Natural Resistance to Japanese Beetle Among *Malus* Taxa: Role of Endogenous Foliar Phenolics

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Nature of Work: Japanese beetles (JB), *Popillia japonica* Newman, are destructive, highly polyphagus herbivores that show a general preference for plants in the Rosaceae family. Although *Malus* taxa are often found to be susceptible to JB there are substantial differences in resistance among *Malus* species and cultivars. (1). The objectives of this study were to compare natural resistance to JB among *Malus* taxa and to evaluate the role of phenolics in host plant resistance. Choice and no-choice feeding assays were performed on 10 taxa grown in a randomized complete block design at the Mountain Horticultural Crops Research Station in Fletcher, NC.

For no-choice feeding trials the beetles were placed in a growth chamber with constant light (PAR 75-80 umol$\cdot m^{-2} \cdot s^{-1}$) at 25°C (77°F) and starved for 24 hr. The following morning 3 branches of a given replicate, of each taxa, were collected and kept with the cut stem in water. Leaves for chemical analysis were frozen at -80°C (-112°F), freeze dried, and then stored at -80°C (-112°F) until needed. The leaves for the feeding study were removed, leaf area was measured (FIN DIAS System #2, Decagon Devices, Inc.), and leaves were set into a petri dish with the petiole in a water filled vial. One female beetle was placed in each petri dish and each dish was set randomly in the growth chamber. After 24 hr the beetles were removed, leaf area was remeasured, and fecal matter was collected and dried for 24 hr at 70°C (158°F). Three separate assays (subsamples) were conducted on each of three replicates (trees) for each taxa.

The choice feeding study was conducted on field grown trees and utilized the natural beetle population. Defoliation ratings were done by two independent observers on August 14, 1995. Observers estimated percentage defoliation based on an 11 point, pretransformed rating scale and data were averaged among observers.

Total phenolics determinations were made using the Folin-Ciocalteu method, adapted from Julkunen-Tiitto (3). Two hundred mg (0.01 oz) of freeze dried leaf tissue was dissolved in 10 ml (0.02 pt) acetone in a centrifuge tube. While extracting, the tissue was shaken in an ice bath for 30 min, then centrifuged for 20 min. A 25ul (0.000053 pt) aliquot was taken and diluted with water to 2 ml (0.004 pt) in a 10 ml (0.02 pt) volumetric flask. One ml (0.002 pt) of Folin - Ciocalteu phenol reagent was added to each flask and shaken. Five ml (0.01 pt) of 20% sodium carbonate was added and the mixture was raised to 10 ml (0.02 pt) with water. The flasks were shaken thoroughly. After 60 min the absorptivity of the mixture was read at 765 nm (Lambda 6 UV/VIS spectrophotometer, Perkin Elmer). The spectrophotometer was zeroed against air. A standard curve was prepared using concentrations of gallic acid ranging from 0 to 2000 ug/L.

Results and Discussion: Leaf area consumption under no-choice conditions ranged from 1.0 cm^2 to 7.6 cm^2 (Table 1). Under this intense feeding pressure *M.* 'Golden Raindrops', *M. baccata* 'Jackii', and *M.* Harvest Gold™ were highly resistant with less than 2 cm^2 leaf area consumption. Six other taxa were intermediate and *M.* 'Radiant' was statistically the most susceptible with 7.6 cm^2 leaf area consumption. Mean fecal weight provided an additional measure of feeding intensity and ranged from 4.70 to 17.4 mg (Table 1). Beetles feeding on *M. baccata* 'Jackii' produced the lowest with their mean fecal weight not being statistically different from zero.

Feeding intensity among *Malus* taxa in the choice test varied from 0 to 73 percent defoliation (Table1). Eight *Malus* taxa had an average feeding defoliation of 10% or less. *Malus* 'Red Splendor' was intermediate with 26% defoliation and *M*. 'Radiant' was the most susceptible with defoliation of 73%. In general results from no-choice and choice assays provided similar rankings for susceptibility with the exception of 'Baskatong' which was relatively more susceptible to feeding under no-choice conditions.

Total phenolic levels in these plants ranged from 7.4 to 17.3% dry weight of leaf tissue and was not correlated with resistance (Table1). Although total phenolic content can sometimes influence insect feeding, the presence and concentration of specific phenolic constituents can be more important than total phenolic content. *Malus* taxa contain several phenolics such as phloridzin, phloretin, quercitin, kaempferol, and catechin (4,5). Research has shown chemical constituents such as phloridzin, and its hydrolysis product phloretin, are highly effective at deterring JB feeding when present in artificial diets (2). Conversely, quercitin was found to be a phagostimulant (2). Variations in phenolic constituents among these plants would explain what otherwise seem to be inconsistencies in total phenolic levels and feeding relationships. Due to the high percentage of total phenolics in leaf tissue and their individual documented relationship with resistance there is a need for additional studies on the isolation and identification of the exact compound or compounds responsible for resistance and their mode of action.

Significance to Industry: Japanese beetles are one of the most damaging insect pests on rosaceous trees. This research documented a broad range of natural resistance to feeding by adult Japanese beetles among taxa of M*alus.* Greater use of pest resistant plants will reduce the need for chemical controls, reduce production and maintenance costs, and aid in the development of more sustainable landscapes. In addition, identification of resistant genotypes provides the basic information needed for breeding new plants that are deliberately designed to have greater pest resistance.

Literature Cited

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Table 1. Resistance to Japanese beetle as measured by leaf area consumption, fecal weight, field defoliation, and total phenolics among crabapples (*Malus* spp.).

Taxon	Cultivar/ Tradename	No choice test: Leaf area consumed (cm^2)	No choice test: Fecal wt (mg)	Choice test: % Field defol.	Total phenolics (%)
'Golden Raindrops'	'Golden Raindrops'	0.99	8.5	1	16.6
M. baccata 'Jackii'	M. baccata 'Jackii'	1.07	4.7	0	8.6
'Hargozam'	Harvest Gold™	1.83	10.7	1	14.8
'Branzam'	Brandywine™	3.29	10.6	1	11.4
M. floribunda	M. floribunda	3.61	15.9	0	16.4
'Naragansett'	'Naragansett'	3.63	11.7	3	15.1
'Robinson'	'Robinson'	4.19	15.0	2	17.3
'Red Splendor'	'Red Splendor'	4.84	16.6	26	7.4
'Baskatong'	'Baskatong'	5.05	17.4	9	7.6
'Radiant'	'Radiant'	7.62	14.7	73	8.7
$LSD_{0.05}$		2.01	4.8	10	2.2