Effect of Greenbug (Hemiptera: Aphididae) from Resistant Sorghum on Developmental Rates of Convergent Lady Beetle (Coleoptera: Coccinellidae)

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Abstract. Understanding tritrophic effects of resistant crops on natural enemies feeding on pests is important for developing resistant plants while conserving natural enemies. The effect on convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, fed greenbugs, *Schizaphis graminum* (Rondani), reared on resistant sorghum, *Sorghum bicolor* (L.) Moench, was assessed. Convergent lady beetles from sorghum fields were paired and allowed to mate. F_1 progeny were reared at a photoperiod of 14:10 light:dark hours at 23 or 30°C and fed daily with a known number of biotype I greenbugs from resistant PI550607 or susceptible RTx430 sorghum. Lady beetle eggs hatched 1 day later at 23°C (3.0 days) than at 30°C. Larvae completed development in 0.75 more day and pupae developed in 6.0 versus 3.0 days at 23 than at 30°C. Lady beetles consumed similar numbers of greenbugs from either genotype of sorghum, but larvae and adults consumed 1.7 and 2.5 times more greenbugs at 23 than at 30°C, respectively. Each lady beetle adult at 23°C consumed an average of 17,124 greenbugs. Adult lady beetles were paired in four combinations depending on the sorghum source of greenbugs from which they had been fed. Most eggs were produced (2,893 eggs) and hatched (2,657 or 91.0%) per lady beetle fed greenbugs from susceptible sorghum at 23°C.

Introduction

Resistant plants and conservation of natural enemies such as convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, are used to manage greenbug, *Schizaphis graminum* (Rondani), a major aphid pest of small grains and sorghum, *Sorghum bicolor* (L.) Moench (Teetes and Pendleton 2000). But, tritrophic effects of resistant sorghum on natural enemies feeding on pests from resistant plants have not been studied adequately.

Chan et al. (1978), Orr et al. (1985), Rogers and Sullivan (1986), and Shepard and Dahlman (1988) found plant changes reduced prey quality of insects feeding on plants. Karner and Manglitz (1985) reported that convergent lady beetles consumed more pea aphids, *Acyrthosiphon pisum* (Harris), on resistant than susceptible alfalfa, *Medicago sativa* L. Starks et al. (1972) determined that greenbug-resistant barley, *Hordeum vulgare* L., and sorghum worked in conjunction

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with the parasitic wasp *Lysiphlebus testaceipes* (Cresson) to reduce abundance of greenbugs. Fuentes-Granados et al. (2001) found that wheat containing *Gb3* or *Gb6* resistance genes did not limit the impact of parasitoids on greenbug. Giles et al. (2005) found no difference in survival or development of sevenspotted lady beetle, *Coccinella septempunctata* L., fed greenbugs from biotype E-resistant or susceptible wheat, but lady beetles fed greenbugs from resistant wheat weighed less.

The goal of this study was to assess tritrophic effects on the life cycle of convergent lady beetle fed biotype I greenbugs from resistant PI550607 versus susceptible RTx430 sorghum at 23 and 30°C. The objectives of the experiment were to assess the effects of resistant versus susceptible sorghum and temperature on the number of days required for convergent lady beetles to develop from egg to adult; number of greenbugs consumed per day by lady beetle larvae and adults; number of eggs produced per day, total number of eggs produced, and percentage of eggs hatched per female lady beetle.

Materials and Methods

An aspirator was used to collect lady beetle adults from sorghum fields near Canyon, TX. The gender of each adult was determined by the presence (male) or absence (female) of an indentation on the ventral side of the fifth abdominal sternite at the medial portion of the segment (Chedester 1979). Adults were paired in 300-cc plastic condiment cups closed with paper lids (Bio-serve, Frenchtown, NJ). The lady beetles were discarded after they produced eggs that were allowed to hatch at room temperature. A camel-hair brush was used to transfer each first-instar larva to a condiment cup with lid. The larvae were kept at 23 or 30°C and a photoperiod of 14:10 light:dark hours in a Precision model 818 Microprocessor Controlled Low Temperature Illuminated Incubator (Precision, Winchester, VA). One hundred fifty and 100 lady beetle larvae were used at 23 and 30°C, respectively. Each larva was considered a replication.

Greenbug biotype I-susceptible RTx430 or resistant PI550607 sorghum plants were grown in Miracle-Gro Enriched Potting Mix with Miracle-Gro Plant Food (Miracle-Gro Lawn Products, Inc., Port Washington, NY) in 23-cm diameter plastic pots in a greenhouse at West Texas A&M University, Canyon. Plants at the five true-leaf stage were infested with biotype I greenbugs from a culture. Daily, a known number of biotype I greenbugs reared on RTx430 sorghum was fed to half of the lady beetle larvae, and the same number of greenbugs reared on resistant PI550607 sorghum was fed to the other half. A camel-hair brush and an aspirator were used to collect greenbugs from the culture plants, and a camel-hair brush was used to transfer greenbugs to each condiment cup. Greenbugs remaining in the condiment cup the following day were counted and discarded. Each condiment cup was checked daily for the presence of exuvia from the larva.

The gender was determined of each lady beetle adult that emerged. Adults were paired in one of four ways depending on the sorghum source of greenbugs: female and male fed greenbugs from susceptible sorghum, female fed greenbugs from susceptible sorghum with a male fed greenbugs from resistant sorghum, female fed greenbugs from resistant sorghum with a male fed greenbugs from susceptible sorghum, or female and male fed greenbugs from resistant sorghum. The male was removed when the female began producing eggs. The females were fed greenbugs from the original sorghum source from which they previously were fed. Daily numbers of eggs laid and hatched per female were recorded until the beetle died or until 90 days after emergence of the adult.

A completely randomized design was used. The General Linear Model (GLM) procedure (SAS Institute 2000) was used to assist in analyzing data on the average number of greenbugs consumed per day by lady beetle larvae and adults, number of days required for the lady beetle to develop from egg to adult, and number of eggs laid and percentage of eggs that hatched per lady beetle female. Data were analyzed by factorial analysis. Data collected for all larvae and adults at each temperature and from each sorghum source of greenbugs were analyzed, and the Least Significant Difference test at $P = 0.05$ was used to separate means.

Results and Discussion

The sorghum source of biotype I greenbugs did not significantly affect the number of days (2.6) required before lady beetle eggs hatched (Table 1). First- and

Table 1. Effect of Temperature and Sorghum Source of Biotype I Greenbugs on Duration (Days) ± SE of Life Stages of Convergent Lady Beetle.

Life stage of lady beetle	Temperature (°C)	Sorghum source of greenbugs: PI550607 (resistant)	RTx430 (susceptible)	Combined
Egg	23	3.0 ± 0.00 aA	3.0 ± 0.00 aA	3.0 ± 0.00 a
	30	2.0 ± 0.00 bA	2.0 ± 0.00 bA	2.0 ± 0.00 b
	Combined	2.6 ± 0.00 A	2.6 ± 0.00 A	
1st instar	23	2.8 ± 0.04 aA	2.4 ± 0.06 aB	2.6 ± 0.04 a
	30	1.8 ± 0.06 bA	1.7 ± 0.06 bA	1.8 ± 0.04 b
	Combined	2.4 ± 0.04 A	2.1 ± 0.04 B	
2nd instar	23	2.1 ± 0.06 aA	1.3 ± 0.05 aB	1.7 ± 0.04 a
	30	1.1 ± 0.03 bA	1.1 ± 0.05 bA	1.1 ± 0.04 b
	Combined	1.7 ± 0.03 A	1.2 ± 0.04 B	
3rd instar	23	2.3 ± 0.05 aA	3.1 ± 0.09 aB	2.7 ± 0.05 a
	30	1.4 ± 0.07 bA	1.7 ± 0.07 bB	1.6 ± 0.05 b
	Combined	1.9 ± 0.05 B	2.6 ± 0.06 A	
4th instar	23	3.2 ± 0.07 aA	3.7 ± 0.10 aB	3.5 ± 0.06 a
	30	3.1 ± 0.09 aA	2.9 ± 0.09 bB	3.0 ± 0.06 b
	Combined	3.2 ± 0.06 A	3.4 ± 0.07 B	
Pupa	23	6.0 ± 0.00 aA	6.0 ± 0.00 aA	6.0 ± 0.00 a
	30	3.0 ± 0.00 bA	3.0 ± 0.00 bA	3.0 ± 0.00 b
	Combined	4.8 ± 0.00 A	4.8 ± 0.00 A	

Means followed by the same lower-case letter in a column or upper-case letter in a row are not significantly different (LSD, $P = 0.05$).

second-instar larvae required 0.3 and 0.5 day longer, whereas third and fourth instars required 0.7 and 0.2 fewer day to develop when fed greenbugs from resistant rather than susceptible sorghum. The pupal stage lasted an average of 4.8 days. Giles et al. (2005) also found no differences in survival or development of sevenspotted lady beetle fed biotype E greenbugs from resistant or susceptible wheat.

Although the sorghum source had no significant effect on lady beetles, temperature did affect development. Eggs hatched in 3.0 and 2.0 days at 23 and 30°C, respectively (Table 1). At 30°C, 0.8, 0.6, 1.1, and 0.5 fewer days were required for larvae to develop during the first, second, third, and fourth instars, respectively, than at 23°C. Pupae required 6.0 and 3.0 days to develop at 23 and 30°C, respectively. These findings are in agreement with those of Butler and Dickerson (1972) who found that development of convergent lady beetles fed pea aphid or cotton aphid, *Aphis gossypii* Glover, was faster at 30°C than at cooler temperatures. They found eggs, larvae, and pupae at 30°C developed in 1.8, 9.9, and 3.0 days, respectively.

The number of greenbugs consumed per lady beetle larva did not differ by the genotype of sorghum on which the greenbugs had been reared (Table 2). But, the number of greenbugs consumed per larva differed significantly by temperature (F = 1,813.77; df = 1, 246; P < 0.0001). For both sorghum sources, approximately 1.7 times more greenbugs were consumed per lady beetle at 23 than at 30°C. The number of greenbugs consumed per lady beetle larva was not affected by interaction between temperature and sorghum source of greenbugs. At 23 and 30°C, most greenbugs were consumed by lady beetle larvae 10 and 7 days old, respectively.

Table 2. Effect of Temperature and Sorghum Source of Biotype I Greenbugs on the Average Total Number ± SE of Greenbugs Consumed per Convergent Lady Beetle.

Sorghum source of greenbugs	Temperature (°C)	n	Greenbugs per larva	Greenbugs per adult
PI550607 (resistant)	23	75	747.2 ± 7.5 aA	17,124.0 ± 431.9 aA
	30	50	422.7 ± 9.1 bB	5,568.2 ± 511.0 bB
RTx430 (susceptible)	23	75	730.7 ± 7.3 aA	16,324.5 ± 457.7 aA
	30	50	435.0 ± 9.0 bB	8,267.5 ± 770.4 bB

Means followed by the same lower-case letter in a column or upper-case letter in a row are not significantly different (LSD, P = 0.05).

A total of 72 convergent lady beetles was paired, with nine pairs used for each combination of temperature and sorghum source of greenbugs. The number of eggs produced per lady beetle female differed significantly by the combination in which the females and males were paired (F = 5.20; df = 3, 68; P = 0.001) and by temperature (F = 22.96; df = 1, 70; P < 0.0001). At 23°C, more eggs were produced per female (total of 2,893) when male and female lady beetles fed greenbugs from susceptible sorghum were paired than when lady beetles were paired in any other combination (Table 3). At 23°C, two females laid eggs for 89 days. Only 66.7, 33.3, and 22.2% of females produced eggs when females fed greenbugs from susceptible sorghum were paired with males fed greenbugs from resistant sorghum, females fed greenbugs from resistant sorghum were paired with males fed greenbugs from susceptible sorghum, and females and males fed greenbugs from resistant sorghum were paired, respectively.

Table 3. Effect of Temperature and Sorghum Source of Greenbugs on the Mean Number of Eggs Laid and Number and Percentage of Eggs Hatched ± SE per Convergent Lady Beetle Female.

Pair[a]	n	No. (%) females producing eggs	Average no. eggs produced/female	Average no. eggs that hatched/ female	Average % eggs that hatched/female	% of eggs that hatched/female that produced eggs
			23°C			
SxS	9	9 (100%)	2,893.1 ± 218.3 aA	2,656.8 ± 251.1 aA	91.0 ± 11.6 aA	91.0 ± 5.2 aA
SxR	9	6 (66.7%)	1,209.6 ± 315.6 bA	763.8 ± 196.3 bA	42.9 ± 11.2 bA	64.4 ± 6.4 bcA
RxS	9	3 (33.3%)	414.0 ± 208.7 cA	196.8 ± 130.9 bA	14.7 ± 9.8 cA	44.2 ± 9.0 cA
RxR	9	2 (22.2%)	352.4 ± 254.5 cA	290.7 ± 212.6 bA	18.1 ± 12.0 cA	81.3 ± 11.1 abA
			30°C			
SxS	9	2 (22.2%)	144.7 ± 96.4 aB	108.3 ± 71.7 aB	16.8 ± 11.1 bB	75.4 ± 15.2 aB
SxR	9	4 (44.4%)	221.3 ± 122.4 aB	88.0 ± 53.3 aB	15.6 ± 7.9 bB	34.8 ± 10.7 bB
RxS	9	7 (77.7%)	250.3 ± 78.1 aA	88.4 ± 33.8 aA	26.1 ± 8.2 aA	33.8 ± 7.5 bB
RxR	9	3 (33.3%)	114.1 ± 63.7 aA	20.6 ± 18.5 aA	7.3 ± 6.3 bA	21.8 ± 12.4 bB

Within a temperature regime, means followed by the same lower-case letter in a column are not significantly different (LSD, P = 0.05). Between temperatures, means followed by the same upper-case letter in a column are not significantly different (LSD, P = 0.05).
[a]S = convergent lady beetle fed greenbugs from susceptible RTx430 sorghum; R = convergent lady beetle fed greenbugs from resistant PI550607 sorghum. Females are listed first in a pair.

At 30°C, females from crosses between lady beetles fed greenbugs from susceptible sorghum, females fed greenbugs from susceptible sorghum and males fed greenbugs from resistant sorghum, females fed greenbugs from resistant sorghum and males fed greenbugs from susceptible sorghum, and lady beetles fed greenbugs from resistant sorghum laid eggs for 74, 57, 41, and 46 days, respectively. From 1.7 to 20.0 times more eggs were laid per female of the same cross at 23 than 30°C. Twenty times more eggs were produced when lady beetles fed greenbugs from susceptible sorghum at 23°C were paired versus beetles fed greenbugs from resistant sorghum at 30°C. Interaction between temperature and combination in which the lady beetles were paired significantly affected the total number of eggs produced per lady beetle female (F = 21.64; df = 7, 64; P < 0.0001).

The total number of eggs hatched per lady beetle female differed significantly by the combination in which the lady beetles were paired (F = 7.04; df = 3, 68; P < 0.0001) and by temperature (F = 21.28; df = 1, 70; P < 0.0001). At 23°C, 3.5 times more eggs hatched (2,656.8 total) per female when lady beetles fed greenbugs from susceptible sorghum were paired than when paired in any other combination (Table 3). Significantly more eggs hatched at 23 than 30°C from females fed greenbugs from susceptible sorghum. The total number of eggs hatched per female differed significantly by interaction between temperature and combination in which the lady beetles were paired (F = 31.67; df = 7, 64; P < 0.0001).

The percentage of eggs that hatched per female differed significantly by the combination in which lady beetles from susceptible or resistant sorghum were paired (F = 3.84; df = 3, 68; P = 0.0072) and by temperature (F = 9.81; df = 1, 70; P = 0.0025). At 23°C, 91.0% of the eggs hatched that were produced by pairing females and males fed greenbugs from susceptible sorghum (Table 3). At 23°C, only 14.7 and 18.1% of the eggs hatched that were produced per lady beetle female fed greenbugs from resistant sorghum and paired with males fed greenbugs from susceptible and resistant sorghum, respectively. At 30°C, most eggs hatched (26.1%) per female lady beetle fed greenbugs from resistant sorghum and paired with a male fed greenbugs from susceptible sorghum. A significantly greater percentage of eggs hatched per female at 23 than 30°C when lady beetle females were fed greenbugs from susceptible sorghum. The percentage of eggs that hatched per female differed significantly by interaction between the temperature and combination in which lady beetles were paired (F = 7.83; df = 7, 64; P < 0.0001).

At 23°C, the percentage of eggs that hatched per female that produced eggs was significantly greatest when the pair of lady beetles had been fed greenbugs from susceptible sorghum (91.0%) or from resistant sorghum (81.3%) (Table 3). At 30°C, the percentage of eggs that hatched per female that produced eggs was significantly greatest (75.4%) when lady beetles fed greenbugs from susceptible sorghum were paired. A significantly greater percentage hatched of the eggs produced per pair of lady beetles of the same cross at 23 than at 30°C.

Temperature affected feeding and development of convergent lady beetle larvae and adults. Greenbug-resistant sorghum did not directly affect feeding and development but perhaps affected the number and viability of eggs produced by lady beetles. More research is needed using more pairs of lady beetles than nine, many of which died before producing any eggs or viable eggs. However, lady beetles are mobile and might prefer feeding on aphids from wild or other kinds of cultivated hosts instead of on greenbug-resistant sorghum.

Acknowledgment

This study was supported in part by the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP) sponsored by the U.S. Agency for International Development, under the terms of Grant No. LAG-G-00-96-90009-00.

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