

Optimal *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) Density and Time of Introduction for Suppression of Bruchid Progeny in Stored Legumes

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ABSTRACT The influences of both predator density and elapsed time between initial infestation and introduction of predators were determined for suppression of bruchids infesting stored grain legumes by *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae). Predator density treatments consisted of zero, one, two, three, or five male:female pairs of adult *X. flavipes* added to experimental arenas 0, 24, or 120 h after bruchid introduction. Suppression of *Acanthoscelides obtectus* approached eradication with all predator treatments. For all other bruchid species evaluated (*Callosobruchus analis*, *C. chinensis*, *C. maculatus*, and *Zabrotes subfasciatus*), the most effective predator density and addition time (five predator pairs at 0 h) resulted in an ~50% reduction of emerging F₁ bruchids compared with progeny produced in arenas without predators. The predator addition time of 0 h, when predators were added to experimental arenas simultaneously with the pest species, was determined to be the universally most efficacious treatment time. Predator density above one pair but lower than five pairs was less influential overall when *X. flavipes* was added 24 or 120 h after initial bruchid infestation; however, maximum suppression was achieved at approximately two predator pairs and not significantly improved on with increased predator density. Our results indicate that the most effective biological control of pest bruchids would occur when *X. flavipes* is added as soon as possible after legumes are stored.

KEY WORDS stored products, biological control, *Acanthoscelides obtectus*, *Callosobruchus* spp., *Zabrotes subfasciatus*

Bruchids (Coleoptera: Bruchidae) are the primary pests of stored food legumes (Southgate 1978, Cardona 2004). Advantageous immigration has allowed many pest bruchid species to become cosmopolitan in distribution and entrenched at every level in the pulse ecosystem: in the field, in farm and household storages, at processing sites, during local, regional and international commodity transportation, and in domestic and foreign storage (Pedersen 1978, Hill 2002). Biological control of pest bruchids may provide an important management strategy for reducing the incidence and intensity of stored grain legume infestation. Previous research has focused on the potential of parasitoids to control bruchids (Alebeek et al. 1996a, 1996b, van Huis and de Rooy 1998, Mbata et al. 2005), whereas the role of generalist predators in this application has not yet been extensively explored.

Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae), commonly known as the warehouse pirate bug, is a cosmopolitan generalist predator of coleopteran and lepidopteran stored-product pests (Gross 1954,

Arbogast 1979). This predaceous bug most successfully controls small, externally developing prey, particularly readily accessible eggs and early instars that are neither heavily sclerotized nor overly hirsute (LeCato and Davis 1973, Sing 1997, Sing and Arbogast 2007).

The biocontrol efficacy of the anthocorids *Lyctocoris campestris* (F.) (Parajulee et al. 1994) and *X. flavipes* (LeCato 1976, LeCato et al. 1977, Brower and Press 1992, Brower et al. 1996, Donnelly and Phillips 2001) against stored-product pests under a variety of environmental conditions is well-documented, although evaluations with bruchid prey have been comparatively limited. Sing (1997) and Sing and Arbogast (2007) quantified prey stage-specific rates of predation by *X. flavipes* on five bruchid species, reporting the predatory response to be low but consistent on the adult stage of all prey species and much higher on the eggs and neonate larvae of *Acanthoscelides obtectus* (Say). Attack on bruchid eggs and larvae was limited to *A. obtectus* prey because this species' eggs are deposited loosely throughout the bulk of stored host seeds, leaving the eggs and first instars susceptible to mortality by predation and desiccation until larvae enter a host seed (Hill 2002; Howe and Currie 1964). In contrast, the eggs of the other four species evalu-

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Table 1. Influence of experiment (replicate performed in time) on mean density of bruchid progeny by bruchid/legume combination

Bruchid/legume combination	df	F	Pr > F
<i>A. obtectus</i> -black-eyed pea	1,120	17.2	<0.01
<i>A. obtectus</i> -white navy bean	1,120	0.0	0.92
<i>C. analis</i> -black-eyed pea	1,120	2.5	0.12
<i>C. chinensis</i> -black-eyed pea	1,120	12.5	<0.01
<i>C. maculatus</i> -black-eyed pea	1,120	219.0	<0.01
<i>C. maculatus</i> -chickpea	1,120	19.6	<0.01
<i>Z. subfasciatus</i> -black-eyed pea	1,120	0.8	0.37
<i>Z. subfasciatus</i> -white navy bean	1,120	30.1	<0.01

ated are adhered to the outer testa of the host seed with an ovipositional exudate that hardens into a protective coating, and hatching larvae bore directly into the host seed where all development is completed

(Southgate 1979; Hill 2002; Rees 2004). Those experiments (Sing 1997; Sing and Arbogast 2007) evaluated *X. flavipes* predation under highly simplified conditions that excluded assessing the potential for the stored commodity, predator density, and the lag between infestation and predator introduction to confound host finding and attack success. Intraspecific competition and prey-predator population oscillations, as influenced by predator density and the timing of predator introduction to the stored commodity, would also play an important role in the development of an effective treatment protocol for any operational use of *X. flavipes* to manage bruchid infestations of stored legumes.

This study was undertaken to determine if the suppression of bruchid adult progeny would be affected

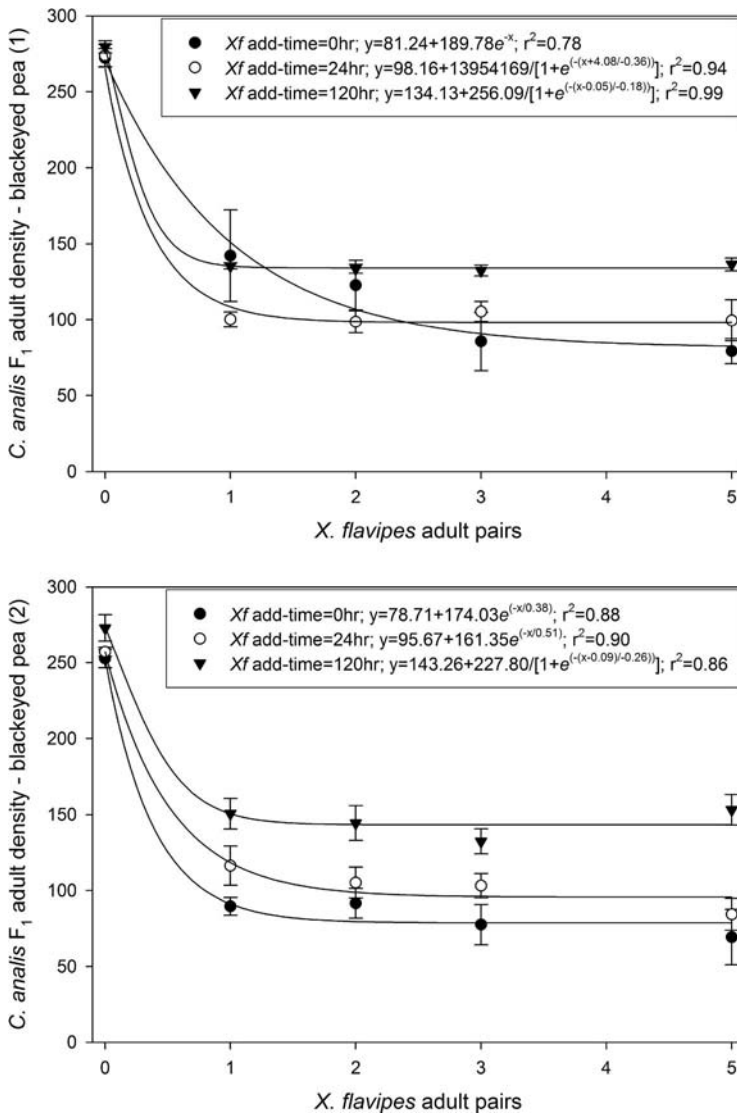


Fig. 1. Influence of predator density on F₁ adult density of *C. analis* on black-eyed pea when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

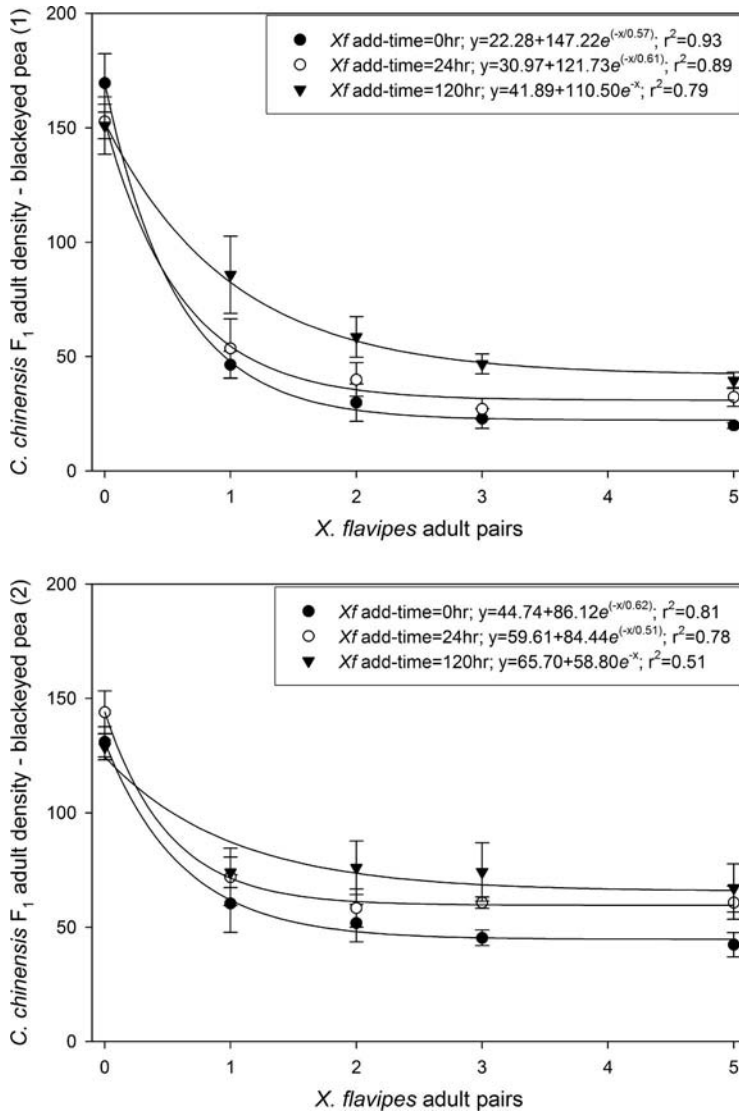


Fig. 2. Influence of predator density on F₁ adult density of *C. chinensis* on black-eyed pea when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

by the number and timing of predators introduced to stored legumes. Experiments varying both the treatment density of predators and the interval between bruchid infestation of the grain legumes and the time predators were added (predator add-time) were undertaken to provide a more realistic assessment of *X. flavipes*' potential to control specific bruchid species.

Materials and Methods

Bruchid Prey. All bruchid species were maintained in continuous culture, and experiments were performed under identical environmental conditions of 12:12-h L:D and 29 ± 2°C, 65 ± 5% RH. Experiments evaluated *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) reared on both black-eyed pea (*Vigna un-*

guiculata L. Walp.) and white navy bean (*Phaseolus vulgaris* L.), and *Callosobruchus analis* (F.) and *Callosobruchus chinensis* L. reared on black-eyed pea only, all from continuous cultures started in 1981 with stock received from the Pest Infestation Control Laboratory, Slough, UK, and maintained at the Stored-Product Insects Research and Development Laboratory (SPIRDL), Savannah, GA. *Callosobruchus maculatus* (F.) used in the study were reared on black-eyed pea and garbanzo bean or chickpea (*Cicer arietinum* L.), obtained from a SPIRDL continuous culture that originated from USDA-ARS research facilities in Fresno, CA. Preliminary studies indicated that bruchid fecundity, survival, and longevity had the potential to vary significantly with host legume; therefore, experiments were expanded to evaluate and compare the response of the same species when it had

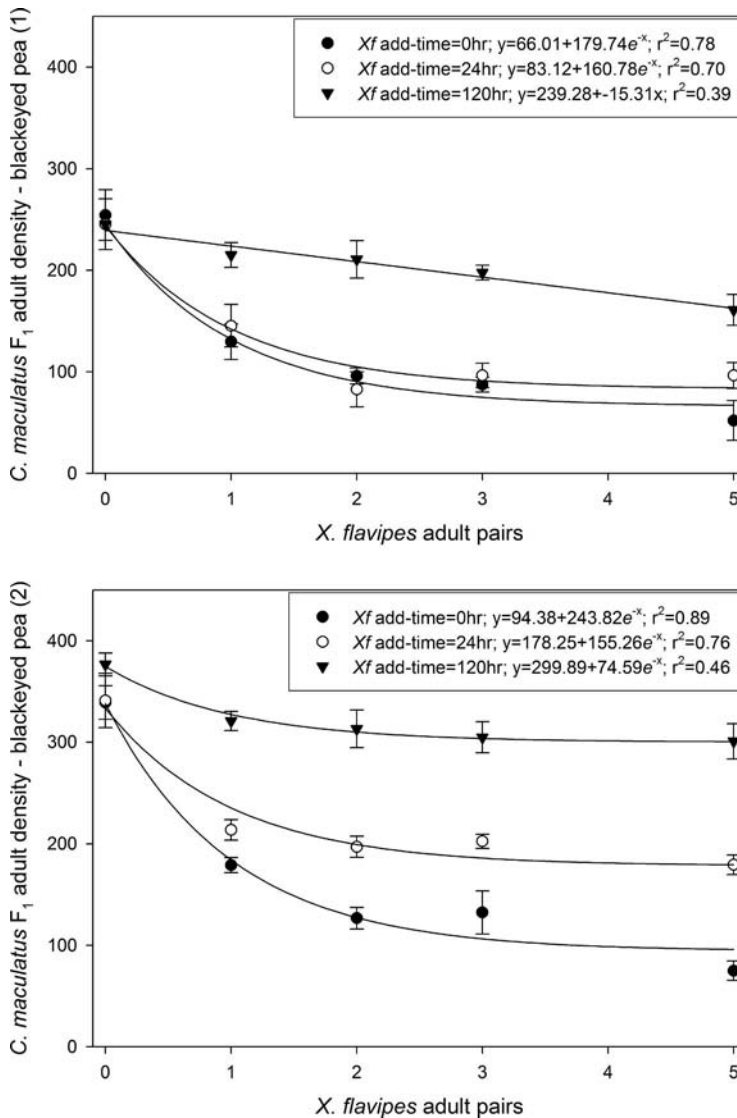


Fig. 3. Influence of predator density on F₁ adult density of *C. maculatus* on black-eyed pea when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

been continuously cultured on more than one commodity.

Experimental subjects were collected from culture jars that had been sifted through a U.S. no. 6 standard sieve 24 h previously to ensure that all individuals were 0–24 h after emergence from host seeds. Individuals were sexed according to authoritative keys (Southgate et al. 1957, Southgate 1958, Halstead 1963) and collected and retained in groups of five male:female pairs in 18.3-ml plastic shell vials (7 cm high by 2.5 cm diameter).

Organically produced dried grain legumes used in this study were purchased locally in bulk 11.4-kg bags, held below 0°C for at least 2 wk to ensure disinfestation, and acclimated under culture/experimental conditions in 0.95-liter Mason jars until the legumes reached environ-

mental equilibrium, usually after 1 wk. Equilibration was verified by repeated dry weight determination of grain legume moisture content (ASAE 1987).

Predators. Individuals used in this study were reared from a continuous culture of *X. flavipes* started with specimens collected in 1977 from a purposely infested experimental warehouse facility at SPIRDL. The continuous culture was maintained under the environmental conditions stated above and reared in 3.78-liter glass jars provided with Hexcell paperboard harborage and previously frozen *Plodia interpunctella* (Hübner) eggs as a food source. Culture jars were cleared of all adult predators, and experimental subjects were collected from a pool of adults emerging 0–6 d after initial sorting. Subjects were sexed according to Arbogast et al. (1971) and retained indi-

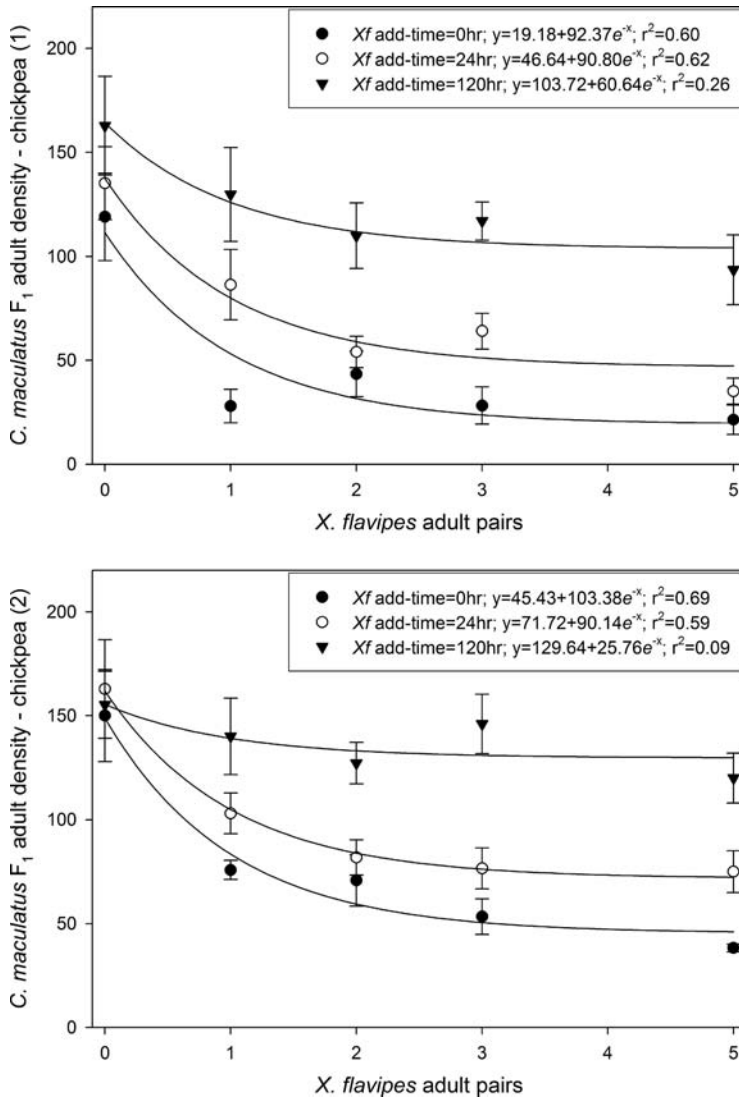


Fig. 4. Influence of predator density on F₁ adult density of *C. maculatus* on chickpea when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

vidually in gelatin capsules to prevent cannibalism and reduce stress.

Predator Density/Add-Time. Experimental arenas consisted of half pint (240 ml) Mason jars filled with 100 g of grain legumes conditioned as described above. Five newly emerged (0–24 h) male:female pairs of bruchids were added to each of five replicate arenas per treatment. Predator density treatments of zero, one, two, three, or five male:female pairs of 0- to 6-d-old adult *X. flavipes* were added to arenas at three different predator introduction intervals: 0, 24, or 120 h after bruchid introduction, and totaled 75 arenas per bruchid species/commodity combination. The entire experiment was performed twice. To ensure that only the F₁ adults were counted, each bruchid species experiment was terminated according to the formula, $T = 2(D) - 10$,

where T was the expected time to terminate the experiment, and D was based on the empirically determined number of days to F₁ adulthood for individual bruchid species under the specific environmental conditions of these experiments. The number of days to F₁ adulthood varied within species by commodity, with the developmental period consistently shorter for cultures on black-eyed pea, where applicable, compared with alternate hosts: *A. obtectus* on black-eyed pea, 52 d; *A. obtectus* on white navy bean, 54 d; *Z. subfasciatus* on black-eyed pea, 32 d; *Z. subfasciatus* on white navy bean, 36 d; *C. maculatus* on black-eyed pea, 32 d; *C. maculatus* on chickpea, 36 d; *C. analis* on black-eyed pea, 42 d; *C. chinensis* on black-eyed pea, 32 d. The termination time for experiments 1 and 2 of each bruchid/commodity combination was the same. Arenas were

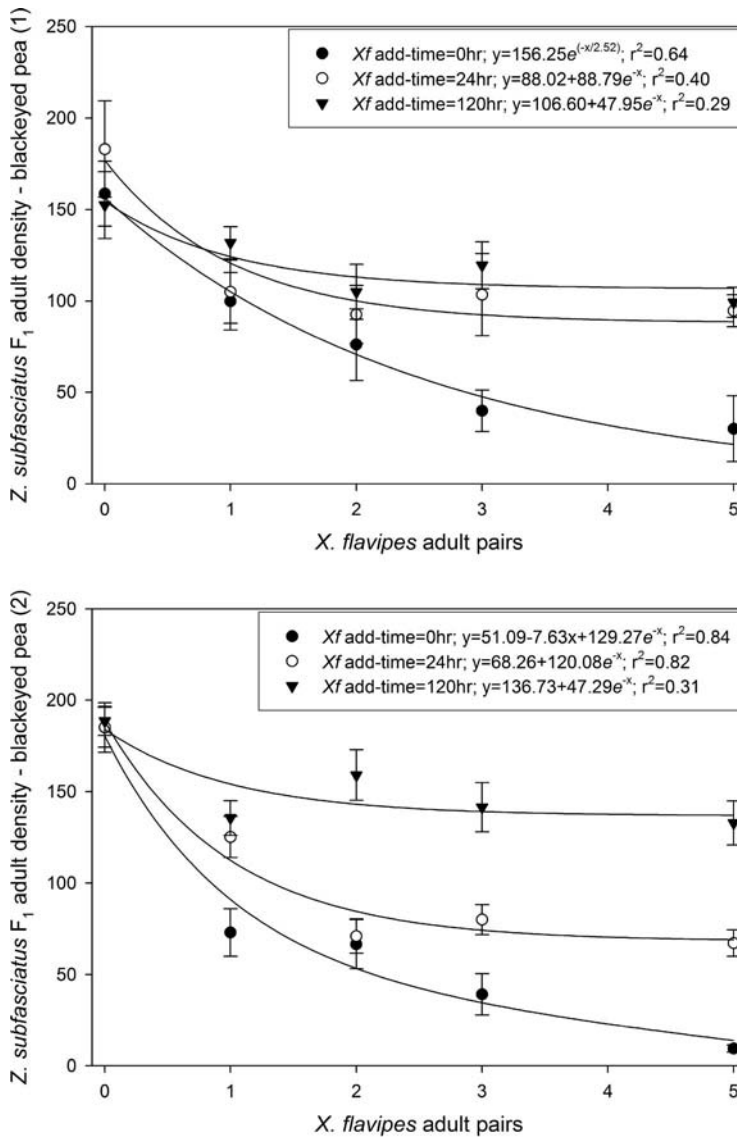


Fig. 5. Influence of predator density on F₁ adult density of *Z. subfasciatus* on black-eyed pea when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

frozen for 2 wk at the termination of the experiment, and the contents of each arena jar were sifted through a series of sieves, and the yield of adult bruchid progeny was recorded.

Statistical Analyses. Raw data were adjusted to take into account the number of adult bruchids used in the initial infestation. Adjusted data were analyzed using the mixed models procedure (PROC MIXED) of SAS (SAS Institute, Cary, NC) Fixed effects were assessed using type 3 tests in an analysis of variance (ANOVA). This determined the contribution of experiment (study replication in time), predator density, time of predator addition, and their multiple interactions to the total variation observed in bruchid F₁ progeny produced for each bruchid/legume combination

treatment. Replicate experimental arenas within each treatment of the two total experiments (jar 1 though jar 5 for each bruchid/predator add-time combination) was the random effect in this model. Means for significant effects or their interactions were separated using a Student's *t*-test ($\alpha = 0.05$) for pairwise comparisons of the least-square means of the pooled data from experiments 1 and 2.

Replicate experiment or the interaction of replicate experiment with predator density or add-time was significant for five of the eight bruchid/legume combinations. Therefore, optimal regression equations for the effect of predator density or add-time for both experiments were plotted for each bruchid/legume combination using Table Curve 2D curve-fitting soft-

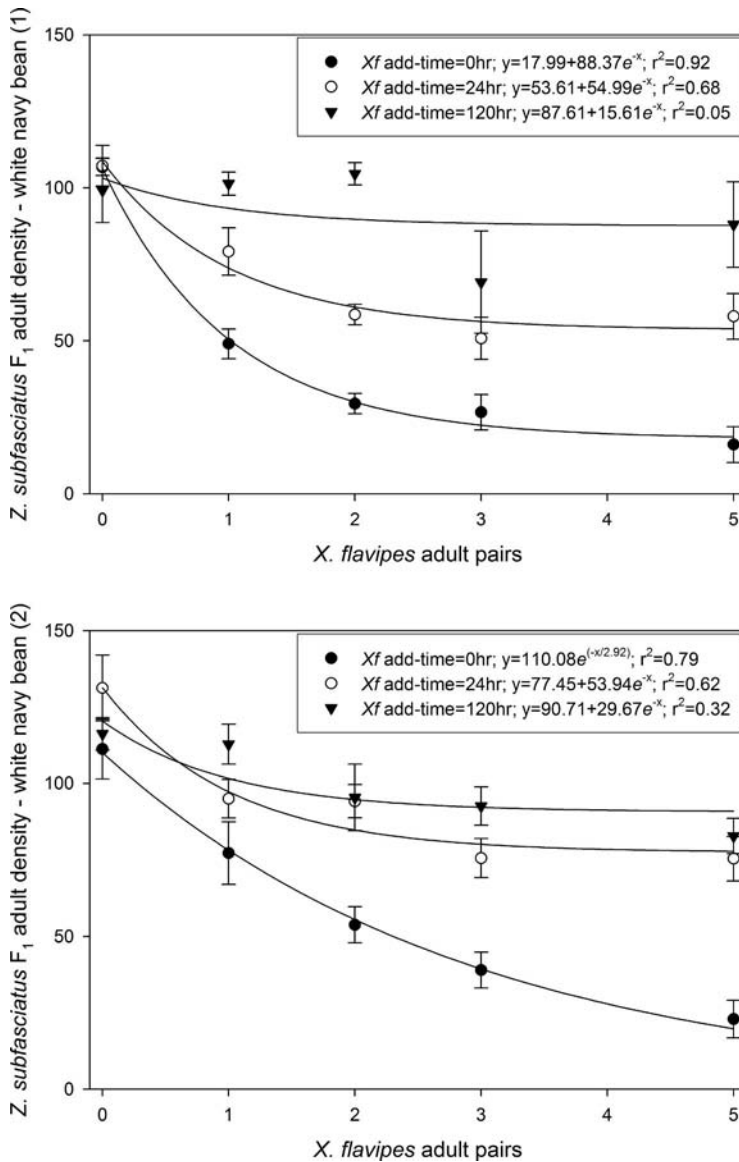


Fig. 6. Influence of predator density on F₁ adult density of *Z. subfasciatus* on white navy bean when *X. flavipes* adult pairs were added 0 (●), 24 (○), or 120 h (▼) after prey in experiment 1 (top) or 2 (bottom).

ware (Systat Software, Point Richmond, CA). Data were analyzed in this manner to capture the intensity of main effects (predator density and add-time) that might be lost by presenting graphical results based on pooled data. Candidate equations were initially sorted by the *F*-statistic to provide a range of simple equations that described the data well, and the pattern and magnitude of the residuals and lack-of-fit tests were scrutinized. Selected equations were chosen based on percentage of variation explained (*R*²).

Results

The results of our initial ANOVA indicated that there was a significant difference in the densities of

emerging F₁ bruchids because of replication in time (i.e., progeny produced in experiment 1 versus experiment 2) for *A. obtectus* on black-eyed pea, *C. chinensis* on black-eyed pea, *C. maculatus* on both black-eyed pea and chickpea, and *Z. subfasciatus* on white navy bean (Table 1). This was evident in the plotted data for the separate experiments, when no predators (density = 0) were added (Figs. 1–6). However, overall trends with regard to the influence of predator pair density, predator pair add-time, and the interaction of these two factors on the density of emerging F₁ bruchids were consistent between experiments 1 and 2 for most bruchid/commodity combinations evaluated (Table 2). Figures 1–6 further show the overall similarity of ex-

Table 2. Results of ANOVA type 3 test of fixed effects to determine the influence of predator density, predator add-time, and the interaction of predator density and add-time on the density of bruchid progeny by bruchid/legume combination for experiment 1 or 2

Source	df	F	Pr > F	df	F	Pr > F	
		<i>A. obtectus</i> -black-eyed pea-1				<i>A. obtectus</i> -black-eyed pea-2	
<i>XF</i> ^a	4,60	7.48	<0.0001	4,56	27.45	<0.0001	
<i>TIME</i> ^b	2,60	0.32	0.7279	2,56	0.63	0.5382	
<i>XF</i> × <i>TIME</i> ^c	8,60	0.31	0.9599	8,56	0.47	0.8724	
		<i>A. obtectus</i> -white navy bean-1				<i>A. obtectus</i> -white navy bean-2	
<i>XF</i> ^a	4,60	15.55	<0.0001	4,60	26.33	<0.0001	
<i>TIME</i> ^b	2,60	1.75	0.1828	2,60	0.60	0.5514	
<i>XF</i> × <i>TIME</i> ^c	8,60	1.58	0.1488	8,60	0.47	0.8755	
		<i>C. analis</i> -black-eyed pea-1				<i>C. analis</i> -black-eyed pea-2	
<i>XF</i> ^a	4,56	122.80	<0.0001	4,56	129.97	<0.0001	
<i>TIME</i> ^b	2,56	8.66	0.0005	2,56	36.45	<0.0001	
<i>XF</i> × <i>TIME</i> ^c	8,56	2.19	0.0421	8,56	1.55	0.1615	
		<i>C. chinensis</i> -black-eyed pea-1				<i>C. chinensis</i> -black-eyed pea-2	
<i>XF</i> ^a	4,60	111.00	<0.0001	4,56	41.47	<0.0001	
<i>TIME</i> ^b	2,60	6.36	0.0031	2,56	5.47	0.0067	
<i>XF</i> × <i>TIME</i> ^c	8,60	1.72	0.1126	8,56	0.73	0.6680	
		<i>C. maculatus</i> -black-eyed pea-1				<i>C. maculatus</i> -black-eyed pea-2	
<i>XF</i> ^a	4,60	30.77	<0.0001	4,60	61.07	<0.0001	
<i>TIME</i> ^b	2,60	34.54	<0.0001	2,60	142.18	<0.0001	
<i>XF</i> × <i>TIME</i> ^c	8,60	2.83	0.0098	8,60	6.21	<0.0001	
		<i>C. maculatus</i> -chickpea-1				<i>C. maculatus</i> -chickpea-2	
<i>XF</i> ^a	4,60	16.09	<0.0001	4,56	15.24	<0.0001	
<i>TIME</i> ^b	2,60	33.43	<0.0001	2,56	27.24	<0.0001	
<i>XF</i> × <i>TIME</i> ^c	8,60	0.82	0.5662	8,56	1.85	0.0863	
		<i>Z. subfasciatus</i> -black-eyed pea-1				<i>Z. subfasciatus</i> -black-eyed pea-2	
<i>XF</i> ^a	4,56	53.77	<0.0001	4,60	13.81	<0.0001	
<i>TIME</i> ^b	2,56	66.19	<0.0001	2,60	8.88	0.0004	
<i>XF</i> × <i>TIME</i> ^c	8,56	6.28	<0.0001	8,60	1.48	0.1842	
		<i>Z. subfasciatus</i> -white navy bean-1				<i>Z. subfasciatus</i> -white navy bean-2	
<i>XF</i> ^a	4,60	23.45	<0.0001	4,56	29.58	<0.0001	
<i>TIME</i> ^b	2,60	43.95	<0.0001	2,56	40.66	<0.0001	
<i>XF</i> × <i>TIME</i> ^c	8,60	4.61	0.0002	8,56	2.57	0.0183	

^a *XF*, density of *X. flavipes* added to experimental arena (zero, one, two, three, or five male:female pairs).

^b *TIME*, time predators added to experimental arenas relative to bruchid prey (0, 24, or 120 h after addition of adult bruchids).

^c *XF* × *TIME*, interaction of predator density and time predators added to experimental arenas relative to bruchid prey.

periments 1 and 2 in response to treatment effects detected.

Inconsistencies between experiments 1 and 2 (significance and nonsignificance) between trials were detected only in the potential role of the interaction term (predator pair density × predator add-time) on adult F_1 progeny produced by *C. analis* and *Z. subfasciatus* on black-eyed pea (Table 2). Data for each bruchid species/commodity treatment from experiments 1 and 2 were therefore pooled, and resulting means were used in all subsequent ANOVA analyses. All treatment comparisons reported reflect those cases where significance at the $\alpha = 0.05$ level was detected consistently for data from experiment 1 and 2 when analyzed separately, and also when pooled.

ANOVA results listed in Table 2 indicate that predator pair density influenced the number of adult F_1 bruchid progeny produced for all evaluated bruchid/legume combinations. Significant differences in the mean density of adult bruchid progeny produced in response to predator density are reported in Table 3. Suppression of *A. obtectus* approached eradication in all treatments; no variation in effect of time of predator addition or predator density higher than one pair was observed (Tables 3 and 4). The addition of a single pair of *X. flavipes* to experimental arenas reduced the density of emerging F_1 adult bruchid progeny on all

bruchid/legume combinations evaluated ($P < 0.0001$; Table 3). The density of adult progeny produced when one pair of *X. flavipes* versus five pairs were added to treatment jars was significantly higher for all bruchid/legume combinations other than *A. obtectus* on black-eyed pea or white navy bean and *C. chinensis* on black-eyed pea (Table 3). We detected a significant difference in the density of emerging F_1 *Z. subfasciatus* on white navy bean ($P < 0.0001$) when one versus three pairs of *X. flavipes* were added to experimental arenas (Table 3). *C. maculatus* on black-eyed pea was the combination most sensitive to small changes in predator pressure with significant differences in adult progeny produced when one versus two and one versus three predator pairs were introduced to experimental arenas (Table 3). Otherwise, we found that the density of adult F_1 bruchids for predator pair density treatments of at least one pair but <5 pairs were not significantly different overall.

A. obtectus on black-eyed pea or white navy bean was the only evaluated bruchid/legume combination that was unaffected by predator add-time (Table 2). The least effective predator add-time of those evaluated in this study was 120 h after initial bruchid infestation of the legumes (Table 4; Figs. 1–6). The most obvious contrast in bruchid suppression resulted when *X. flavipes* were added to experimental arenas at

Table 3. Pairwise comparisons of differences in mean density of adult F₁ bruchid progeny in response to predator density (zero, one, two, three, or five male:female pairs)

<i>X.f.</i> density	Bruchid progeny ^a (mean ± SE)	<i>X.f.</i> density	Bruchid progeny (mean ± SE)	<i>P</i> value ^b (α = 0.05)
<i>A. obtectus</i> -black-eyed pea				
0	24.73 ± 5.02	1	0.60 ± 0.23	<0.0001
0	24.73 ± 5.02	2	0.50 ± 0.21	<0.0001
0	24.73 ± 5.02	3	0.27 ± 0.19	<0.0001
0	24.73 ± 5.02	5	0.07 ± 0.05	<0.0001
<i>A. obtectus</i> -white navy bean				
0	17.33 ± 2.72	1	0.40 ± 0.27	<0.0001
0	17.33 ± 2.72	2	0.10 ± 0.06	<0.0001
0	17.33 ± 2.72	3	0.17 ± 0.10	<0.0001
0	17.33 ± 2.72	5	0.20 ± 0.10	<0.0001
<i>C. analis</i> -black-eyed pea				
0	268.17 ± 2.93	1	122.40 ± 6.75	<0.0001
0	268.17 ± 2.93	2	116.07 ± 5.26	<0.0001
0	268.17 ± 2.93	3	106.10 ± 5.66	<0.0001
0	268.17 ± 2.93	5	103.70 ± 7.17	<0.0001
1	122.40 ± 6.75	5	103.70 ± 7.17	0.0043
<i>C. chinensis</i> -black-eyed pea				
0	146.20 ± 4.36	1	65.37 ± 5.05	<0.0001
0	146.20 ± 4.36	2	52.47 ± 4.28	<0.0001
0	146.20 ± 4.36	3	46.23 ± 4.05	<0.0001
0	146.20 ± 4.36	5	43.73 ± 3.75	<0.0001
<i>C. maculatus</i> -black-eyed pea				
0	298.77 ± 13.11	1	200.57 ± 12.68	<0.0001
0	298.77 ± 13.11	2	171.10 ± 15.70	<0.0001
0	298.77 ± 13.11	3	170.17 ± 14.69	<0.0001
0	298.77 ± 13.11	5	144.10 ± 16.38	<0.0001
1	200.57 ± 12.68	2	171.10 ± 15.70	0.0035
1	200.57 ± 12.68	3	170.17 ± 14.69	0.0026
1	200.57 ± 12.68	5	144.10 ± 16.38	<0.0001
<i>C. maculatus</i> -chickpea				
0	146.83 ± 8.47	1	93.83 ± 8.81	<0.0001
0	146.83 ± 8.47	2	81.20 ± 6.87	<0.0001
0	146.83 ± 8.47	3	80.87 ± 8.25	<0.0001
0	146.83 ± 8.47	5	63.93 ± 7.55	<0.0001
1	93.83 ± 8.81	5	63.93 ± 7.55	0.0002
<i>Z. subfasciatus</i> -black-eyed pea				
0	175.47 ± 6.80	1	111.73 ± 6.28	<0.0001
0	175.47 ± 6.80	2	95.03 ± 8.04	<0.0001
0	175.47 ± 6.80	3	87.20 ± 8.82	<0.0001
0	175.47 ± 6.80	5	72.20 ± 8.75	<0.0001
1	111.73 ± 6.28	5	72.20 ± 8.75	<0.0001
<i>Z. subfasciatus</i> -white navy bean				
0	111.97 ± 3.56	1	85.77 ± 4.59	<0.0001
0	111.97 ± 3.56	2	72.67 ± 5.52	<0.0001
0	111.97 ± 3.56	3	58.97 ± 5.32	<0.0001
0	111.97 ± 3.56	5	57.20 ± 6.02	<0.0001
1	85.77 ± 4.59	3	58.97 ± 5.32	<0.0001
1	85.77 ± 4.59	5	57.20 ± 6.02	<0.0001

^a Bruchid progeny: mean ± SE pooled density of adult F₁ bruchid progeny from experiments 1 and 2.

^b *P* value: Student's *t*-test, α = 0.05. Only significantly different pairwise comparisons are reported.

0 versus 120 h after the parental bruchids (Table 4). The density of emerging F₁ bruchids was 60–70% lower than levels observed in the control arenas (Table 3) when predator and prey were added at the same time (0 h; Table 4), but the difference between treatment and control levels became minimal when the predator was added 120 h (Table 4) after the infesting bruchids.

We detected a significant difference in adult bruchid progeny produced in the 0 versus 24 h predator add-time treatments for *C. maculatus* on black-eyed pea and chickpea and *Z. subfasciatus* on black-eyed pea and white navy bean. Densities of adult F₁ progeny were higher for all bruchid species except *A.*

Table 4. Pairwise comparisons of differences in mean density of adult F₁ bruchid progeny in response to variation in predator add-time (0, 24, or 120 h after bruchid adults added to experimental arenas)

<i>X.f.</i> add-time	Bruchid progeny ^a (mean ± SE)	<i>X.f.</i> add-time	Bruchid progeny (mean ± SE)	<i>P</i> value ^b (α = 0.05)
<i>A. obtectus</i> -black-eyed pea				
0	5.80 ± 2.68	120	6.18 ± 2.35	0.8746
0	5.80 ± 2.68	24	3.72 ± 1.42	0.3884
120	6.18 ± 2.35	24	3.72 ± 1.42	0.3080
<i>A. obtectus</i> -white navy bean				
0	2.86 ± 1.20	120	3.94 ± 1.45	0.4311
0	2.86 ± 1.20	24	4.12 ± 1.40	0.3586
120	3.94 ± 1.45	24	4.12 ± 1.40	0.8955
<i>C. analis</i> -black-eyed pea				
0	128.34 ± 10.96	120	167.10 ± 8.14	<0.0001
0	128.34 ± 10.96	24	134.42 ± 9.79	0.2244
120	167.10 ± 8.14	24	134.42 ± 9.79	<0.0001
<i>C. chinensis</i> -black-eyed pea				
0	62.00 ± 7.00	120	80.20 ± 5.54	<0.0001
0	62.00 ± 7.00	24	70.20 ± 6.36	0.0595
120	80.20 ± 5.54	24	70.20 ± 6.36	0.0220
<i>C. maculatus</i> -black-eyed pea				
0	146.26 ± 12.66	120	264.58 ± 10.28	<0.0001
0	146.26 ± 12.66	24	179.98 ± 11.75	<0.0001
120	264.58 ± 10.28	24	179.98 ± 11.75	<0.0001
<i>C. maculatus</i> -chickpea				
0	62.44 ± 6.71	120	130.16 ± 5.53	<0.0001
0	62.44 ± 6.71	24	87.40 ± 6.34	<0.0001
120	130.16 ± 5.53	24	87.40 ± 6.34	<0.0001
<i>Z. subfasciatus</i> -black-eyed pea				
0	77.74 ± 8.67	120	136.52 ± 5.02	<0.0001
0	77.74 ± 8.67	24	110.72 ± 7.17	<0.0001
120	136.52 ± 5.02	24	110.72 ± 7.17	<0.0001
<i>Z. subfasciatus</i> -white navy bean				
0	53.20 ± 5.00	120	96.22 ± 3.28	<0.0001
0	53.20 ± 5.00	24	82.52 ± 3.92	<0.0001
120	96.22 ± 3.28	24	82.52 ± 3.92	0.0001

^a Bruchid progeny: mean ± SE pooled density of adult F₁ bruchid progeny from experiments 1 and 2.

^b *P* value: Student's *t*-test, α = 0.05. All pairwise comparisons are reported.

obtectus (*P* < 0.0001) when predators were added 120 versus 24 h after the parental bruchids (Table 4).

ANOVA results reported in Table 2 indicate that the interaction of predator pair density and predator add-time were clearly significant across the majority of treatments for only *C. maculatus* on black-eyed pea and *Z. subfasciatus* on white navy bean. Significant differences in F₁ adult bruchids produced as influenced by specific predator pair density/add-time interactions are reported in Table 5. Through this analysis, we determined that predator pair density/add-time interactions played no detectable role in the density of adult progeny produced by *A. obtectus* on either black-eyed pea or white navy bean or *C. chinensis* on black-eyed pea. For all other bruchid/legume combinations, predator treatments of five pairs at the 0 h add-time were universally most effective (Table 5; Figs. 1–6).

Reproduction of *X. flavipes* was observed in many 0 h experimental arenas, although it is not quantitatively reported here (S.E.S., unpublished data). Significant predator population growth in treatment arenas was not possible because the numbers of prey available were low and of about the same age/stage.

Table 5. Pairwise comparisons of differences in mean density of adult F_1 bruchid progeny in response to variation in the interaction of predator pair density (zero, one, two, three, or five adult male:female pairs) and add-time (0, 24, or 120 h after adult bruchids added to arena)

<i>X. flavipes</i> pairs/add-time treatment ^a (treatment 1 versus treatment 2)	Bruchid progeny for treatment 1 ^b (mean \pm SE)	Bruchid progeny for treatment 2 (mean \pm SE)	<i>P</i> value ^c ($\alpha = 0.05$)
<i>C. analis</i> –black-eyed pea			
1/120 versus 1/24 h	143.00 \pm 5.47	108.30 \pm 7.10	0.0023
2/120 versus 2/24 h	139.00 \pm 5.85	101.90 \pm 6.03	0.0011
3/0 versus 3/120 h	81.60 \pm 11.12	132.40 \pm 4.20	<0.0001
5/0 versus 5/120 h	74.30 \pm 9.57	144.80 \pm 5.84	<0.0001
5/120 versus 5/24 h	144.80 \pm 5.84	92.00 \pm 8.48	<0.0001
<i>C. maculatus</i> –black-eyed pea			
1/0 versus 1/120 h	154.30 \pm 12.18	267.90 \pm 19.07	<0.0001
1/120 versus 1/24 h	267.90 \pm 19.07	179.50 \pm 15.83	<0.0001
2/0 versus 2/120 h	111.40 \pm 8.09	262.00 \pm 21.05	<0.0001
2/120 versus 2/24 h	262.00 \pm 21.05	139.90 \pm 21.27	<0.0001
3/0 versus 3/120 h	109.90 \pm 12.98	251.20 \pm 19.52	<0.0001
3/120 versus 3/24 h	251.20 \pm 19.52	149.40 \pm 18.85	<0.0001
5/0 versus 5/120 h	63.50 \pm 11.03	230.90 \pm 25.72	<0.0001
5/120 versus 5/24 h	230.90 \pm 25.72	137.90 \pm 15.74	<0.0001
<i>C. maculatus</i> –chickpea			
1/0 versus 1/120 h	51.90 \pm 9.10	134.90 \pm 13.83	<0.0001
1/120 versus 1/24 h	134.90 \pm 13.83	94.70 \pm 9.64	0.0041
2/0 versus 2/120 h	57.10 \pm 9.02	118.60 \pm 9.23	<0.0001
2/120 versus 2/24 h	118.60 \pm 9.23	67.90 \pm 7.07	<0.0001
3/0 versus 3/120 h	40.80 \pm 7.19	131.50 \pm 9.35	<0.0001
3/120 versus 3/24 h	131.50 \pm 9.35	70.30 \pm 6.53	<0.0001
5/0 versus 5/120 h	29.90 \pm 4.50	106.80 \pm 10.67	<0.0001
5/120 versus 5/24 h	106.80 \pm 10.67	55.10 \pm 8.67	<0.0001
<i>Z. subfasciatus</i> –black-eyed pea			
3/0 versus 3/120 h	39.50 \pm 7.54	130.40 \pm 9.50	<0.0001
3/0 versus 3/24 h	130.40 \pm 9.50	91.70 \pm 11.92	0.0005
5/0 versus 5/120 h	19.70 \pm 9.19	116.00 \pm 8.85	<0.0001
5/0 versus 5/24 h	19.70 \pm 9.19	80.90 \pm 7.01	<0.0001
<i>Z. subfasciatus</i> –white navy bean			
1/0 versus 1/120 h	63.10 \pm 7.11	107.10 \pm 4.03	<0.0001
2/0 versus 2/120 h	41.60 \pm 5.17	100.00 \pm 5.61	<0.0001
2/0 versus 2/24 h	41.60 \pm 5.17	76.40 \pm 6.66	<0.0001
3/0 versus 3/120 h	32.80 \pm 4.39	80.90 \pm 9.25	<0.0001
3/0 versus 3/24 h	32.80 \pm 4.39	63.20 \pm 6.06	0.0002
5/0 versus 5/120 h	19.50 \pm 4.17	85.40 \pm 7.17	<0.0001
5/0 versus 5/24 h	19.50 \pm 4.17	66.70 \pm 5.57	<0.0001

^a *X. flavipes* pairs/add-time treatment: specific predator pair density and predator add-time combination.

^b Bruchid progeny: mean \pm SE pooled density of adult F_1 bruchid progeny from experiments 1 and 2.

^c *P* value: Student's *t*-test, $\alpha = 0.05$. Only significantly different pairwise comparisons are reported.

Because all species evaluated here other than *A. obtectus* develop entirely within seeds, predators introduced into experimental arenas were subjected to prolonged periods of starvation and potential cannibalism during the development of F_1 bruchid progeny within the protective confines of their host seeds, once the nutritional resources from parental bruchids were exhausted.

Discussion

Differences in host quality alone affected the density of adult progeny produced by *A. obtectus* on black-eyed pea versus white navy bean, *C. maculatus* on black-eyed pea versus chickpea, and *Z. subfasciatus* on black-eyed pea versus white navy bean (see mean values for controls listed under "*X.f.* density" = 0, Table 3). Significant differences in the density of adult bruchid progeny produced in experiment 1 versus experiment 2 were therefore probably caused by variability in host quality within the same commodity,

which we attribute to inability to stockpile enough of each commodity to ensure that legumes used in the first and second full replication of the study came from the same lot.

The results of this study indicate that *X. flavipes* can reduce the number of adult bruchid progeny when applied at a variety of times and densities after initial bruchid infestation, but because of the inaccessibility of the eggs and developing larvae of all species other than *A. obtectus*, is clearly most effective when it can begin to prey on (Sing and Arbogast 2007), or at least disturb, the mating and oviposition of the parental bruchids as soon as infestation occurs. Significant bruchid damage to stored grain legumes begins in most cases with low level field infestation that quickly grows to catastrophic proportions in the sheltered environs of storage facilities (Southgate 1978, Labeyrie 1981); this study reinforces the urgency of protecting stored legumes as soon after they are stored as possible.

The practicality of producing and introducing the appropriate density of predators within 24 h of the onset of storage infestation, the period when this treatment would be most effective, may be questioned. However, if infestation by many of these bruchid species begins when the pests are first brought into the storage facility from the field with the legumes, planning to have a quantity of predators on hand to treat the stored commodity within 24 h does not seem so far-fetched. In fact, *X. flavipes* could be collected from residue of the previous season's stored legumes if sanitation of the facility took place just before the current season's crop was stored. This type of treatment could play an important role in on-farm or household sized storage, and perhaps in small cooperative storage facilities, but probably would not be suitable for storage associated with large-scale industrial or commercial legume production. Even in cases where smaller or noncommercial producers store legumes in 1,000 bushel-capacity bins at the end of a given growing season, it is doubtful that the entire bulk would be deposited in the storage facility at one time. Gradual filling of the storage would allow for and might even increase efficacy if the initial number of predators were relatively low. Finally, the experimental results reported here relate to the application of a single method of bruchid management; depending on locally available and implemented storage pest management practices, natural enemies could play an important role in an integrated approach to bruchid management.

Another issue related to large scale storage is the question of *X. flavipes*' ability to disperse within a large bulk of stored legumes, which was not experimentally evaluated in the current study. The statement of Arbogast (1979) that this species' movement and predatory capacity were highest in bulks of stored product that have large interstitial spaces, such as peanuts and shelled corn, suggests that it could function well in large-capacity legume storage. Previous studies with this predator have concluded that its best application lies in its shown facility to prophylactically disinfest emptied storage facilities of residual populations of pest insect eggs and early-instar larvae (Arbogast 1979), a major source of contamination in newly stored commodities (LeCato et al. 1977, Brower and Press 1992). This study suggests that *X. flavipes* could play a valuable role in preventive disinfestation of emptied legume storage facilities by reducing the threat of contamination to freshly stored legumes by residual storage populations. The ability of *X. flavipes* to successfully attack large, sclerotized prey when more accessible prey are not available (LeCato 1976) was observed with all bruchid species evaluated here (Sing and Arbogast 2007) and seems to be reiterated in the results of this study. Because bruchids are typically the primary pest of stored legumes, *X. flavipes* predation on this family of pests would not be detracted from by the presence of more favored prey species.

The ability of this predator to reproduce successfully on bruchid prey was not tested in these exper-

iments. In these studies, the uniform age of parental bruchids represents a prey age structure that would ensure a life history refuge from extinction, whereas at the same time forcing *X. flavipes* into starvation once all the parental bruchids had died and their cryptically developing progeny had not yet emerged. However, under field conditions, the continual emergence of low numbers of bruchid adults from newly harvested legumes would provide sustained prey for predator population establishment.

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