High Carbohydrate Content in Snap Bean Leaves Stimulates Chinese Rose Beetle Feeding

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Abstract. Chinese rose beetle (CRB) (*Adoretus sinicus* Burmeister) preferential feeding based on leaf carbohydrate content was determined using 'Hawaiian Wonder' snap bean (*Phaseolus vulgaris* L.) plants exposed to 0%, 40%, or 80% shade for 2 days. Plants exposed to 0% shade before CRBs fed had 8.5% leaf area consumed compared with 2.8% and 3.6% for 40% and 80% shade, respectively. Leaves exposed to 0% shade had a higher carbohydrate content than leaves exposed to 40% and 80% shade. Darkening the apical and subtending leaf with aluminum foil for 1 day before CRBs fed shifted CRB feeding from heavy feeding on the apical leaf and light feeding on the subtending leaves to a reversed feeding pattern—light feeding on the apical leaf and heavy feeding on the subtending leaves. Three snap bean cultivars grown under similar environmental conditions were compared to 'Hawaiian Wonder' using a unifoliate split-leaf technique. Cultivars with a high endogenous carbohydrate content in their leaves had a higher percentage of leaf area consumed by CRBs than cultivars with low endogenous carbohydrates. This report suggests that high endogenous carbohydrate content in leaves stimulates CRB feeding.

In Hawaii, the Chinese rose beetle (CRB) is a common pest of >255 plant species, including many agriculturally important crops (Habeck, 1963). CRBs feed during early evening hours, and their damage is characterized by distinct interveinal defoliation of host plant leaves—damage that can ultimately affect plant maturation and yield (Furutani et al., 1990).

Since CRBs have an extensive host range, Arita et al. (1988) suggested that the stimulus used to initiate and sustain beetle feeding must be a fairly common product found in host plant leaves. Furutani and Arita (1990) suggested that CRBs feed on carbohydrates. In similar *in vitro* studies, Ladd (1986) reported that several common carbohydrates, including sucrose, maltose, fructose, and glucose, were strong phagostimulants for the adult Japanese rose beetle (*Popillia japonica* Newman).

Previous CRB feeding studies demonstrated that CRBs preferred plant material

treated with foliar applications of (2-chloroethyl) phosphonic acid (ethephon) (Arita et al., 1988; Furutani and Nagao, 1986). Furutani and Arita (1990) proposed that, while leaf carbohydrates may be a general phagostimulant, carbohydrate quantity is of major importance in the beetles' selection of a food source. Similarly, McKibben et al. (1985) and Parrott et al. (1989) found that there was a relationship between the quantity of an ester extract in host plants and the feeding response of the cotton boll weevil (*Anthonomus grandis* Boheman).

Thus, the objectives of this study were to observe the effect of shade, leaf position, and leaf carbohydrate content of snap beans on CRB feeding.

Shade treatment. 'Hawaiian Wonder' snap bean (Seed Program, Dept. of Horticulture, Univ. of Hawaii, Honolulu) plants were started from seed in a fiberglass greenhouse (Furutani and Arita, 1990). When plants had three ranks of trifoliate leaves (≈ 25 days after sowing), five plants (one per pot) were placed in a screened cage (1.0 m high $\times$ 0.7 m wide $\times$ 2.0 m long) under full sun or in a shadehouse covered with 40% or 80% polypropylene shade cloth (1500, 810, and 214 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). Light readings were taken at 1200 HR on a cloudless day. The mean air temperature in the cages was $25 \pm 7^\circ\text{C}$.

Two days afterplants were placed in cages, 25 nonsexed CRBs (collected from the field the previous evening) were placed into each cage between 1600 and 1800 HR and left for 24 h. Plants were removed from the cages and the percent leaf area consumed was quantified

with a leaf area meter (LI-3000A; LI-COR, Lincoln, Neb.). The experiment was designed as a randomized complete block with three replicate cages per shade treatment. The experiment was performed seven times; the data from all experiments were pooled. Experiments were conducted between Oct. 1991 and Feb. 1992 at the College of Agriculture Farm Laboratory, Panaewa, Hawaii. Daylight was between 0600 to 1800+0100HR. The data were analyzed by polynomial regression to investigate the trend in percent leaf area consumed by CRBs after shading the plants.

Leaf carbohydrate content was determined for plants grown under each shade treatment as described below. The total carbohydrate content measurement for each treatment was based on 20 replicate plants. The experiment was arranged in a randomized complete block and analyzed by linear regression to investigate the trend in leaf endogenous carbohydrate content resulting from shading.

Foil treatment. Snap bean plants prepared as described above were grown in a fiberglass greenhouse under 0% shade. Plants were grown until four ranks of leaves (order of naming leaves acropetally: unifoliate and trifoliate 1, 2, and 3) were visible. Trifoliate 2 and 3 were kept dark by covering the leaves with a single wrapping of aluminum foil between 0800 and 0900 HR. The unifoliate and first trifoliate leaves were not wrapped in foil. The foil wrappings were removed that evening between 1600 and 1800 HR. CRB preferential feeding was determined by placing a single plant in a wooden-framed cage (38 $\times$ 38 $\times$ 62 cm) screened with organdy cloth. Ten nonsexed CRBs (collected from the field the previous evening) were placed in the cage between 1600 and 1800 HR, and the cage was then placed in a darkened chamber at 25°C. Nonwrapped snap bean plants were used as controls. After 24 h, the unifoliate and trifoliate leaves were removed, and the leaf area consumed by CRBs was determined as described above. Each treatment consisted of 30 replicate plants. Leaf carbohydrate content was determined as described below. The experiment was completely randomized; treatment means and SE values were calculated for percent leaf area consumed and leaf endogenous carbohydrate content after wrapping leaves.

Cultivar trials. 'Kentucky Wonder', 'Green Crop', and 'Blue Lake Bush 274' (W. Atlee Burpee Co., Warminster, Pa.) bean plants were planted in the greenhouse as described under shade treatment and grown unshaded until their unifoliate leaves were fully expanded (≈ 12 days). The unifoliate leaves from each cultivar were excised and sectioned along the midrib with a razor blade. A sectioned unifoliate leaf from each cultivar was placed beside a 'Hawaiian Wonder' section (split-leaf arrangement). The midribs of the two unifoliate leaf sections were matched to simulate a whole leaf. The paired sections were placed in a 9-cm petri dish fitted with a single layer of Whatman no. 2 filter paper moistened with 1.0 ml distilled water. One CRB was placed in the petri dish and the dish was placed in a dark chamber at 24°C for 12 h. The unifoliate leaf sections

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were removed from the dish, and the leaf area consumed by the CRB was quantified with a leaf area meter as already described. Each treatment comparison was replicated 30 times. Ten plants from each cultivar were sampled for the total carbohydrate content measurement. Unifoliate split leaves were prepared and CRBs introduced between 1600 and 1800 HR. The experiment was designed as a randomized complete block; data were analyzed by paired two-group *t* tests to compare percent leaf area consumed and leaf endogenous carbohydrate content between snap bean cultivars.

Total carbohydrate content measurement. Leaf total carbohydrate content was determined by sampling trifoliate and unifoliate leaves. Leaves were analyzed by subsampling all unifoliate and trifoliate leaflets midway from the base to the leaf apex and 5 mm to the left of the midrib with a 3-mm-diameter cork borer. Total carbohydrate content of leaves (expressed as micrograms of sucrose equivalent/5 mm² leaf area) was determined with Dreywood's anthrone reagent as described by Morris (1948).

Influence of shade treatment on feeding. Snap bean plants exposed to 0% shade before CRBs fed had $\approx 8.5\%$ of the leaf area consumed compared to 2.8% to 3.6% for 40% and 80% shade, respectively. The relationship between percent leaf area consumed and percent shade was quadratic ($R^2 = 0.23$, $P = 0.01$) (Fig. 1). Plants grown in 0% shade also had the highest leaf carbohydrate content, expressed as sucrose equivalents (Fig. 2). Trend analysis suggests that there is a linear relationship between leaf carbohydrate content and percent shade ($R^2 = 0.81$, $P = 0.01$).

Effect of foil treatment on feeding. The unifoliate and trifoliate 1 and 2 had only 1.9% to 2.8% feeding damage, while trifoliate 3 had 6.2% feeding damage (Fig. 3). Nonwrapped leaf carbohydrate content for trifoliate 1, 2, and 3 was from 59.2 to 61.2 μg sucrose eq/5mm² leaf area, while the unifoliate had the lowest carbohydrate content—47.1 μg sucrose eq/5 mm² leaf area (Fig. 4). Wrapping trifoliate 2 and 3 in foil significantly decreased leaf feeding at trifoliate 3 compared to feeding at the nonwrapped leaves. The unifoliate and trifoliate 1 had a higher percent leaf area consumed by CRBs when trifoliate 2 and 3 were wrapped. Leaf carbohydrate content for wrapped and nonwrapped snap bean plants was similar, except for trifoliate 2 and 3, which had a $>50\%$ decrease in carbohydrate content compared to the nonwrapped control (Fig. 4). The total percent leaf area consumed of wrapped (13.5 ± 0.73 SE) compared to nonwrapped (13.0 ± 0.82 SE) plants was not significant.

Effect of cultivar on feeding. 'Blue Lake Bush 274' and 'Kentucky Wonder' snap bean cultivars had higher percent leaf area consumed than 'Hawaiian Wonder' (Table 1). Both cultivars also had higher leaf carbohydrate contents than 'Hawaiian Wonder'. There was no difference between leaf carbohydrate content or percent leaf area consumed for 'Green Crop' and 'Hawaiian Wonder'.

Wrapping trifoliate 2 and 3 in foil during

Fig. 1. Relationship between exposing snap bean plants to 0%, 40%, or 80% shade (S) for 2 days and percent leaf area consumed by the Chinese rose beetle. Regression equation: percent leaf area consumed = $9.1 - 0.3(S\%) + 0.0026(S\%)^2$ ($R^2 = 0.23$, $P = 0.01$).

Fig. 2. Relationship between exposing plants to 0%, 40%, or 80% shade (S) for 2 days and accumulated endogenous carbohydrate content of snap bean leaves at 1600 to 1800 HR. Regression equation: accumulated endogenous carbohydrate content = $93.2 - 0.8(S\%)$ ($R^2 = 0.81$, $P = 0.01$).

daylight hours kept the leaves from photosynthesizing. Consequently, during evening hours, when CRBs fed, these leaves were abnormally low in endogenous carbohydrates. In response to the lowered carbohydrates, CRBs shifted their feeding to the lower leaves where the carbohydrate content was higher. Apparently, CRBs are able to assess leaf endogenous carbohydrate content. CRBs may determine carbohydrate content by sampling the leaves, since tiny feeding holes were observed on the wrapped leaves.

Differences in the carbohydrate content of leaves were observed in snap bean cultivars grown under the same environmental conditions. A higher carbohydrate content in the

Fig. 3. Comparison of percent leaf area consumed in relation to leaf position [order of leaves acropetally: unifoliate (U), trifoliate 1, 2, and 3] for nonwrapped and wrapped (trifoliate 2 and 3 were wrapped; trifoliate 1 and unifoliate were not wrapped) snap bean plants. Trifoliate 2 and 3 were wrapped (kept dark) for 1 day; foil was removed just before Chinese rose beetle feeding.

Fig. 4. Comparison of endogenous carbohydrate content in relation to leaf position [order of leaves acropetally: unifoliate (U), trifoliate 1, 2, and 3] for nonwrapped and wrapped (trifoliate 2 and 3 were wrapped; trifoliate 1 and unifoliate were not wrapped) snap bean plants. Trifoliate 2 and 3 were wrapped (kept dark) for 1 day; foil was removed just before carbohydrate analysis.

leaves resulted in a higher percent leaf area consumed by CRBs. Clearly, CRBs select for and feed on those cultivars possessing a high level of leaf carbohydrates. We presume that the CRBs are selecting a mobile carbohydrate source, such as starch grains accumulated via photosynthesis. We also presume that the carbohydrate source is not structural cellulose, because Furutani and Arita (1990) reported

Table 1. Leaf carbohydrate content and percent leaf area consumed by the Chinese rose beetle in split-leaf analyses of three snap bean cultivars compared with 'Hawaiian Wonder'.

Paired cultivars	Percent leaf area consumed (cm ²) x ± SE	Paired t value	Carbohydrate content x ± SE	
			(mg sucrose eq/ g leaf fresh wt)	Paired t value
Blue Lake Bush 274	10.33 ± 1.72	2.12*	28.0 ± 0.98	6.51**
Hawaiian Wonder	5.94 ± 0.97		14.2 ± 1.65	
Kentucky Wonder	15.14 ± 1.56	1.96*	21.0 ± 2.39	2.20*
Hawaiian Wonder	10.35 ± 1.68		14.2 ± 1.65	
Green Crop	7.80 ± 1.46	0.65 ^{NS}	13.0 ± 2.39	-0.767 ^{NS}
Hawaiian Wonder	9.34 ± 1.49		14.2 ± 1.65	

^{NS}, *, **Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively. For all cultivars, $n = 30$ for leaf area consumed and $n = 10$ for carbohydrate content.

higher percent leaf area consumed on snap bean leaf explants treated with exogenous carbohydrates.

Determining plant constituents that cause insects such as CRBs to select and feed on a particular plant can facilitate pest management. Cultural practices, breeding plant selections that can lower the endogenous carbohydrate content in their leaves during early evening hours when CRBs feed, or both may be nonchemical alternatives for controlling

the detrimental feeding effects of this widespread tropical insect pest of leaves.

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