HORTSCIENCE 33(5):862–865. 1998.

Role of Foliar Phenolics in Host Plant Resistance of *Malus* Taxa to Adult Japanese Beetles

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Additional index words. Popillia japonica, Rosaceae, apple, crabapple, pest resistance, antixenosis, antifeedant, secondary metabolites

Abstract. **Japanese beetles (JB), *Popillia japonica* Newman, are destructive, highly polyphagous herbivores that show a general preference for Rosaceous plants. Choice and no-choice feeding assays were conducted to determine the level of resistance among 10 taxa of *Malus* spp. Mill. Under no-choice conditions, *M. baccata* (L.) Borkh. 'Jackii', *M.* x 'Hargozam' Harvest Gold and *M. transitoria* (Balatin) Schneider 'Schmitcutleaf' Golden Raindrops were highly resistant, with <2 cm² leaf area consumed in 24 hours. *M.* x 'Radiant' was highly susceptible, with 7.6 cm² consumed, and the remaining six cultivars were intermediate. Under choice conditions, eight taxa were resistant with <10% defoliation, *M.* x 'Red Splendor' was intermediate with 26%, and *M.* x 'Radiant' was susceptible with 73% defoliation. Feeding responses to eight individual phenolics were tested in artificial diets over a range from 0 to 100 mM. Phloridzin, phloretin, naringenin, and catechin were all feeding deterrents, whereas quercetin and rutin were feeding stimulants. Chlorogenic acid stimulated feeding at low concentrations and deterred feeding at higher concentrations (i.e., a peak response). Kaempferol had no effect. Analysis of endogenous foliar phenolics showed considerable variation in concentrations among taxa. Stepwise multiple regression analysis identified phloridzin as the only endogenous phenolic that was significantly related to resistance under both choice and no-choice feeding conditions.**

Japanese beetles (JB) are highly polyphagous insects, feeding on nearly 300 plant species including a wide diversity of landscape plants (Fleming, 1972; Johnson and Lyon, 1991). Rosaceous plants can be particularly susceptible to foliar feeding by adult JB (Fleming, 1972). However, significant variation in resistance can be observed among closely related taxa. For example, Ranney and Walgenbach (1992) found that defoliation by adult JB varied from 0% to 83% among 33 taxa of crabapples (*Malus* spp.). Spicer et al. (1995) found similar and consistent variation in feeding damage among many of the same cultivars.

Host plant resistance is attributed to many factors, including the presence and concentration of allelochemicals. Japanese beetles are attracted to certain plant volatiles (kairomones), some of which are now commonly used as JB lures (Ahmad, 1982; Ladd and McGovern, 1980; Loughrin et al., 1996a). Recent work has demonstrated that feeding-induced odors may also facilitate host plant location by JB (Loughrin et al., 1995). However, in a study comparing volatile compounds from crabapple cultivars differing in susceptibility to JB, Loughrin et al. (1996b) found that attractiveness of cultivars, as determined in pitfall bioassays, was not related to plant susceptibility in the field. They further proposed that beetles appear to be attracted to certain plant volatiles in search of potential hosts, but that nonvolatile factors ultimately determine host plant suitability.

Some plants contain strong antifeedants (allomones) that can be important in host plant resistance to JB. Neriifolin, a cardiotonic glycoside found in yellow oleander [*Thevetia thevetioides* (HBK) K. Schum.], was identified as an effective antifeedant for JB (Reed et al., 1982). Patton et al. (1997) found that prunasin, herniarin, and coumarin were potent antifeedants for JB and important factors in host plant resistance of *Prunus* L. taxa to JB.

Plants in the genus *Malus* have a diverse complement of phenolic compounds (Williams, 1960). Levels of total and individual phenolics, including chlorogenic acid, rutin, quercitrin, phloridzin, and phloretin, can vary considerably in the foliage of different *Malus* taxa (Garcia et al., 1995). Phloridzin, in particular, is a principal phenolic constituent in the foliage of *Malus* taxa and occurs in concentrations as high as 6.75% of leaf dry mass (Hunter et al., 1994). Phenolics can be important defense chemicals (Bernays and Chapman, 1994); however, Fulcher et al. (1996) did not find a significant relationship between total phenolic content in foliage of crabapple cultivars and resistance to JB feeding. Measurement of total phenolic content may overlook differential effects of specific phenolic compounds on insect feeding. Individual phenolic compounds varied considerably in their antifeedant properties for apple maggot (*Rhagoletis pomonella* Walsh.) when tested in artificial diets (Pree, 1977); suggesting that quantifying individual phenolic compounds may be required in order to elucidate their potential role in host plant resistance.

Our objectives were to determine natural resistance to Japanese beetle among selected *Malus* taxa and to evaluate the role of specific phenolic compounds in host plant resistance.

Materials and Methods

No-choice feeding assay. Japanese beetle feeding assays were performed on 10 *Malus* taxa representing a range of natural resistance based on prior observations. These taxa were *M. baccata* 'Jackii', *M.* x 'Baskatong', *M.* x 'Branzam' (Brandywine), *M. floribunda* Siebold, *M. transitoria* 'Schmitcutleaf' (Golden Raindrops), *M.* x 'Hargozam' (Harvest Gold), *M.* x 'Narragensett', *M.* x 'Radiant', *M.* x 'Red Splendor', and *M.* x 'Robinson'. Trees were grown in a randomized complete block design, n = 3, at the Mountain Horticultural Crops Research Station, Fletcher, N.C., and were planted between Mar. 1990 and Mar. 1991, at a spacing of 6.1 × 4.6 m. Fescue (*Festuca* spp. L.) was planted between rows, leaving strips of bare soil 2.4 m wide within the rows.

Feeding assays were conducted in July 1995, during the peak flight period of the adult JB. Adults were collected from smartweed (*Polygonum* sp. L.) and held in clear plastic containers with moist paper towels and ventilation holes. Beetles were placed in a growth chamber (I-35LL, Percival, Boone, Iowa) with constant light from fluorescent lamps (photosynthetically active radiation 75–80 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 25 °C and starved for 24 h before commencing the feeding trials. The following morning, three branches of a given replicate of each taxa were collected and kept with the cut ends in

Received for publication 11 Oct. 1997. Accepted for publication 22 Jan. 1998. The authors thank Everett Whitman II, Arthur Butler, and Eleanor Maness for technical assistance. This research was funded in part by the North Carolina Agricultural Research Service, the Horticultural Research Institute, and the International Society of Arboriculture Research Trust. Use of trade names does not imply endorsement of the products mentioned nor criticisms of similar ones not named. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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water. The leaves for the feeding study were removed, measured for leaf area (FIN DIAS System #2; Decagon Devices, Pullman, Wash.), and placed in a petri dish with the leaf petiole in a water-filled vial. One female beetle was added to each petri dish, which was then set randomly in the growth chamber. Vials were checked periodically and water was replaced as needed. After 24 h, the beetles were removed and leaf area was re-measured. Leaves for chemical analysis were removed, frozen at –80 °C (Revco ULT; Rheem Mfg. Co, Asheville, N.C.), freeze dried (255RC; Virtis Co., Gardiner, N.Y.), and stored at –80 °C until needed.

Choice feeding assay. The choice feeding study was conducted on field-grown trees (as described previously) and utilized a natural beetle population. Defoliation was rated by two independent observers on 14 Aug. 1995, following the peak flight period of adult JB. Observers estimated percentage defoliation using an 11-point, arcsin pretransformed scale (Little, 1985), and data were averaged among observers.

Artificial diet assays. Beetles were collected and maintained as described previously. Artificial diets were prepared, following methods of Hsiao and Fraenkel (1968), containing sucrose, cellulose, agar, and one of eight test compounds: phloridzin, phloretin, naringenin, kaempferol, rutin, quercetin, catechin, and chlorogenic acid (Sigma Co., St. Louis) at 0, 1.0, 3.2, 10.0, 31.6, and 100.0 mM. Given its high cost, kaempferol was not tested at 100 mM. Ten replications of each compound were used at each concentration. One female beetle was placed in each petri dish with a plug of diet and set randomly in the growth chamber. After 24 h, beetles were removed, feces were collected, dried for 24 h at 70 °C (Isotemp Oven 665F; Fischer Scientific, Pittsburgh), and weighed.

Foliar analysis. Endogenous levels of individual phenolics were analyzed by a reversed-phase HPLC method adapted from Hunter et al. (1994). Freeze-dried tissue (300 mg) was extracted in 3 mL of 85% methanol, homogenized, and centrifuged for 10 min. The supernatant was decanted and collected. The homogenizer was rinsed with an additional 3 mL of 15% methanol, the pellet was re-suspended with the rinsate and re-centrifuged. The supernatant was decanted and mixed with the first supernatant. Samples were diluted 10-fold before injection. Standard curves were prepared of phloridzin, phloretin, kaempferol, naringenin, chlorogenic acid, and catechin from standards (Sigma). An isocratic solvent system of 25% methanol plus 0.1% phosphoric acid : 75% water was used. The column was an Adsorbosphere C18, 250 × 4.6 mm (Alltech Associates, Deerfield, Ill.). Compounds were detected at 254 nm with a UV detector (Millipore Corp., Bedford, Mass.). Concentration on a fresh mass basis was calculated from fresh mass : dry mass ratios of each sample.

Data for feeding intensity and concentration of phenolics were subjected to analysis of variance with least significant differences tested at $P \leq 0.05$. Dose : response curves for individual compounds were evaluated using linear and nonlinear regression analyses (TableCurve 2D; Jandel Scientific, San Rafael, Calif.). Regression models were ranked and selected based on the significance of the F-statistic and the degree-of-freedom adjusted R^2 statistic, provided that the model and all individual terms of the model were significant ($P \leq 0.05$). The effective doses required to reduce feeding by 25% (ED_{25}) and 50% (ED_{50}) were estimated based on regression equations. The influence of endogenous phenolic constituents on host plant resistance was evaluated using stepwise multiple regression analysis.

Results and Discussion

No-choice feeding assay. Leaf area consumption ranged from 1.0 to 7.6 $cm^2 \cdot d^{-1}$ (Table 1). Even under these conditions of intense feeding pressure, *M. baccata* 'Jackii', *M.* x 'Hargozam' Harvest Gold, and *M. transitoria* 'Schmitcutleaf' Golden Raindrops were highly resistant, with less than 2 $cm^2 \cdot d^{-1}$ consumed. Six other taxa were intermediate and *M.* x 'Radiant' was most susceptible with 7.6 $cm^2 \cdot d^{-1}$ consumed.

Choice feeding assay. Feeding intensity on crabapples varied from averages of 0% to 73% defoliation (Table 1). Eight taxa were resis-

Table 1. Resistance to Japanese beetle as measured by leaf area consumption and field defoliation among ten crabapple taxa (*Malus* spp.).

Taxon	No-choice test: Leaf area consumed ($cm^2 \cdot d^{-1}$)	Choice test: Defoliation (%)
M. x 'Schmitcutleaf' Golden Raindrops	0.99	1
M. baccata 'Jackii'	1.07	0
M. x 'Hargozam' Harvest Gold	1.83	1
M. x 'Branzam' Brandywine	3.29	1
M. floribunda	3.61	0
M. x 'Naragansett'	3.63	3
M. x 'Robinson'	4.19	2
M. x 'Red Splendor'	4.84	26
M. x 'Baskatong'	5.05	9
M. x 'Radiant'	7.62	73
$LSD_{0.05}$	2.01	10

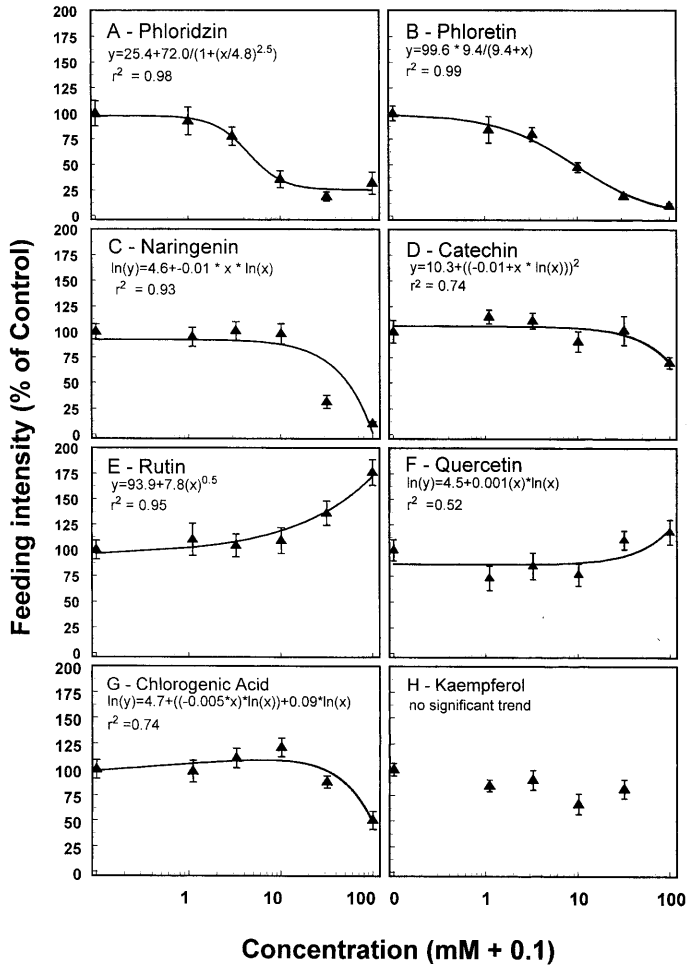

Fig. 1. (**A–H**) Feeding intensity (fecal dry mass) of adult Japanese beetles in response to different concentrations of individual phenolics tested in artificial diets. Symbols represent means, n = 10 ± 1 SEM.

tant, with <10% defoliation. *M.* x 'Red Splendor' was intermediate with 26% defoliation, and *M.* x 'Radiant' was highly susceptible with 73% defoliation. Although feeding intensity was higher in prior years, the field evaluation data were generally consistent with those of Spicer et al. (1995) and Ranney and Walgenbach (1992). Results from no-choice and choice assays provided similar rankings for resistance with the exception of *M.* x 'Baskatong', which was more susceptible to feeding under no-choice than under choice conditions.

Artificial diet assays. Phloridzin, and its aglycone phloretin, were highly effective at deterring JB feeding. The ED_{25} for phloridzin was 3.2 mM and the ED_{50} was 7.1 mM (Fig. 1A). Phloretin had an ED_{25} of 2.9 and an ED_{50} of 9.3 (Fig. 1B). Naringenin also reduced feeding, with an ED_{25} of 17.2 and an ED_{50} of 32.9 (Fig. 1C). Catechin only deterred feeding at high concentrations (Fig. 1D). Conversely, rutin was a phagostimulant, increasing feeding to 174% of the control at the 100 mM concentration (Fig. 1E). Quercetin also stimulated feeding at higher concentrations (Fig. 1F). Chlorogenic acid was mildly stimulating at intermediate concentrations, but deterred feeding at higher concentrations (Fig. 1G). Kaempferol had no effect on feeding (Fig. 1H).

Foliar analysis. The concentrations of endogenous phenolics differed widely among taxa. In general, concentrations of phloridzin, phloretin, and catechin were highest but varied significantly among taxa (Table 2). Variations in individual phenolic constituents among these plants may explain why Fulcher et al. (1996) did not find a significant relationship between total phenolic levels and feeding intensity. This is particularly important when considering the range of responses from the artificial diets. Using stepwise multiple regression analysis, phloridzin was the only endogenous phenolic significantly related to percent defoliation from choice feeding assays and to leaf area consumption from no-choice assays ($P \leq 0.05$). Further regression analysis showed that phloridzin was a more effective deterrent under choice conditions than under no-choice conditions (Fig. 2 A and B). Endogenous levels of feeding stimulants were not measured in this study. Outliers such as *M.* x 'Robinson', which had high levels of phloridzin, yet only had intermediate resistance under no-choice tests (Tables 1 and 2; Fig. 2B), may contain higher levels of feeding stimulants that counterbalanced existing feeding deterrents.

Research by Hunter et al. (1994) did not implicate phloridzin as a feeding deterrent for the tufted apple bud moth (*Playnota idaeusalis* Walker). Instead, they found that diets containing phloridzin decreased larval development time and increased larval mass, suggesting the diets were actually beneficial to the insect. This may occur because the tufted apple bud moth is a pest well adapted to the genus *Malus*, whereas JB is a generalist herbivore and may not have adapted to specific defense chemicals such as phloridzin.

Using plants with natural resistance to insect pests is an important component of integrated pest management that can contribute to development of more sustainable landscapes. This research identified a number of *Malus* taxa with natural resistance to JB and further indicated that phloridzin is an important chemical factor in host plant resistance of *Malus* to JB. In addition to identifying resistant germplasm, information on the chemical nature of pest resistance may aid in further selection and improvement of plants for greater pest resistance.

Table 2. Concentration of individual foliar phenolics (fresh mass basis) among 10 crabapple taxa (*Malus* spp.).

	Phenolic concn (mmol·kg^{-1})				
Taxon	Phloridzin	Phloretin	Naringenin	Chlorogenic acid	Catechin
M. x 'Schmitcutleaf' Golden Raindrops	154	27	11.5	0.41	66
M. baccata 'Jackii'	144	15	5.7	0.36	62
M. x 'Hargozam' Harvest Gold	143	26	6.9	1.02	146
M. x 'Branzam' Brandywine	177	10	0.5	0.46	35
M. floribunda	146	20	5.0	0.46	75
M. x 'Naragansett'	80	6	4.0	0.30	55
M. x 'Robinson'	170	32	11.2	0.65	67
M. x 'Red Splendor'	34	21	5.5	0.31	48
M. x 'Baskatong'	96	9	4.3	0.39	59
M. x 'Radiant'	34	21	3.4	0.31	50
$LSD_{0.05}$	36	9	4.3	0.21	38

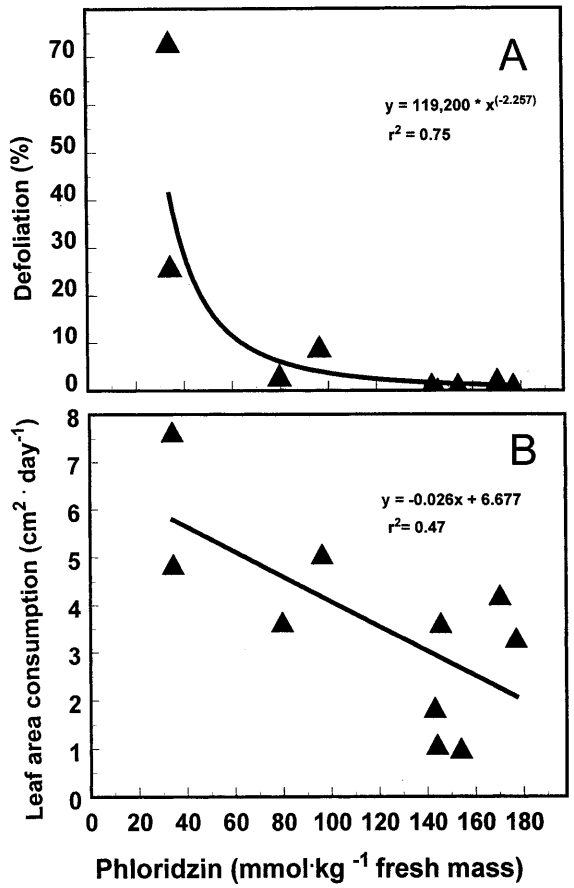

Fig. 2. Relationship between endogenous phloridzin concentration and (**A**) percent defoliation from a choice feeding assay and (**B**) leaf area consumption from a no-choice feeding assay. Symbols represent means, n = 3 (each with three subsamples).

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