

Comparative Feeding by Three Chrysomelid (Coleoptera: Chrysomelidae) Species on Eight Soybean Genotypes¹

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Abstract The feeding rates on soybean, *Glycine max* (L.) Merr., by three beetle species, *Cerotoma trifurcata* (Forster), *Diabrotica balteata* LeConte, and *Colaspis louisianae* Blake, were compared in bioassays in the laboratory. Eight soybean genotypes, including two resistant standards (PI171451 and PI229358), a resistant line (D89-9121), four resistant commercial cultivars (Lamar, Lyon, Crockett, and Shore), and a susceptible standard (Centennial) were planted on the research farm at St. Gabriel, LA. Beetles were collected from the field throughout central and south Louisiana, separated by gender, and placed in Petri dishes with leaflets of known leaf area. Feeding, mortality, and oviposition were monitored daily for 5 d. Per capita daily consumption by *C. louisianae* and *D. balteata* averaged between 3.5 and 2.5 mg dry weight, and *C. trifurcata* consumed 7.6 mg dry weight. Beetles of all species consumed more Centennial foliage than foliage from PI229358, PI171451, Lyon, and Shore. Consumption of other soybean genotypes varied among beetle species. Number of eggs deposited by *C. trifurcata* and *C. louisianae* did not vary significantly among soybean genotypes. *Diabrotica balteata* females were not observed ovipositing on leaves of any cultivar. Mortality varied significantly among beetle species, but not among soybean genotypes. *Diabrotica balteata* suffered the highest mortality and *C. trifurcata* the lowest. The results suggest cultivars resistant to *C. trifurcata* and *D. balteata* should also be effective against *C. louisianae*.

Key Words *Cerotoma*, *Diabrotica*, *Colaspis*, *Glycine max*, bean leaf beetle, banded cucumber beetle

Feeding injury by bean leaf beetle, *Cerotoma trifurcata* (Forster), banded cucumber beetle, *Diabrotica balteata* LeConte, and to a lesser extent striped blister beetle, *Epicauta vittata* (F.), can adversely affect soybean yields in the mid-South (Kogan et al. 1980). Consequently, different soybean cultivars and plant introductions have been screened for resistance or tolerance to foliar feeding damage by these species (Layton et al. 1987, Clark et al. 1972). However, in 1997 in Louisiana, *Colaspis* species populations were high, and concern was expressed about feeding impact. A preliminary experiment demonstrated that *Colaspis* species consumed less foliage than *C. trifurcata*, but more foliage than *D. balteata*. Therefore, this study was conducted to compare consumption rates of *Colaspis* species to that of *C. trifurcata* and *D. balteata* on several susceptible and resistant soybean cultivars and plant introductions.

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Materials and Methods

Beetles used for studies were collected from Opelousus (St. Landry Parish), Alexandria (Rapides Parish), Jeanerette (Iberia Parish), and St. Gabriel (Iberville Parish). Two species of *Colaspis* are found in Louisiana soybeans: *C. brunnea* (F.) (less common) and *C. louisianae* (Blake) (more common) (Chapin 1979). To determine the relative abundance of the three species, 30 males were dissected and species identified (Chapin 1979). From those dissections, only *C. louisianae* males were found. Therefore, we assumed that the large majority of *Colaspis* beetles used in experiments were *C. louisianae*. Eight soybean genotypes were planted at St. Gabriel in May 1998. Soybeans used included two plant introductions (PI171451 and PI229358), 5 commercial cultivars (Centennial, Crockett, Lamar, Lyon, and Shore) and the advanced breeding line D89-9121. The two PI lines are considered resistant standards for coleopteran and lepidopteran defoliators (Boethel 1999). PI171451 is the source of resistance in Crockett and D89-9121 (Kilen and Lambert 1994, Bowers 1990). PI229358 is the source of resistance in Lamar and Lyon (Hartwig et al. 1994, 1990). Shore does not derive its resistance to Mexican bean beetle, *Epilachna varivestis* Mulsant, from either of the two PI lines listed above (Smith et al. 1975). Centennial is considered a susceptible standard (Rowan et al. 1991, Layton et al. 1987). Seeds were provided by the USDA-ARS Soybean Research Unit in Stoneville, MS.

Beetles collected from the field were separated by gender and placed (single sex, 5 to a dish) in 10-cm Petri dishes lined with wetted filter paper. Beetles were sexed according to the color of the frons (*C. trifurcata*) (Kogan et al. 1980), the shape of the apex of the abdomen (*C. louisianae*) (Chapin 1979), and the relative size of second and third antennal segments (*D. balteata*). At least 20 beetles of each species and each gender were dissected and genitalia examined to confirm that the characters listed above adequately separated the genders. Leaves from soybean plants at stages R2-R4 (Fehr and Caviness 1977) were removed from the middle to top of the soybean canopy and returned to the laboratory. Single leaflets of known leaf area were placed in Petri dishes and removed 24 h later. Area of leaflets was again measured using a LiCor LI3000 leaf area meter following feeding, and the difference between before and after measurements was used as a consumption estimate. Leaflets were replaced each day for 5 d. In addition, filter paper was moistened and dead beetles replaced daily. Four replicates of males and females were conducted for *C. trifurcata* and *D. balteata*; eight were conducted for each sex for *C. louisianae*. Beetle mortality and oviposition also were recorded.

Consumption values measured in cm^2 were converted to dry weight values by measuring the leaf area of undamaged leaflets of all eight genotypes, drying the leaflets at 50 C for 24 h, and measuring their dry weight. This provided a factor (mg/cm^2) that was used to convert consumption values. Consumption (mg dry weight) data were expressed as either total daily consumption by all beetles in a Petri dish or a per capita daily consumption. Per capita data were calculated by taking the total consumption and dividing it by the number of surviving beetles at the end of the 24-h period [total number of beetles (5)—mortality for that 24 h period]. Total and per capita consumption data were analyzed by factorial model ANOVA for all species (including beetle species and sex, and soybean genotype as factors), and then separately for each species (including beetle sex, soybean genotype, and day as factors) (GLM,

SAS Institute 1994). Mortality and oviposition data were analyzed separately for each species by Kruskal-Wallis test (Proc NPar 1 Way, Stokes et al. 1997).

Results and Discussion

Total and per capita daily consumption by *C. trifurcata* were significantly higher than that by either *C. louisianae* or *D. balteata*, and per capita daily consumption by *C. louisianae* was significantly higher than *D. balteata*. However, both total and per capita daily consumption varied significantly among the different soybean genotypes and beetle species ($F = 2.5$ for total consumption and 2.9 for per capita daily consumption; $df = 14, 1232$; $P < 0.01$). Total daily consumption by *C. trifurcata*, averaged across all soybean genotypes, was 40 ± 0.3 mg, while *D. balteata* averaged 16 ± 0.2 and *C. louisianae* average 19 ± 0.1 mg dry weight. Per capita daily consumption averaged 7.6 ± 0.1 mg dry weight for *C. trifurcata*, 3.5 ± 0.1 mg dry weight for *C. louisianae*, and 2.5 ± 0.1 mg dry weight for *D. balteata*. Consumption rates reported here were nearly twice that observed by Layton et al. (1987) where four *C. trifurcata* consumed 10 to 25 mg dry weight of soybean foliage in 2 d and *D. balteata* consumed 4 to 13 mg dry weight. In the Layton et al. (1987) study, several of the collection dates (late-July to late-August) were after the time when adult *C. trifurcata* may enter diapause, and beetles in this state may consume less foliage than actively reproducing adults. In the present study, collections of beetles were made from mid-June to mid-July, a time when none of the beetle species should be entering diapause.

The pattern of feeding preference by *C. louisianae* was indicative of patterns observed for *C. trifurcata* and *D. balteata*. More Centennial foliage was consumed than PI229358 and PI171451 by *C. louisianae* (Table 1). Although consumption of PI171451 by *C. trifurcata* and consumption of PI229358 by *D. balteata* was higher than expected, consumption of Centennial was higher than any other soybean ge-

Table 1. Mean ($\pm$ S.E.M.) *Colaspis louisianae* daily consumption (either total or per capita), oviposition, and daily mortality on various soybean genotypes

Soybean genotype	N	Total daily leaf consumption (mg dry weight)*	Per capita daily leaf consumption (mg dry weight)*	Oviposition per five days per five females	Number dead per day
Crockett	80	21.7 ± 1.6 a	4.2 ± 0.3 a	41.1 ± 26.9	0.12 ± 0.04
D89-9121	80	20.0 ± 1.4 ab	3.8 ± 0.3 ab	41.1 ± 26.9	0.17 ± 0.05
Lamar	80	20.0 ± 1.2 ab	3.8 ± 0.2 ab	0.0 ± 0.0	0.18 ± 0.05
Centennial	80	19.2 ± 1.1 ab	3.7 ± 0.2 ab	13.9 ± 13.9	0.13 ± 0.04
Shore	80	18.9 ± 1.1 abc	3.6 ± 0.2 abc	6.4 ± 6.4	0.18 ± 0.05
PI 229358	80	18.5 ± 1.0 abc	3.5 ± 0.2 abc	21.4 ± 14.5	0.23 ± 0.05
PI 171451	80	15.7 ± 1.2 bc	2.9 ± 0.3 bc	14.9 ± 14.9	0.25 ± 0.06
Lyon	80	14.3 ± 0.7 c	2.6 ± 0.2 c	16.8 ± 11.3	0.28 ± 0.07

* Means within columns followed by the same letter are not significantly ($P > 0.05$) different using Tukey's studentized range test (HSD).

notype (Table 2, 3). Lyon was among the least fed upon by all beetle species. Consumption of the other cultivar possessing resistance from PI229358, Lamar, varied among beetle species. Foliage from soybeans with resistance derived from PI171451 (Crockett and D89-9121) was fed upon more by *C. louisianae* than those cultivars with resistance derived from PI229358 (Lamar and Lyon). A similar pattern of consumption was not apparent among soybeans possessing resistance from PI229358 and those with resistance from PI171451 for *C. trifurcata* or *D. balteata*. Consumption by *C. trifurcata* and *D. balteata* of all soybean genotypes possessing resistance from either PI229358 or PI171451 was significantly lower than Centennial. Ranking of soybean genotypes was identical using either total or per capita daily consumption rates (Table 1).

Total daily consumption by females and males differed significantly among species (species by gender interaction term: $F = 2.0$; $df = 7, 1232$; $P < 0.05$). Female *C. louisianae* consumed significantly more soybean foliage (22.2 mg dry weight) than males (15.8 mg dry weight). Consumption was equal for female and male *C. trifurcata* (41.2 and 38.3 mg dry weight, respectively) and for female and male *D. balteata* (14.8 and 17.1 mg dry weight, respectively). Layton et al. (1987) reported that female *C. trifurcata* consumed significantly more foliage than males when beetles were collected early in the summer, but that difference was not evident later in the year. They suggested that beetles collected in August may have entered diapause thereby explaining the lower consumption of females. Layton et al. (1987) also reported that male *D. balteata* consumed more than females, but they also observed higher mortality for females than males. They used the differential mortality between the two sexes as a possible explanation for the differences in consumption.

The daily feeding pattern differed significantly among soybean genotypes for *C. louisianae* (soybean genotype by day interaction term: $F = 1.9$; $df = 28, 560$; $P < 0.01$). Feeding remained fairly constant over the 5 d for all soybean genotypes except

Table 2. Mean ($\pm$ S.E.M.) *Ceratoma trifurcata* daily consumption (either total or per capita), oviposition, and daily mortality on various soybean genotypes

Soybean genotype	N	Total daily leaf consumption (mg dry weight)*	Per capita daily leaf consumption (mg dry weight)*	Oviposition per five days per five females	Number dead per day
Centennial	40	46.0 $\pm$ 4.5 a	9.2 $\pm$ 0.9 a	20.6 $\pm$ 10.8	0.03 $\pm$ 0.03
PI 171451	40	42.0 $\pm$ 5.8 ab	8.4 $\pm$ 1.1 ab	12.7 $\pm$ 12.7	0.00 $\pm$ 0.00
Crockett	40	41.1 $\pm$ 4.7 ab	8.2 $\pm$ 0.9 ab	32.9 $\pm$ 18.5	0.05 $\pm$ 0.03
Lamar	40	37.7 $\pm$ 3.5 ab	7.5 $\pm$ 0.7 ab	3.7 $\pm$ 2.5	0.03 $\pm$ 0.03
D89-9121	40	36.6 $\pm$ 4.7 ab	7.3 $\pm$ 0.9 ab	23.9 $\pm$ 10.1	0.00 $\pm$ 0.00
Lyon	40	35.7 $\pm$ 3.9 ab	7.1 $\pm$ 0.8 ab	11.7 $\pm$ 7.7	0.00 $\pm$ 0.00
Shore	40	32.7 $\pm$ 4.6 b	6.5 $\pm$ 0.9 b	4.7 $\pm$ 4.2	0.03 $\pm$ 0.03
PI 229358	40	31.7 $\pm$ 3.5 b	6.3 $\pm$ 0.7 b	15.9 $\pm$ 9.8	0.00 $\pm$ 0.00

* Means within columns followed by the same letter are not significantly ($P > 0.05$) different using Tukey's studentized range test (HSD).

Table 3. Mean ($\pm$ S.E.M.) *Diabrotica balteata* daily consumption (either total or per capita), and daily mortality on various soybean genotypes

Soybean genotype	N	Total daily leaf consumption (mg dry weight)*	Per capita daily leaf consumption (mg dry weight)*	Oviposition per five days**	Number dead per day
Centennial	40	24.1 $\pm$ 2.3 a	4.7 $\pm$ 0.5	—	0.15 $\pm$ 0.06
PI 229358	40	17.7 $\pm$ 1.5 ab	3.3 $\pm$ 0.3	—	0.27 $\pm$ 0.08
Shore	40	17.3 $\pm$ 4.4 ab	3.2 $\pm$ 0.9	—	0.27 $\pm$ 0.09
Crockett	40	13.6 $\pm$ 1.8 b	2.3 $\pm$ 0.4	—	0.40 $\pm$ 0.10
Lyon	40	13.1 $\pm$ 1.4 b	2.3 $\pm$ 0.3	—	0.27 $\pm$ 0.09
Lamar	40	12.6 $\pm$ 1.7 b	2.1 $\pm$ 0.4	—	0.40 $\pm$ 0.11
D89-9121	40	9.7 $\pm$ 1.1 b	1.4 $\pm$ 0.3	—	0.57 $\pm$ 0.14
PI 171451	40	8.2 $\pm$ 0.9 b	0.9 $\pm$ 0.2	—	0.72 $\pm$ 0.14

* Means within columns followed by the same letter are not significantly ($P > 0.05$) different using Tukey's studentized range test (HSD).

** No eggs found.

D89-9121, PI171451, and Shore, and in these three cases, consumption increased on the last day. Beetles of both *C. trifurcata* and *D. balteata* generally ate more on days 1 and 2 than days 3 to 5 ($F = 110$ for *C. trifurcata* and 3.7 and *D. balteata*; $df = 4, 240$; $P < 0.05$).

Oviposition did not differ significantly among the eight soybean genotypes ($\chi^2 = 7.6$ for *C. louisianae* and 4.4 for *C. trifurcata*; $df = 7$; $P > 0.4$). Despite the non-significant differences, the pattern of ovipositional preferences of the two species was nearly identical (Tables 1 and 2). Both species oviposited primarily on Crockett and D89-9121. They deposited fewer eggs on PI229358 and PI171451 and only very few eggs on Shore and Lamar. The two species differed in the number of eggs deposited on Centennial and Lyon. *Colaspis louisianae* deposited more eggs on leaflets of Lyon than Centennial. *Cerotoma trifurcata* deposited more eggs on leaflets of Centennial than Lyon. *Cerotoma trifurcata* are known to oviposit in soybean, and larvae cause damage to roots and root nodules (Kogan and Turnipseed 1987). Currently, it is not known if *C. louisianae* oviposits in soybean fields. If they do, root and nodule feeding by larvae may be an important determinant in the economic thresholds for *C. louisianae* in soybeans.

Mortality of *C. louisianae* and *D. balteata* was significantly higher than that for *C. trifurcata* ($\chi^2 = 85$; $df = 2$; $P < 0.001$), but mortality did not vary significantly among soybean genotypes ($\chi^2 = 10.3$; $df = 7$; $P > 0.2$). Despite the non-significant differences in mortality among soybean genotypes, the following pattern was observed. *Colaspis louisianae* and *D. balteata* mortality tended to increase as consumption decreased. *Cerotoma trifurcata* mortality overall was very low. Plotting data from all three species and all soybean genotypes, there was a strong relationship between daily consumption and daily mortality (Fig. 1). However, the analyses comparing consumption among chrysomelid species were nearly identical using either total daily consumption (not corrected for mortality) or per capita daily consumption (corrected for mortality).

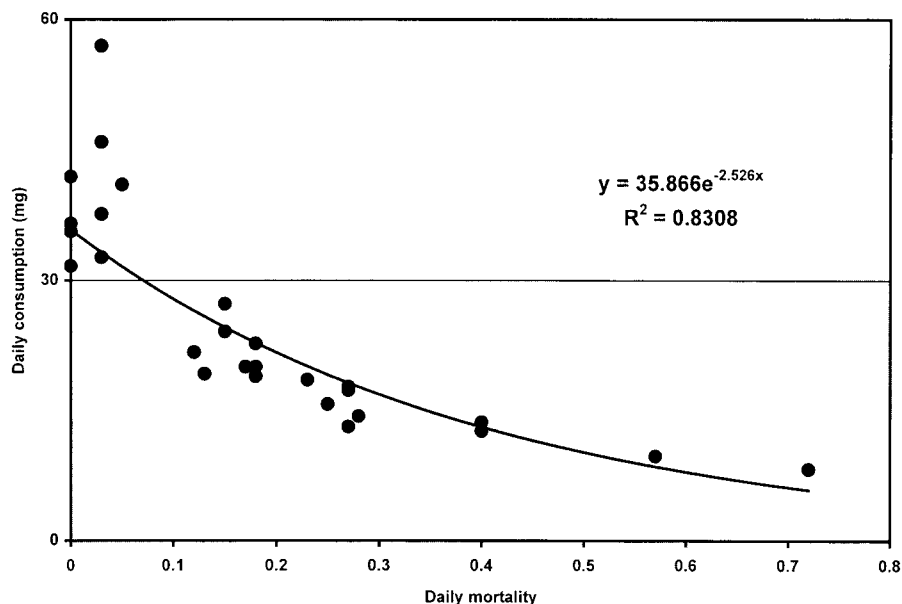


Fig. 1. Comparison between daily beetle mortality and total daily consumption (mg dry weight). Shown are average values from *C. louisianae*, *C. trifurcata*, and *D. balteata* and all eight soybean cultivars.

The only difference observed between these two analyses was a significant difference between *C. louisianae* and *D. balteata* per capita daily consumption. Therefore, consumption rates were not so much affected by mortality rates, but rather, species differences were primarily responsible for the significant relationship between mortality and consumption rates.

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