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Predation rate and development of *Coccinella septempunctata* L. influenced by *Neozygites fresenii*-infected cotton aphid prey

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Abstract

Laboratory studies were conducted to determine the effect of cotton aphids [*Aphis gossypii* Glover (Homoptera: Aphididae)] infected with *Neozygites fresenii* (Nowakowski) Batko (Entomophthorales: Neozygitaceae), on the number of prey attacked by and development of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). A diet of *N. fresenii*-infected aphids (in early stages of infection) did not have a significant effect on predation rate by either the fourth-stage larvae or adults of *C. septempunctata*. Second-, third- and fourth-stage larvae of *C. septempunctata* reared on fungus-infected aphids had significantly longer stadia than those reared on uninfected aphids. Mortality of *C. septempunctata* larvae reared on fungus-infected aphids increased between the second and the fourth instars, whereas mortality for those reared on uninfected aphids was significantly lower than those reared on infected aphids. Feeding on *N. fresenii*-infected aphids resulted in significantly smaller body size of *C. septempunctata* adults and a corresponding reduction in the number of eggs oviposited during a 29-day period relative to those fed a diet of uninfected aphids. Although our findings suggest that a diet of *N. fresenii*-infected aphids had no effect on the number of prey consumed by *C. septempunctata*, it had a significant effect on the development of the predator and its capacity to reproduce. These fitness costs could alter the capacity of the predator population to reduce subsequent pest populations in cotton or adjacent crops.

Keywords: *Coccinella septempunctata*; *Neozygites fresenii*; Entomopathogenic fungus; Cotton aphid; Fungus; Entomophthoralean fungus; Intraguild predation; Interaction; Development

1. Introduction

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), remains as a significant, economic pest of cotton (*Gossypium hirsutum* L.) throughout the cotton belt of the United States (Williams, 2006). Increased insecticide resistance, the destruction of natural enemies by insecticides (which permits aphids to escape parasitism and predation) and the complex interaction that occurs between the cotton plant and the environment are among the most important factors that increase the frequency of *A. gossypii* outbreaks (Kerns and Gaylor, 1992, 1993). Evaluation and integration of various natural enemies and the interactions among them should be undertaken in a bid to maintain *A. gossypii* below economic thresholds.

Insecticides are typically the first management tool selected by producers, but insecticide resistance has been reported for several classes of insecticides, including carbamates (Furk et al., 1980), organophosphates (Saito, 1990; Kerns and Gaylor, 1992), pyrethroids (Kerns and Gaylor, 1992) and the newer neonicotinoids (Wang et al., 2002). Furthermore, increases in cotton aphid densities in insecticide-treated fields are due to direct and/or indirect stimulation of aphid reproduction (Slosser et al., 2004).

The cotton aphid is attacked by numerous natural enemy species. Peak aphid densities often coincide with peak parasitism by *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae), but this parasitoid is likely not responsible for aphid density declines in the Mississippi

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delta region (Kerns and Gaylor, 1993). Green and brown lacewings, araneids, coccinellids, *Geocoris* spp., *Nabis* spp. and *Orius* spp. are all active predators of cotton aphids (Kerns and Gaylor, 1993). Among these predator groups, the coccinellids are generally most abundant, peaking at 12 per 20 sweeps, and are most synchronized with cotton aphid density (Weathersbee and Hardee, 1994; Wells et al., 2001; Conway et al., 2006). The exotic seven-spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), is established in most states (Angalet et al., 1979) and is among the important coccinellids preying upon aphid species in several agricultural crops. Cotton aphid densities in seedling cotton may be significantly reduced by *C. septempunctata* predation (Wells et al., 2001; Conway et al., 2006). A threshold was developed in Arkansas that incorporates the number of beneficial insects, particularly coccinellids, in treatment decisions early in the production season (pre-bloom) (Conway et al., 2006). This threshold was later modified and implemented as part of the insecticide recommendations for the state of Arkansas (Greene, 2006).

An entomopathogen identified by Steinkraus et al. (1991) as *Neozygites fresenii* (Nowakowski) Batko (Entomophthorales: Neozygitaceae) is an important regulatory factor causing epizootics in cotton aphid populations in 11 states in the cotton belt (Steinkraus, 2000). *N. fresenii* caused epizootics of cotton aphid in cotton fields in these states since 1989 (Steinkraus and Boys, 2005). *N. fresenii* may cause two epizootics each year, one during the 3rd week of July and a second during the 3rd week of August, both occurring 1 week after peak aphid abundance (Weathersbee and Hardee, 1994). Kerns and Gaylor (1993) noted that this entomopathogenic fungus was the only mortality factor that demonstrated a well-defined cause–effect relationship with cotton aphid densities both in insecticide-treated and untreated plots.

Both *N. fresenii* and coccinellids (including *C. septempunctata*) simultaneously contribute to declines of *A. gossypii* densities in cotton fields. Once epizootics begin, they progress very rapidly, resulting in almost all aphids within a field becoming infected during a period of 1–2 weeks (Hollingsworth et al., 1995). Peak aphid population densities tend to coincide with *N. fresenii* prevalence, a scenario that may leave *C. septempunctata* with few options: it has to feed on fungus-infected aphids, switch to non-aphid prey, or emigrate (in case of adults). Therefore, during an epizootic, the acceptability and suitability of the *N. fresenii*-infected cotton aphid to *C. septempunctata* may not only influence predator development to maturity but also its subsequent seasonal population dynamics. The effect of a diet of *N. fresenii*-infected cotton aphid on development of *C. septempunctata* has not been examined.

Starved *C. septempunctata* feed on *Acyrthosiphon pisum* (Harris) killed by *Pandora* (=*Erynia*) *neoaphidis* Remaudiere and Hennebert. Although they rarely consume the entire cadaver, levels of transmission are comparable with partially consumed and intact cadavers (Pell et al., 1997; Roy et al., 1998). This predation of fungal-infected aphids by *C. septempunctata* larvae and adults is considered a form of intraguild predation (Roy et al., 1998).

We examined the effect of a diet of *N. fresenii*-infected cotton aphid on predation by and development of *C. septempunctata*. The objectives of our study were to: (1) compare the predation rates of *C. septempunctata* larvae and adults feeding on uninfected versus *N. fresenii*-infected cotton aphids; (2) determine the effect of a diet of *N. fresenii*-infected cotton aphid on duration of immature stages and mortality of *C. septempunctata*; and (3) determine the effect of a diet of *N. fresenii*-infected cotton aphid on body size and fecundity of *C. septempunctata* adults. This study was not designed to address the impact of predation of fungus-infected aphids on epizootic development.

2. Materials and methods

Aphis gossypii were collected from cotton and reared on cotton plants at the Margaret McClendon Insect Rearing Laboratory at the Arkansas Agricultural Research and Extension Center. Cotton plants were grown in the greenhouse at approximately 26 °C and plants in the three-leaf stage were infested with aphids. Aphids were reared in $60 \times 80 \times 44$ cm cages containing five cotton plants under fluorescent lights (14:10 L:D) in the laboratory (28 ± 3 °C). To infest the new plants, leaves were removed from previously-infected plants and placed on new ones for 24 h. New plants were placed in cages every 4 days.

Coccinella septempunctata adults were collected from crimson clover, *Trifolium incarnatum* L., and reared on cotton aphids in the laboratory under the same temperature and light regimen. Individual adults were housed in small (5 cm) petri dishes. When eggs were observed, the adults were removed. At eclosion, *C. septempunctata* larvae were separated and reared individually in 5-cm petri dishes throughout their development to avoid cannibalism. Aphid prey were brushed off the cotton leaf into the dish using a soft natural-hair brush.

Neozygites fresenii-killed aphids (referred to as "cadavers") were initially obtained from the insect pathology laboratory at the Arkansas Agricultural Research and Extension Center. Cotton aphids were infected with *N. fresenii* according to the methods described by Steinkraus and Slaymaker (1994). To induce sporulation, five "cadavers" (cotton aphids previously infected with *N. fresenii*, then dried and frozen at the hyphal body stage) were placed in a 10-cm petri dish at ~100% RH, 16:8 L:D and 27 °C for 24 h. During that period, the hyphal bodies within aphid cadavers formed conidiophores that forcibly discharged primary conidia. Primary conidia germinated to form capilliconidiophores, on which capilliconidia (infective secondary conidia) were formed. Nine h after the induction of sporulation, about 100 aphids were infected by enclosing them in a 10-cm dish containing infective capilliconidia for 6 h. Newly infected aphids were transferred onto an excised cotton leaf and were exposed to