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Influence of *Rhopus nigroclavatus* (Hymenoptera: Encyrtidae) on the Mealybugs *Tridiscus sporoboli* and *Trionymus* sp. (Homoptera: Pseudococcidae)

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ABSTRACT This research investigated parasitism of the buffalograss mealybugs *Tridiscus sporoboli* (Cockerell) and *Trionymus* sp. by *Rhopus nigroclavatus* (Ashmead) through rearing and dissection studies, paired comparison tests, and a field survey. Rates of parasitism reported in this article reflect combined parasitism of the 2 mealybug species. In the rearing study, parasitism of adult female mealybugs by *R. nigroclavatus* was 48.6%, with an average emergence of 1.77 parasitoids per parasitized mealybug. A maximum of 7 *R. nigroclavatus* adults emerged from a single mealybug female. Mealybug dissections documented parasitism rates by *R. nigroclavatus* of 78.5, 67.5, and 4.3%, respectively, for adult females, 3rd and 4th (male pupae) instars, and 1st- and 2nd-instar mealybugs. Results revealed the preference of *R. nigroclavatus* for adult female mealybugs and later instars, and suggested a potential regulating effect of *R. nigroclavatus* on buffalograss mealybug populations. Paired comparison tests with and without *R. nigroclavatus* demonstrated the effectiveness of this parasitoid as a biological control agent for buffalograss mealybugs under greenhouse conditions. Significant differences were detected in the number of nonparasitized mealybugs between treatments containing only mealybugs and treatments containing both mealybugs and parasitoids. Sticky trap captures in the field suggested a functional relationship between the seasonal abundance of *R. nigroclavatus* and its mealybug hosts.

KEY WORDS *Rhopus nigroclavatus, Tridiscus sporoboli,* buffalograss, mealybug, parasitoid

THE DEVELOPMENT OF turfgrass species requiring less water, mowing, and fertilization has received considerable attention over the last decade. Buffalograss, *Buchloë dactyloides* (Nuttall) Engelmann, is an appealing, alternative turfgrass species because of its low maintenance requirements and relative freedom from diseases and arthropod pests (Riordan et al. 1993). Few arthropods are considered as serious pests of buffalograss. Recently, however, 2 grass-feeding mealybugs, *Tridiscus sporoboli* (Cockerell) and *Trionymus* sp., were identified as potentially serious insect pests of buffalograss in Nebraska (Baxendale et al. 1994). These 2 mealybugs are similar in appearance and can be distinguished only after specimens have been cleared, stained, and examined for a series of microscopic morphological features (Ferris 1950, 1953; McKenzie 1967).

Tridiscus sporoboli and *Trionymus* sp. were first observed in buffalograss evaluation plots at the John Seaton Anderson Turfgrass and Ornamental Research Facility (JSA Facility), University of Nebraska Agricultural Research and Development Center, near Mead, NE, in 1988, and had caused extensive damage to the buffalograss by 1989 (Baxendale et al. 1994). In 1990, however, only low mealybug numbers were detected in these same plots. A proposed explanation for the dramatic decline in mealybug numbers was the natural regulating effect of beneficial arthropods, specifically parasitoid wasps (Baxendale et al. 1994). Subsequent research identified *Rhopus nigroclavatus* (Ashmead) as a parasitoid of *T. sporoboli* and *Trionymus* sp. (Heng-Moss 1997); however, little information was available on the potential regulatory effectiveness of this parasitoid on these buffalograss-inhabiting mealybugs.

Accordingly, the objective of this research was to better understand the biology of *R. nigroclavatus* as a parasitoid of *T. sporoboli* and *Trionymus* sp. by evaluating, under controlled greenhouse conditions, age-specific parasitoid preferences, methods for assessing rates of parasitism, and the potential of *R. nigroclavatus* to regulate mealybug populations. A field survey also was conducted to further evaluate possible regulating effects of the parasitoid on mealybugs in buffalograss turf.

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Materials and Methods

Rearing and dissection studies served to evaluate age-specific rates of parasitism by *R. nigroclavatus* under greenhouse conditions. Mealybugs were obtained by collecting fifty 10.6-cm sod plug samples of NE-85-97, a mealybug-susceptible buffalograss selection (Johnson-Cicalese et al. 1998), from buffalograss evaluation plots at the JSA Facility, near Mead, NE. Buffalograss plugs, containing both mealybugs and naturally occurring parasitoids, were planted in 15-cm pots and placed under 400-W high-intensity discharge lamps with a photoperiod of 14:10 (L:D) h. These mealybug-infested plants were maintained in a greenhouse at the University of Nebraska, Lincoln, for the duration of the rearing and dissection studies to ensure a continuous supply of mealybugs and parasitoids.

Rearing Study. Adult female mealybugs were collected from the leaf sheaths of mealybug-infested NE 85-97 buffalograss plants (described above) on 20 May, 6 June, 19 June, 9 July, and 23 July 1996. Adult female mealybugs were placed individually in small plastic cups (28.35 g) and maintained in a growth chamber at 30°C with a photoperiod of 14:10 (L:D) h until parasitoid emergence. Parasitoids were reared from a total of 350 mealybugs.

Dissection Study. Earlier observations had shown that parasitoid levels build rapidly in the greenhouse (T.M.M., unpublished data). Therefore, this study was conducted over a 4-mo period to document the increase in parasitoid abundance under greenhouse conditions. One hundred mealybugs from each of the 3 age groups 1st and 2nd instars, 3rd and 4th (male pupae) instars, and similarly aged adult females were collected on 27 May, 24 June, 29 July, and 26 August 1996 from the previously described NE 85-97 buffalograss. These mealybugs were dissected to detect internal parasitoids. Analysis of variance (ANOVA) was conducted on the logit of the probability of parasitism using the CATMOD analysis procedure (SAS Institute 1990).

Paired Comparison Tests. Paired comparison tests were conducted in the greenhouse to evaluate the regulatory effectiveness of *R. nigroclavatus* on *T. sporoboli* and *Trionymus* sp. Cylindrical clear acetate cages (12 cm diameter, 30 cm high) served to exclude extraneous parasitoid wasps. Cage tops were covered with organdy fabric.

Buffalograss plants were started from 'Texoka' (Stock Seed Farm, Murdock, NE) seed to ensure initial freedom from mealybugs and parasitoids. Plants were maintained in the greenhouse and were caged to prevent accidental mealybug and/or parasitoid contamination. Once seedlings were well established, 40 nymphal mealybugs were introduced into each cage to initiate a mealybug infestation. Because *R. nigroclavatus* prefers to parasitize late instars (3rd and 4th) and adult female mealybugs (Heng-Moss 1997), plants were inoculated with early-instar (1st and 2nd) mealybugs to minimize the likelihood of introducing previously parasitized mealybugs. The experiment was initiated ≈3 wk later when white cottony masses produced by late-instar and adult female mealybugs signaled the presence of suitable mealybug life stages for parasitism by *R. nigroclavatus*.

The mealybug-infested pots were randomly assigned to 1 of 2 treatments: cages containing only mealybugs (MB only), and cages containing both mealybugs and parasitoids (MB + Para). At the start of the experiment, 3 parasitized female mealybugs were introduced into the cages of the MB + Para treatments. Parasitized mealybugs rather than adult parasitoids were introduced because of the difficulty associated with transferring the highly active wasps. The study was repeated 2 times.

Treatments were arranged in a completely randomized design with 6 replications (study 1), and 4 replications (study 2). Plants were harvested 4 wk after introduction of the parasitized female mealybugs. Every buffalograss tiller (≈25) in each pot was examined and the number of parasitized and nonparasitized mealybugs recorded. A mealybug was considered parasitized when 1 or more parasitoid larvae or adult emergence holes were detected.

Analysis of variance (ANOVA) was conducted to determine if there was a significant difference in the number of nonparasitized mealybugs between the 2 studies. Because there was no significant difference between studies, the data were pooled and analyzed using the GLM procedure (SAS Institute 1990), and means were separated using the least significant difference (LSD) test (SAS Institute 1990).

Seasonal Abundance of *R. nigroclavatus* and Its Mealybug Hosts. As a 1st step in evaluating *R. nigroclavatus* as a biological control agent for *T. sporoboli* and *Trionymus* sp. under field conditions, adhesive-covered yellow sticky traps (15 by 30 cm) (Dowell and Cherry 1981, Neuenschwander 1982) were used to monitor simultaneously the seasonal abundance of *R. nigroclavatus* and its mealybug hosts. This study served to identify seasonal abundance relationships between mealybugs and parasitoids. Male mealybugs were used to reflect the seasonal abundance of the mealybug population because, like *R. nigroclavatus*, they are attracted to yellow sticky traps (Heng-Moss et al. 1997).

Traps were constructed by rolling a sticky strip into a cylinder, then stapling it to a 30-cm wooden stake. Ten yellow sticky traps were placed randomly in a mealybug-infested buffalograss evaluation plot at the JSA Facility. Traps were located in buffalograss selections where large numbers of mealybugs (mean = 54.3 mealybug per 230 cm^2) had been collected the previous season (1994). Every 14 d, traps were collected and replaced with new traps. Collected traps were returned to the laboratory where mealybugs and parasitoids were counted. Monitoring began the 1st week in May and continued through the last week in October during 1995 and 1996.

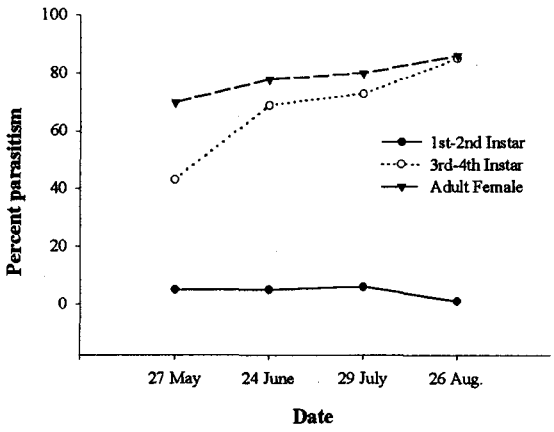

Fig. 1. Parasitism rates of 3 mealybug age groups by *R. nigroclavatus* as determined by dissection studies. Percentage parasitism was significantly (χ^2 = 185.48, df = 2, P < 0.0001) affected by age class. Date did not significantly (χ^2 = 7.36, df = 3, P < 0.06) affect percentage parasitism and there was a significant (χ^2 = 13.35, df = 6, P < 0.04) age class by date interaction.

Results and Discussion

High rates of parasitism were recorded in both rearing and dissection studies, suggesting that *R. nigroclavatus* can parasitize a relatively high proportion of the mealybug population, at least under greenhouse conditions. Because *T. sporoboli* and *Trionymus* sp. are indistinguishable except after being cleared, stained, and examined for a series of microscopic features, rates of parasitism reported in this article reflect the combined parasitism of the 2 mealybug species.

Rearing Study. In the rearing study, 48.6% (170/350) of the adult female mealybugs were parasitized by *R. nigroclavatus*. A total of 301 *R. nigroclavatus* emerged from the 170 parasitized mealybugs, producing an average of 1.77 parasitoids per mealybug. A maximum of 7 *R. nigroclavatus* adults were reared from a single female mealybug.

Dissection Study. The dissection study documented parasitism of both immature and adult female mealybugs. Parasitism rates for the adult females, 3rd and 4th (male pupae) instars, and 1st- and 2nd-instar mealybugs were 78.5, 67.5, and 4.3%, respectively. The high percentage parasitism of adults and later instars suggests this parasitoid may have an important regulating effect on mealybug populations, at least under greenhouse conditions.

There was a significant interaction between age class and date (χ^2 = 13.35, df = 6, P < 0.04) (Fig. 1). Parasitism rates for the 3rd and 4th (male pupae) instars and adult females increased during the study, resulting in high rates of mealybug parasitism by the 4th mo, whereas rates of parasitism for the 1st and 2nd instars remained nearly the same during this period. The main effect of age was highly significant (χ^2 = 185.48, df = 2, P < 0.0001), but the main effect of date was not significant (χ^2 = 7.36, df = 3, P < 0.06). The coefficients obtained from estimating the model parameters indicate that the probability of parasitism increased with age.

Table 1. Paired comparison studies (mean ± SE) conducted to evaluate the parasitism to mealybugs by *R. nigroclavatus*

Treatment	n	No. mealybugs		Parasitism rate
		Nonparasitized	Parasitized	
Mealybug only[a]	10	49.8 ± 11.6a	0.6 ± 0.3	1.1 ± 0.8
Mealybug + parasitoid[b]	10	8.8 ± 6.5b	16.3 ± 4.6	77.5 ± 5.7

Significant differences were detected (T = 3.1; df = 18, 0; P < 0.007) in the number of nonparasitized mealybugs between the mealybug only and the mealybug + parasitoid treatments.

[a] Treatment isolating only mealybugs.

[b] Treatment isolating both mealybugs and parasitoids.

Because the mealybug dissections were conducted at times different from the rearing studies, statistical comparison of the 2 methods was not appropriate. However, a nonstatistical comparison of the 2 methods revealed a higher rate of adult female mealybug parasitism (78.5%) for the dissection method than for the rearing method (48.6%). Other researchers (Chamberlin 1926, Cartright et al. 1984, Day 1994) have also reported observing higher rates of parasitism from dissection studies. Premature death of the host and subsequently of the endoparasitoids, parasitoid mortality from host defense mechanisms, reduced survival of mealybugs because of less than optimal conditions in the rearing containers, and/or different sampling dates, may help explain this discrepancy.

Paired Comparison Tests. Paired comparison tests documented the effectiveness of *R. nigroclavatus* as a biological control agent for *T. sporoboli* and *Trionymus* sp. under greenhouse conditions (Table 1). Significant differences were detected (T = 3.1; df = 18, 0; P < 0.007) in the number of nonparasitized mealybugs between the MB only (mean = 49.8) and the MB + Para (mean = 8.8) treatments. The mean rate of parasitism for the MB + Para treatments was 77.5%. The buffalograss in these treatments sustained only minimal mealybug damage.

The ability of *R. nigroclavatus* to locate and parasitize a mealybug host was further demonstrated when, despite best efforts at exclusion, parasitoids managed to enter the MB only pots, resulting in a mean parasitism rate of 1.1%. However, this contamination was not considered to have confounded the experiment because only 6 of the 498 mealybugs collected from this treatment were parasitized. These studies further demonstrated the regulating potential of *R. nigroclavatus* on mealybug populations.

Seasonal Abundance of *R. nigroclavatus* and Its Mealybug Hosts. Yellow sticky trap captures suggested a functional relationship between the seasonal abundance of *R. nigroclavatus* and its mealybug hosts under field conditions. In 1995, male mealybugs captured on sticky traps steadily increased from May to July, then decreased through-

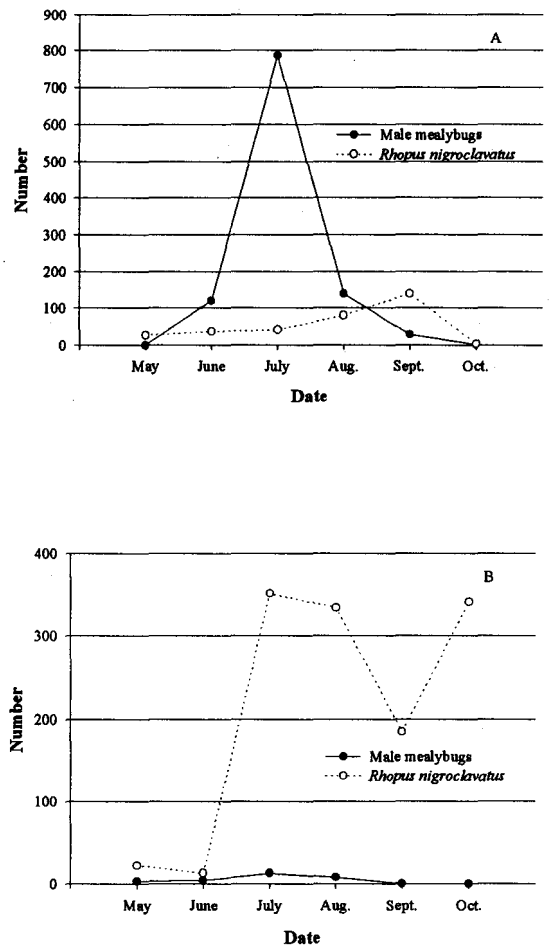

Fig. 2. Seasonal abundance of male mealybugs and *R. nigroclavatus* as sampled by sticky traps placed in buffalograss evaluation plots at the JSA Facility, near Mead, NE, during 1995 (A) and 1996 (B).

out the remainder of the season (Fig. 2A). The number of *R. nigroclavatus* captured on the sticky traps remained relatively low throughout the 1995 season. Both mealybug and *R. nigroclavatus* populations dropped to low levels with the onset of freezing temperatures in October.

Although male mealybugs were much more abundant than *R. nigroclavatus* during 1995, the opposite occurred in 1996. Male mealybugs remained at low levels throughout 1996, whereas parasitioids were very abundant, especially late in the season (Fig. 2B). These fluctuations in mealybug population levels were similar to those reported by Baxendale et al. (1994). In both cases, the natural regulatory effect of *R. nigroclavatus* may have been responsible for the decline in mealybug numbers. The reason for the late-season increase in *R. nigroclavatus* in 1996 is unclear. Additional seasonal abundance studies, conducted at shorter sampling intervals, are needed to characterize further the seasonal activity of both *R. nigroclavatus* and its mealybug hosts and confirm the regulating potential of *R. nigroclavatus* on mealybug populations under field conditions.

Biological control offers an attractive, alternative approach for managing insect pests affecting buffalograss. Information obtained from these studies will be valuable in developing a biological control program using *R. nigroclavatus* to manage *T. sporoboli* and *Trionymus* sp. infestations in buffalograss.

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