

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: Neozygiteaceae), 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.

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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 *Acyrtosiphon 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 × 80 × 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

75% RH, 27 °C and 16:8 L:D. The leaf petiole was inserted into a 50 ml water-filled flask and was tightly held using a cotton ball while enclosed in a plastic container. After a 3-day incubation period, infected live aphids began to show symptoms of *N. fresenii* infection; i.e., they were pale green and swollen (Steinkraus et al., 1993). Aphids in this stage of infection were used in all subsequent experiments because dead aphids in the late stages of infection were not recognized as viable prey by *C. septempunctata*. Five percent of the infected aphids from each population were selected at random, squashed on microscope slides and viewed under a 100× phase microscope to confirm *N. fresenii* infection (as shown by the presence of protoplasts or hyphal bodies) (Steinkraus et al., 1991). Once studies were initiated, these subsamples confirmed that efficiency of selecting infected aphids exceeded 95%.

2.1. Predation of infected aphids by fourth-stage larvae and adult *C. septempunctata*

Comparisons were made of the predation rates of both fourth-stage and adult *C. septempunctata* on uninfected and *N. fresenii*-infected cotton aphids. Adults and larvae used in experiments were starved for 24 h before being provided aphids. Coccinellids typically undergo periods of satiation followed by long periods of inactivity where they do not feed (Wells et al., 2001). Starvation of the insects in this study also assured a comparison of the diets within the observable time period of the no-choice tests. Two fourth-stage *C. septempunctata* were enclosed in separate 10-cm petri dishes containing freshly excised cotton leaves. One larva was provided 160 uninfected aphids, while the other was given the same number of *N. fresenii*-infected aphids. Predation during the dark phase of the day was not observed; as a result, the data are presented as the total number of aphids consumed based only on the 14-h light phase of the day. The number of aphids consumed by each larva was recorded hourly within the 14-h period. The same procedure was followed when comparing predation rates by *C. septempunctata* adults on uninfected and *N. fresenii*-infected aphids. Tests were conducted for each diet with 12 larvae and 12 adults, each in individual dishes.

2.2. Effect of a diet of infected aphids on *C. septempunctata* development and survival

The suitability of *N. fresenii*-infected cotton aphids as prey for *C. septempunctata* was determined. Duration of each immature stage, mortality during the course of development, and body size and partial fecundity of the resulting adults were considered as parameters of prey suitability. For the subsequent experiments, two experimental groups, each consisting of 20 beetles, were reared on different diets. One group was reared on uninfected aphids while the other on *N. fresenii*-infected aphids. Beetles and larvae were moved daily to new dishes containing

excess prey of each diet type for all evaluations. The test was repeated three times (60 beetles per treatment).

The duration of each developmental stage (in days) of *C. septempunctata* immatures reared on uninfected and *N. fresenii*-infected aphids was measured beginning after eclosion. Mortality and ecdysis were assessed daily for each insect. Total developmental period from first-stage larva to pupa was evaluated and compared between the diets. Mortality of each stage of *C. septempunctata* immatures was also recorded in each group.

2.3. Effect of a diet of infected aphids on body size and fecundity of *C. septempunctata* adults

The effects of a diet of *N. fresenii*-infected aphids on body size (tibial length and abdominal width) and fecundity of *C. septempunctata* were determined. Tibial length and abdominal width are often highly correlated to each other and adult body size (Omkar et al., 2005; de Sassi et al., 2006). While body size is an adequate predictor of fecundity, lady beetles produce fewer eggs as they age (Dixon and Agarwala, 2002). From each of the two experimental groups reared through on different diets (uninfected or *N. fresenii*-infected aphids) as previously described, the sizes of 25 *C. septempunctata* adults were measured using an ocular micrometer. Comparisons of hind tibial lengths and abdominal widths (at abdominal segment II) were made between *C. septempunctata* adults reared on the two prey types. Male and female adults were measured separately after being sexed according to the methods of Bauggaard (1980).

The effect of a diet of *N. fresenii*-infected aphids on partial fecundity of *C. septempunctata* was evaluated for the surviving females from the two groups reared on different diets (uninfected or *N. fresenii*-infected aphids) as previously described. Females of each group were fed *ad libitum* with their respective (uninfected or *N. fresenii*-infected aphids) diets throughout their 8-day preoviposition period and a 29-day oviposition period. After the 8-day preoviposition period, *C. septempunctata* females were allowed to mate with males reared on the same diet. The number of eggs oviposited by each female was recorded daily for 29 days after their preoviposition period ended. The daily oviposition of *C. septempunctata* over its entire reproductive longevity (66 days) has been previously described (Kawachi, 1985) and reaches a general plateau in daily production from 21 to 56 days (Phoofolo and Obrycki, 1995).

2.4. Statistical analysis

Data were analyzed by analysis of variance procedures with means separated (when appropriate) using the Duncan's Multiple Range test to detect significant differences among means of *C. septempunctata* larvae and adults feeding on *N. fresenii*-infected and uninfected aphids (SAS Institute, 1999). Student's *t*-tests were used to detect significant differences between body sizes of *C. septempunctata*

adults and partial fecundity of *C. septempunctata* females reared on uninfected and those reared on infected aphids.

3. Results and discussion

Preliminary studies determined that both fourth-stage and adult *C. septempunctata* would accept and feed on fresh, symptomatic, *N. fresenii*-killed aphids (3–4 days after infection). However, fungus-killed aphids appeared to desiccate rapidly or were soon attacked by saprophytes, after which they were avoided by both larvae and adults. Therefore, in our study only, living infected aphids showing the symptoms of infection (pale green and swollen) were used as food in the experiments.

3.1. Predation of infected aphids by fourth-stage and adult *C. septempunctata*

No deposition of progeny by either infected or uninfected aphids was observed during the course of the feeding trials (14 h), presumably due to crowding, the disruption caused by aphid handling, and activity of the foraging predator in each dish. Thus, we assumed that aphid consumption was accurately assessed by enumerating prey remaining at the end of each trial period. Predation of uninfected and *N. fresenii*-infected aphids was significantly influenced by the predator's developmental stage (fourth-stage or adult) and aphid infection status ($F = 6.27$; $df = 3, 44$; $P = 0.0012$), but not their interaction ($P = 0.6357$). Infection status of aphids did not have a significant effect on the number of aphids consumed during the observation period (a single 14 h day) by either fourth-stage larvae or adults ($F = 1.76$; $df = 1, 44$; $P = 0.192$) (Table 1). *C. septempunctata* larvae consumed significantly more aphids in this period than adults, irrespective of aphids' state of infection ($F = 16.83$; $df = 1, 44$; $P = 0.0002$) (Table 1). Each *C. septempunctata* larva consumed an average of 9.7 ± 2.5 uninfected and 9.7 ± 2.5 *N. fresenii*-infected aphids per hour during daylight hours (14 h) (Fig. 1). Fifty-three percent of uninfected and 54% of *N. fresenii*-infected aphids were consumed by *C. septempunctata* larvae during the first 3 h of exposure. Consumption of a larger number prey at the initiation of the study was expected due to the prior starvation of the

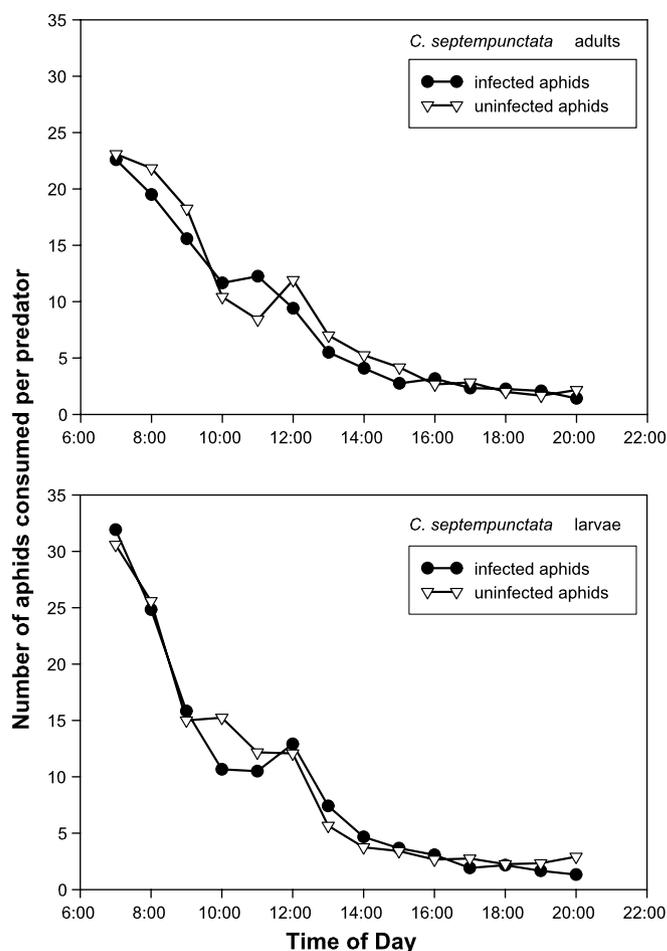


Fig. 1. Mean number of uninfected and *Neozygites fresenii*-infected cotton aphids consumed hourly by *Coccinella septempunctata* larvae ($n = 12$) and adults ($n = 12$) over a 14-h period.

coccinellids and because the predators became satiated as the 14 h test continued.

Coccinella septempunctata adults consumed 121.7 ± 5.0 uninfected and 114.6 ± 4.0 *N. fresenii*-infected aphids per observation period. Individual adult *C. septempunctata* consumed ~ 40 *Aphis fabae* Scopoli per day (Homoptera: Aphididae), a much larger aphid (Honek, 1985). In our study, adults consumed 52% of the total consumed uninfected or *N. fresenii*-infected aphids, respectively, during the first 3 h of exposure. Adults consumed 8.7 ± 2.0 uninfected and 8.2 ± 1.9 *N. fresenii*-infected aphids per observation period. Neither larvae nor adults were observed feeding during the dark phase of the day between 20:00 and 06:00 h. As in our studies, *C. septempunctata* larvae and adults consume more than half of their total diet during the first 3 h when fed *Lipaphis erysimi* Kalt (Shukla et al., 1990).

Coccinella septempunctata spends a relatively shorter time handling *P. neoaphidis*-infected bean aphids (*A. pisum*) than uninfected ones, eventually consuming more uninfected aphids than infected ones (Roy et al., 1998). In our study, neither fourth-stage larvae nor adults of *C. septempunctata* showed any obvious difference

Table 1

Mean (\pm SEM) number of uninfected and *Neozygites fresenii*-infected aphids consumed by individual *Coccinella septempunctata* fourth-stage larvae and adults in a 14 h observation period ($n = 12$)^a

Stage	Diet		Average
	Infected aphids	Uninfected aphids	
Larva	132.6 ± 2.6	135.9 ± 3.7	$134.3 \pm 3.2A$
Adult	114.6 ± 4.0	121.7 ± 5.0	$118.1 \pm 2.3B$
Average	$123.6 \pm 3.0a$	$128.8 \pm 3.4a$	

^a Means followed by the same letter of the same case are not significantly different ($P \leq 0.05$).

between the times spent handling either prey type (infected or uninfected). However, this may have been partially due to our use of 24 h starved individuals. *C. septempunctata* increases its selectivity for uninfected aphids as the prior starvation period decreases (Pell et al., 1997). Fourth-stage *C. septempunctata* and adults partially consume *P. neoaphidis*-infected bean aphids, allowing the fungus in the abandoned portion of the aphid to sporulate and thus spatially distribute the pathogen to other aphids (Roy et al., 1998). Partial feeding by both stages of *C. septempunctata* on either prey was not recorded in our study, but likely occurred.

3.2. Effect of a diet of infected aphids on *C. septempunctata* development and survival

Neozygites fresenii-infected aphids had a significant effect on stadia of some instars of *C. septempunctata* (Table 2). Stadia of first instars fed with either uninfected or fungus-infected aphids did not differ significantly ($F = 2.79$; $df = 1, 107$; $P = 0.099$). However, there was a significant increase in the stadia of second, third and fourth instars reared on infected aphids compared to those reared on uninfected aphids. The infection status of aphids did not have a significant effect on pupal stadia ($F = 0.2$; $df = 1, 84$; $P = 0.6594$). The total developmental period of immatures reared on *N. fresenii*-infected aphids was significantly longer (~10% increase) than of those reared on uninfected aphids ($F = 41.76$; $df = 1, 84$; $P = 0.0001$).

Total development time of immatures of *C. septempunctata* reared on *A. fabae* at 26.6 °C was 13.4 days (Awadallah and Khalil, 1970), which is consistent for the stadia of *C. septempunctata* fed uninfected aphids in our study. The extended development of immature *C. septempunctata* reared on *N. fresenii*-infected aphids may be attributed to the condition of the fungus-infected aphids in the petri dish, which deteriorated with time during the 24 h between feedings. If so, the suitability of infected aphids to larvae of *C. septempunctata* may be lessened due to a decrease in prey attractiveness and/or nutritive value. No significant differences between diets were detected in the stadia of first

Table 2
Mean (\pm SEM) number of days for each stage of *Coccinella septempunctata* reared on uninfected and *Neozygites fresenii*-infected aphids^a

Stage	Number of days in each stage			
	Diet			
	Uninfected aphids		Infected aphids	
	<i>n</i>	Mean \pm SEM	<i>n</i>	Mean \pm SEM
1st	55	1.7 \pm 0.06a	54	1.9 \pm 0.06a
2nd	55	1.1 \pm 0.03a	50	1.3 \pm 0.06b
3rd	55	2.0 \pm 0.04a	47	2.2 \pm 0.08b
4th	53	3.2 \pm 0.07a	40	3.9 \pm 0.11b
Pupa	51	3.2 \pm 0.06a	35	3.3 \pm 0.07a
Total	51	11.3 \pm 0.08a	35	12.2 \pm 0.12b

^a Means followed by the same letter within a row are not significantly different ($P \leq 0.05$).

instars or pupae. If infection by *N. fresenii* decreases the nutritive value of aphids, both first instars and pupae should be unaffected, likely because the former would consume limited infected prey and the latter consumes none.

Mortality of immatures reared on *N. fresenii*-infected aphids was greater than those reared on uninfected aphids, reaching a cumulative percentage of 36.4% versus 5.4% for immatures reared on uninfected aphids (Fig. 2). A rapid increase in mortality was observed for second instars and reached a peak of 12.3% at the fourth-stage for *C. septempunctata* reared on fungus-infected aphids versus only 1.9% for the same stage reared on uninfected aphids.

A quality food source is essential for *C. septempunctata* second through fourth instars, as larvae develop very rapidly. Relative to this study, lower cumulative percentage mortality should be expected in the field during the early stages of a *N. fresenii* epizootic, as *C. septempunctata* would be exposed to a wider choice of aphid prey, including both fungus-infected and uninfected aphids. Additionally, *C. septempunctata* is a generalist predator and may switch to other, non-aphid hosts as suitable prey become scarce. This switching behavior needs to be evaluated before conclusions concerning effects on predator populations in the field could be drawn.

Excessive predation pressure may hamper the epizootic nature of an aphid pathogen and thus reduce its spatial distribution (Roy et al., 1998). The impact of coccinellid predation on epizootic development of *N. fresenii* was not addressed in our study. Although adults and fourth-instar larvae of *C. septempunctata* are potential intraguild predators of the fungus *N. fresenii*, this interaction may not be important during epizootics where a large number of

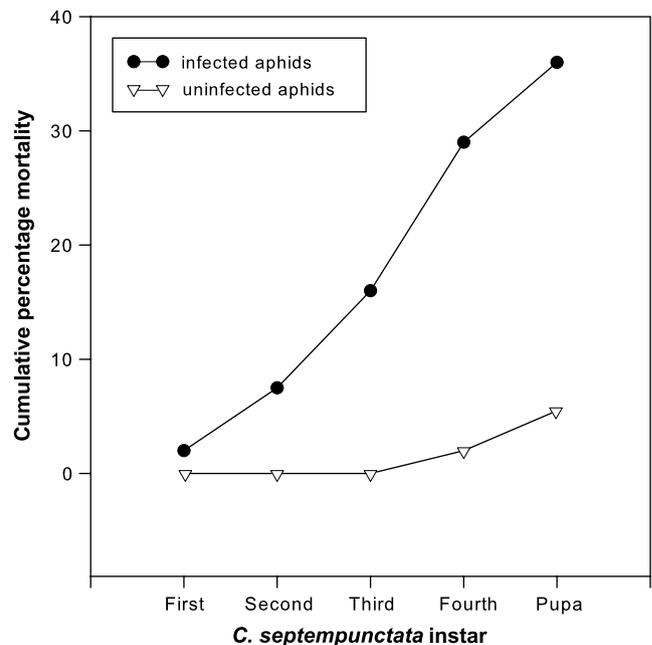


Fig. 2. Cumulative percentage mortality of larval and pupal stages of *Coccinella septempunctata* reared on uninfected and *Neozygites fresenii*-infected cotton aphids ($n = 55$).

aphids are killed by the fungus. *P. neoaphidis*-killed *A. pisum* (cadavers) are not likely to be consumed by *C. septempunctata* (Pell et al., 1997) but aphids partially consumed in earlier stages of infection remain on the plant and subsequently produce infective conidia (Roy et al., 1998). Furthermore, the presence of foraging coccinellids greatly increases local transmission and dispersal of *P. neoaphidis*, and thus may partially compensate for the reduction in inoculum caused by ingestion of the pathogen by the predator (Roy et al., 1998; Roy and Pell, 2000). Though not evaluated, we expect a similar relationship in the cotton aphid-*N. fresenii* system.

3.3. Effect of a diet of infected aphids on body size and fecundity of *C. septempunctata* adults

Both male and female of *C. septempunctata* reared on *N. fresenii*-infected aphids were smaller than those reared on uninfected aphids (Table 3). Tibiae of *C. septempunctata* males and females reared on *N. fresenii*-infected aphids were significantly shorter than those reared on uninfected aphids ($P \leq 0.01$). Likewise, the abdominal widths of *C. septempunctata* males and females reared *N. fresenii*-infected aphids were significantly narrower than those reared on uninfected aphids ($P \leq 0.01$). A diet of *P. neoaphidis*-infected tobacco aphid and *P. neoaphidis*-infected bean aphid had no impact on body size of selected predators (Roy and Pell, 2000).

Abdominal widths of *C. septempunctata* reared on corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) and pea aphid (*A. pisum*) were measured at $23 \pm 2^\circ\text{C}$; 16:8 L:D by Obrycki and Orr (1990). They found that females reared on *R. maidis* and *A. pisum* were 5.2 ± 0.1 and 5.9 ± 0.1 mm wide, respectively, whereas males reared on the same prey species were 4.9 ± 0.1 and 5.6 ± 0.1 mm wide, respectively. In our study, *C. septempunctata* adults reared on uninfected *A. gossypii* were significantly smaller ($P \leq 0.05$, *t*-test, Table 3).

Coccinella septempunctata reared on uninfected aphids produced significantly more eggs per day during the 29-day observation period than those reared on fungus-

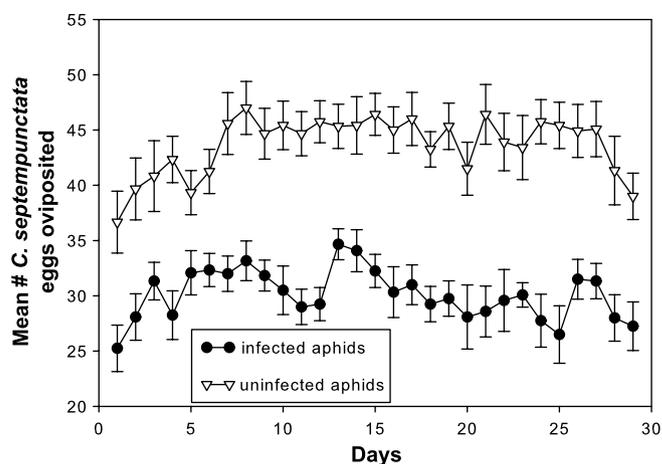


Fig. 3. Mean number of eggs produced daily by *Coccinella septempunctata* reared on uninfected and *Neozygites fresenii*-infected cotton aphids ($n = 16$).

infected aphids ($t = 27.75$; $df = 28$; $P \leq 0.01$) (Fig. 3). The mean number of eggs oviposited daily by females reared on uninfected aphids was 43.7 ± 0.5 versus 30.1 ± 0.4 by females reared on *N. fresenii*-infected aphids. *C. septempunctata* reared on uninfected aphids oviposited an average of 1266.7 ± 45.1 per individual compared to 873.1 ± 11.3 eggs produced by *C. septempunctata* reared on fungus-infected aphids during the entire 29-day period. These data are reasonable given that in other studies at 25°C ; 14:10 L:D, *C. septempunctata* oviposited a mean of 1660 eggs over a total oviposition period of 66 days (Kawauchi, 1985). The cumulative oviposition rate was almost linear in both treatments throughout the initial oviposition period, as noted in other studies of this species (Phoofolo and Obrycki, 1995). However, 11% of the females of *C. septempunctata* reared on fungus-infected aphids stopped oviposition within about a week and later died. No cessation in oviposition was observed on those reared on uninfected aphids during the same period. General observation suggested that only ~40% of males reared on fungus-infected aphids would actually mate, compared to 100% reared on uninfected aphids.

Females of *C. septempunctata* reared on *N. fresenii*-infected aphids were significantly smaller than those reared on uninfected aphids and oviposited significantly fewer eggs. Decreased nutrient quality of the *N. fresenii*-infected aphids is likely to have contributed directly to the reduced size and therefore fecundity of *C. septempunctata*. Only fresh *N. fresenii*-killed aphids were recognized as prey by *C. septempunctata*, so it is important to reiterate that these studies reflect the suitability only of live infected aphids (<3 days after inoculation). The stage of infection at the time of predation has an impact not only on prey selection, but also on the ability of the fungus to continue development (sporulation) in studies with *C. septempunctata* feeding on *P. neoaphidis*-infected pea aphids (Roy et al., 1998). Intraguild predation studies such as theirs are clearly warranted for the cotton aphid system.

Table 3
Mean tibial lengths and abdominal widths (\pm SEM) of *Coccinella septempunctata* reared on uninfected and *Neozygites fresenii*-infected aphids

Sex	Body part	Diet ^a		
		<i>n</i>	Infected aphids	Uninfected aphids
Male	Tibial length (mm)	55	$1.6 \pm 0.01\text{a}$	$1.7 \pm 0.01\text{b}$
Male	Abdominal width (mm)	55	$3.6 \pm 0.02\text{a}$	$3.8 \pm 0.02\text{b}$
Female	Tibial length (mm)	55	$1.8 \pm 0.01\text{a}$	$1.8 \pm 0.01\text{b}$
Female	Abdominal width (mm)	55	$4.0 \pm 0.03\text{a}$	$4.1 \pm 0.02\text{b}$

^a Means followed by the same letter within a row are not significantly different ($P \leq 0.05$).

Infection of cotton aphids with *N. fresenii* did not affect the rate of predation by fourth-stage larvae or adults of *C. septempunctata*. However, a diet of *N. fresenii*-infected aphids significantly extended the duration of stadia of *C. septempunctata*, particularly for second-, third- and fourth-stage larvae. Greater mortality of the same stages reared on *N. fresenii*-infected aphids indicated the lack of suitability of fungus-infected aphids for *C. septempunctata*. A diet of *N. fresenii*-infected aphids significantly reduced the fitness of *C. septempunctata* as indicated by a smaller body size and reduced fecundity. The difference in fecundity may be related to the nutrient quality of fungus-infected aphids and their effect on female size. A rapidly developing *N. fresenii* epizootic in a cotton field may negatively affect *C. septempunctata* that are unable to emigrate from a field (e.g., larvae), particularly when alternative prey are unavailable. A negative impact on coccinellids could result from the suboptimal nutritional value of *N. fresenii*-infected aphids and/or a failure of the predators to utilize fungus-killed aphids as prey. The extent of any potential effects of predation of fungus-infected aphids on the development of field epizootics by *N. fresenii* is unknown and may be the focus of future studies.

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