working with billbug larvae that are difficult to overcome. First, they cannot be identified to species, so tests conducted with field-collected larvae would be a mixture of species. Reared, identified larvae from this project were saved for future work on a larval key. Second, conducting a test with field-collected larvae would be time-consuming because there is no efficient way to collect them. In our preliminary work with rearing billbugs, we had only limited success (Johnson-Cicalese et al., 1989), but additional work on rearing techniques may yield enough specimens to run replicated trials.

In conclusion, *Acremonium* endophytes in both tall fescue and perennial ryegrass appear to affect the adult survival of all four billbug species in laboratory trials, further corroborating the effectiveness and usefulness of endophyte-enhanced pest resistance in turfgrasses.

Literature Cited

- Ahmad, S., J.M. Johnson-Cicalese, W.K. Dickson, and C.R. Funk. 1986. Endophyte-enhanced resistance in perennial ryegrass to the bluegrass billbug, *Sphenophorus parvulus*. Entomol. Expt. Appl. 41:3-10.
- Barker, G.M., R.P. Pottinger, and P.J. Addison. 1983. Effect of tall fescue and ryegrass endophytes on Argentine stem weevil, p. 216-219. In: Proc. 36th New Zealand Weed Pest Control Conf.
- Barker, G.M., R.P. Pottinger, and P.J. Addison. 1984. Effect of *Lolium* endophyte fungus infections on survival of larval Argentine stem weevil. N.Z.J. Agr. Res. 27:279-281.
- Funk, C.R., P.M. Halisky, S. Ahmad, and R.H. Hurley. 1985. How endophytes modify turfgrass performance and response to insect pests in turfgrass breeding and evaluation trials, p. 137-145. In: F. Le-maire (ed.). Proc. 5th Intl. Turf Res. Conf., Avignon, France. INRA Publishers, Versailles, France.
- Johnson-Cicalese, J.M. 1988. Billbug pests of turfgrass: Biology, host range, and effects of endophyte-infected grasses. MS Thesis, Rutgers Univ., New Brunswick, N.J.
- Johnson-Cicalese, J.M., G.W. Wolfe, and C.R. Funk. 1990. Biology, distribution and taxonomy of billbug turf pests. Environ. Entomol. (In press.)
- Lawrence, K.O. 1982. A linear pitfall trap for mole crickets and other soil arthropods. Sci. Notes, Fla. Entomol. 65:376-377.
- Ott, L. 1984. An introduction to statistical methods and data analysis. 2nd ed. Duxbury Press, Boston.
- Pottinger, R.P., G.M. Barker, and R.A. Prestidge. 1985. A review of the relationships between endophytic fungi of grasses (*Acremonium* spp.) and Argentine stem weevil (*Listronotus bonariensis* (Kuschel)), p. 322-331. In: R.B. Chapman (ed.). Proc. 4th Australasian Conf. on Grassland Invertebrate Ecol., Canterbury, New Zealand.
- Prestidge, R.A., R.P. Pottinger, and G.M. Barker. 1982. An association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil. Proc. 35th New Zealand Weed Pest Control Conf. p. 119-122.
- Siegel, M.R., G.C.M. Latch, and M.C. Johnson. 1987. Fungal endophyte of grasses. Annu. Rev. Phytopathol. 25:293-315.