Levels of Endogenous Phenolics in *Malus* Taxa and Their Possible Role in Resistance to Fire Blight

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Nature of Work: Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow, *et al*., is one of the most important diseases of rosaceous plants, particularly those members in the subfamily Maloideae. This disease can be especially problematic in both the eastern and mid-western regions of the United States where environmental conditions are favorable for the pathogen. Although many taxa are susceptible to fire blight, flowering crabapples (*Malus* Mill. spp.) vary considerably in resistance to this disease thus providing opportunities to study host plant resistance (1, 5, 11).

In many instances, host plant resistance can be attributed to the action of secondary plant metabolites. The role of specific secondary compounds in resistance to fire blight among taxa of pears (*Pyrus* L. spp.) has been investigated (7, 8). Among selected taxa, fire blight susceptible cultivars of pears contained lesser amounts of arbutin, a phenolic glycoside, compared to moderately resistant and resistant cultivars (7). Correlations between resistance to fire blight and the distribution of specific compounds or high levels of total phenolic compounds have been reported (7, 8). Others, however, have suggested that there is no direct connection between phenolic constituents of *Pyrus* and disease resistance (3).

In *Malus* spp., a relationship between total phenolic levels and resistance to fire blight has been reported (10). This preliminary study of the systemic movement of *E. amylovora* in apple suggested that accumulating phenolic acids and simple phenols were inhibiting the migration of the bacteria in host tissue (10). Furthermore, levels of phenolic acids were up to ten times higher in resistant taxa than in those samples that exhibited pronounced symptoms.

Although a correlation between total phenolic concentration and resistance to fire blight may exist, total phenolic levels may not be as important as having high levels of one specific compound. Phloridzin, a major phenolic constituent in *Malus* spp., and its breakdown products (e.g. phloretin, phloroglucinol, and phloretic acid) exhibit significant antibacterial properties *in vitro* towards *E. amylovora* (2, 9). Other phenolic constituents in *Malus* spp. also have been reported as potent inhibitors of *E. amylovora in vitro* (2). The objective of this study was to identify and measure levels of specific endogenous phenolic compounds among ten taxa of flowering crabapples that represent a wide range of resistance to fire blight.

Ten *Malus* taxa, in a field plot arranged as a randomized complete block experimental design with three replications, were screened for fire blight resistance at the Mountain Horticultural Crops Research Station, Fletcher, NC in May 2001. Disease screening was conducted as previously reported (1) . *E. amylovora* strain 2002A at a concentration of approximately 1 x 10^8 cfu/mL was used as the inoculum. For chemical analysis, four healthy actively growing shoots (subsamples) were collected from each plant (shoots ~25 cm long). Tissue storage and preparation followed a published protocol (6). Chemical analysis was conducted in Fall 2002. Sample extraction and HPLC methodology was adapted from a published protocol (4).

Results and Discussion: There was a wide range in resistance and levels of individual phenolic constituents among the taxa included in this study (Tables 1 and 2). *M.* 'Adams' was most resistant with a lesion length of 8% while *M. tschonoskii* was the most suspectible with 100% lesion length (Tables 1 and 2). In general, phloroglucinol and phloridzin were found in high concentrations in both stem and leaf tissue. Chlorogenic acid was present in low concentrations in stem tissue; however, it could not be definitely identified in leaf tissue. Chlorogenic acid is most likely present in *Malus* leaf tissue as previously reported (4). The identification of a few phenolic constituents was not possible. An unidentified constituent, component X, was present in both leaf and stem tissue. Unidentified components L and S were present in leaf and stem tissue, respectively. Preliminary analysis of these samples using [spell out LC] LC-Mass Spectrometry suggested that component X is possibly an isomer of phloridzin. Identification of components L and S was inconclusive.

Regression analysis identified component X in stem tissue as being correlated to disease resistance: Lesion Length (%) = 96.831 - 0.896X, $r^2 = 0.7126$. In leaf tissue, component X and component L were both correlated with disease resistance and there was a significant interaction: Lesion Length (%) = 156.75 - 0.97X - 1.398L + 0.012XL, $r^2 = 0.6122$. Although, phloridzin and phloroglucinol were present in high concentrations, and have exhibited antibacterial activity towards *E. amylovora in vitro*, no correlation to resistance was found.

Significance to the Industry: This research determined a correlation between two unidentified phenolic compounds in flowering crabapples and resistance to fire blight. These results provide justification for further study of phenolic constituents as potential defense compounds in fire blight resistance. Flowering crabapples are one of the most economically important flowering ornamental trees produced in the United States. Fire blight continues to be a major problem in nursery and landscape plantings. A greater understanding of host plant resistance can provide significant opportunities for the development of enhanced disease resistant taxa of flowering crabapples.

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Table1. Lesion length on shoots of crabapple cultivars (*Malus* spp.) inoculated with *Erwinia amylovora* and levels of endogenous phenolic compounds in leaf tissues from inoculated shoots.

	Phenolic Constituents (mmol/kg Dry Wt)					**Lesion Length (%)**
Taxa	Phloroglucinol	Phloridzin	Rutin	Component X	Component L	
M. 'Adams'	193.65	293.41	10.01	139.88	55.78	7.99[Z]
M. 'Centurion'	217.19	275.56	10.06	103.71	106.59	14.79
M. 'David'	150.14	343.22	7.56	98.78	92.79	24.61
M. floribunda	155.04	365.59	7.34	85.01	108.11	27.16
M. 'Canary'	248.16	232.50	12.93	173.45	67.64	39.06
M. 'Red Splendor'	275.58	169.68	11.54	78.85	88.74	45.00
M. 'Harvest Gold'	166.84	291.11	8.31	69.52	62.69	45.11
M. 'Mary Potter'	214.50	333.03	5.48	61.22	63.68	77.02
M. 'Candy Mint'	250.09	442.24	8.24	16.52	44.99	78.65
M. tschonoskii	245.57	193.37	7.04	42.42	37.06	100.00
$LSD_{0.05}$	190.77	72.99	4.33	31.56	27.71	28.37

[Z]% of total shoot length diseased.

Table 2. Endogenous levels of individual phenolic compounds in stem tissue among ten crabapples (*Malus* spp.)

	Phenolic Constituents (mmol/kg Dry Wt)						Lesion Length (%)
Taxa	Phloroglucinol	Chlorogenic Acid	Phloridzin	Rutin	Component X	Component S	
M. 'Adams'	188.96	5.09	172.00	3.39	89.50	49.80	7.99[z]
M. 'Centurion'	193.33	3.64	168.97	1.92	45.84	37.15	14.79
M. 'David'	140.61	1.57	218.25	1.04	59.44	38.93	24.61
M. floribunda	124.68	NA	281.63	1.68	58.81	29.07	27.16
M. 'Canary'	213.67	2.59	138.31	2.28	74.44	28.38	39.06
M. 'Red Splendor'	134.33	6.85	140.06	3.33	62.12	47.32	45.00
M. 'Harvest Gold'	154.26	NA	197.99	0.56	37.43	13.21	45.11
M. 'Mary Potter'	159.48	3.10	199.03	2.43	25.38	41.18	77.02
M. 'Candy Mint'	208.98	5.82	250.54	5.06	9.14	31.92	78.65
M. tschonoskii	167.34	9.58	202.84	1.77	6.76	52.33	100.00
$LSD_{0.05}$	71.68	2.68	66.28	1.85	16.56	21.35	28.37

[z]% total shoot length diseased

NA = Data not available