Role of Endogenous Phenolics in Resistance to Fire Blight Among Flowering Crabapples (*Malus* spp.)

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Nature of Work: Fire blight, caused by the bacterium *Erwinia amylovora*, is one of the most significant diseases of taxa in the Rosaceae subfamily Maloideae. This subfamily includes several genera that exhibit considerable genetic diversity and interspecific crossability. Plant breeders have exploited these opportunities to produce numerous cultivars with enhanced ornamental characteristics that have become economically important to the horticultural industry. Crabapples (*Malus* spp.) are among the most widely planted flowering trees in landscapes. However, the incidence of disease, particularly fire blight, remains a problem. Significant variation in resistance to fire blight has been reported for many taxa of flowering crabapples (1,2,3,9). A better understanding of host plant resistance to this disease could allow for the systematic selection and development of resistant plants through a targeted improvement program.

Host plant defense mechanisms often involve secondary compounds that are pre-formed or that are synthesized once the infection process begins. Several studies have shown that some pre-existing phenolic compounds and their metabolic products can play a significant role in resistance to fungal and bacterial pathogens (5). Inhibition of *Erwinia amylovora* by phenolic compounds *in vitro* and in cortex tissue of *Malus* taxa has been documented (6,7). Stem tissue of fire blight resistant taxa contained levels of phenolic acids that were up to ten times higher than in those taxa that exhibit pronounced symptoms(7).

Levels of phenolic compounds have been measured in numerous taxa of *Malus* and a correlation between high levels and resistance to fire blight has been reported several times (7,8). However, conflicting information exists as to whether high levels of phenolic compounds are related to fire blight resistance (4). Total phenolic levels may not be as important as presence or concentration of specific compounds. The objective of this study was to test specific phenolic compounds found in *Malus* spp. and to evaluate their effects on growth of *E. amylovora*.

Erwinia amylovora strain 2002A was grown on nutrient agar for 18 hours at 26°C. Under asceptic conditions, 50 µl of a suspension (~ 10^8 cfu/mL) was added to a 1.5 mL cuvette containing 1 mL of a nutrient broth amended with 0, 1, 2.5, 5, 10, or 20 mM of a test compound. Cuvettes were arranged in a completely randomized design with six replications, cuvettes without *E. amylovora* served as controls. All cuvettes were capped and place on a shaker and maintained at 26°C. Absorbance measurements were taken initially and again at 8 hours. The phenolic compounds tested were catechin, chlorogenic acid, coumaric acid, phloretic acid, and phloroglucinol (Sigma-Aldrich, Co.).

Results and Discussion: Coumaric acid was the most effective compound included in this study (Fig. 1). The 1 mM concentration reduced *E. amylovora* growth by 67% compared to the 0 mM control. The 2.5 and 5 mM concentrations were not significantly different from the 1 mM concentration. Bacterium growth was reduced by 98% with the 10 and 20 mM concentrations. All concentrations were significantly different from the control. Chlorogenic acid was the least effective compound tested at reducing the growth of *E. amylovora*. Although all concentrations differed significantly from the control, no concentration inhibited growth by more than 50%. The 20 mM concentration reduced growth by 38%.

Strain 2002A of *E. amylovora* behaved similarly to phloretic acid and phloroglucinol where greater than 50% reduction in growth was achieved only at 10 and 20 mM concentrations. Growth was inhibited by 99 and 95% at 20 mM for phloretic acid and phloroglucinol, respectively. There was no significant reduction in growth for 1, 2.5, 5, and 10 mM of catechin, however bacterial growth was greatly reduced at the 20 mM concentration.

These results demonstrated that a number of phenolic compounds found in *Malus* spp. are potent inhibitors of *E. amylovora in vitro*. Furthermore, phenolic compounds varied considerably in efficacy as a function of concentration.

Significance to the Industry: This research investigated individual phenolic compounds of *Malus* taxa and their effects on the growth of *E. amylovora*, the causal agent of fire blight. Fire blight still remains a significant problem in cultivating flowering crabapples. Understanding host plant resistance allows for more focused breeding and selection efforts to develop resistant plants. Fire blight resistant plants can make a significant contribution to the industry by lowering production and maintenance costs and by allowing growers to offer superior plants.

Literature Cited:

1. Aldwinckle, H. S. and J. L. Preczewski. 1979. Reaction of terminal shoots of apple cultivars to invasion by *Erwinia amylovora. Phytopathology* 66:1439-1444.

2. Bell, A. C., et al. 2000. Controlled Screening for fire blight resistance among flowering crabapples (*Malus* spp.). Proc. SNA Research Conference 45:244-248.

3. Bonn, W. G. and D. C. Elfving. 1990 Evaluation of crabapple cultivars and selections for resistance to fire blight. *Acta Horticulturae* 273:311-315.

4. Challice, J. S. and M. N. Westwood. 1972. Phenolic compounds of the genus *Pyrus*. *Phytochemistry* 11:37-44.

5. Ebel, J. 1986. Phytoalexin synthesis: the biochemical analysis of the induction process. *Annual Review of Phytopathology* 24:235-264.

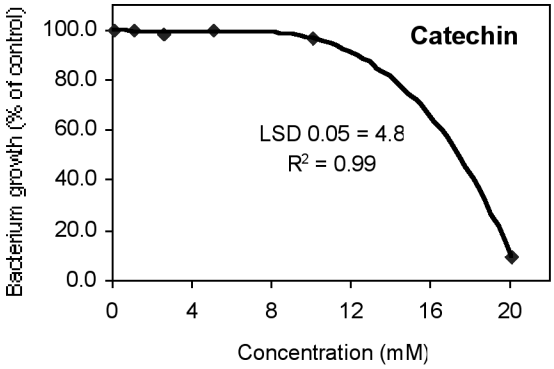
6. Plurad, S. B. 1967. Apple aphids and the etiology of fire blight disease. University of Missouri Ph. D. Dissertation.

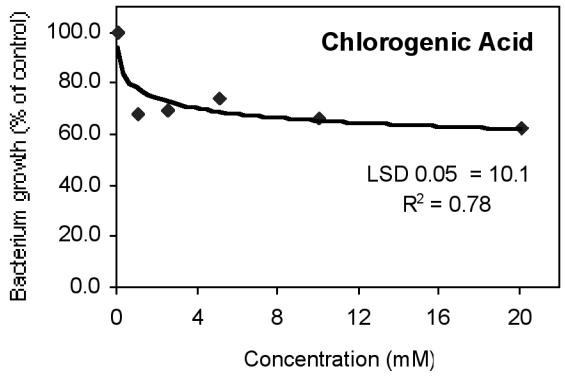
7. Roemmelt, S. et al. 1999. Defense reaction of apple against fire blight: histological and biochemical studies. *Acta Horticulturae* 489:335-336.

8. Williams, A. H. 1960. The distribution of phenolic compounds in apple and pear trees. P. 3-7. *In*: J.

9. Pridham (ed.) Phenolics of plants in health and disease. Pergamon Press, New York, NY.

10. Windham, M. T., et al. 1997. Fire blight and frog eye leaf spot resistance in crabapple cultivars. *Proc. SNA Research Conference* 42:232-233.





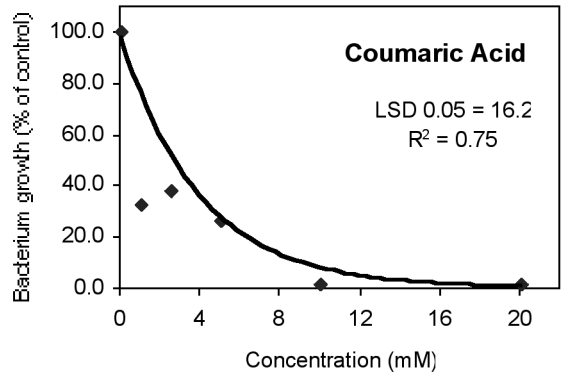
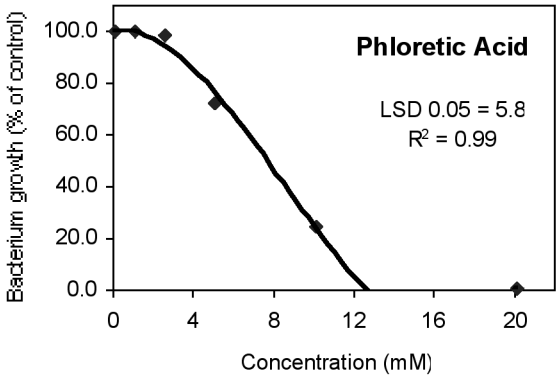
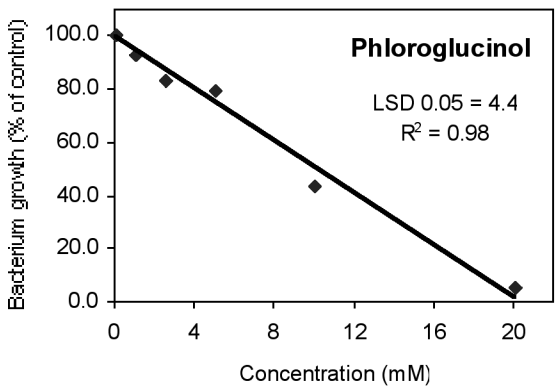


Figure 1: Dose response curves for phenolic compounds found in *Malus* spp. on the growth of *Erwinia amylovora* strain 2002A.

% of control- Relative bacterium growth compared to the control with no compound added.

Figure 1: *(continued)*





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