MANAGING WOUND-ASSOCIATED DISEASES BY UNDERSTANDING WOUND HEALING IN THE BARK OF WOODY PLANTS

by Alan R. Biggs[1]

Abstract. New research findings have changed the way plant pathologists look at wound healing in the bark of woody plants. Research has shown that suberin in wounds has an important role in disease resistance. Its production is under genetic control and can be used to predict the susceptibility of breeding material to canker-causing microbes. In addition, studies on the influence of temperature and soil moisture have identified how these environmental factors affect the generation of new, suberized bark tissues. Management of wound-associated plant diseases through the improved understanding of wound responses may soon be a reality.

Résumé. De novelles découvertes de recherches ont changé la façon qu'avait les pathologistes de considérer la cicatrisation d'une blessure à l'écorce d'un végétal ligneux. La recherche a démontré que la subérine dans les blessures a un rôle important dans la résistance aux maladies. Sa production est contrôlée génétiquement et peut être utilisée pour prédire la susceptibilité du matériel de reproduction aux microbes causals du chancre. En plus, les études sur l'influence de la température et de l'humidité du sol ont identifié comment ces facteurs environnementaux affectent la formation de nouveaux tissus subérisés de l'écorce. La gestion de blessures associées aux maladies des plantes, par la compréhension améliorée de la réponse aux blessures, peut bientôt être une réalité.

Several new findings about how wounded trees respond to injury have the potential to improve our ability to manage diseases associated with wounds. This research is of interest to arborists and comes from studies of a fungal disease of the bark of peach *(Prunus persica)* trees called Cytospora or perennial canker. The disease is caused by two fungi, *Leucostoma cincta* and L. persoonii, which initiate infections through wounds in the tree's outer bark (2). As the infections become established, perennial cankers are formed on the major branches and trunk of the tree. Economic losses occur through the loss of bearing limbs and early tree death. Peach production in some areas may be increased by 50% if this disease could be controlled.

In orchards, wounds to the trees are unavoidable and, in many cases, desirable. Peach trees must be pruned annually in order to stimulate the vigorous growth that is necessary for the production of a high quality fruit crop. Over 25% of new infections by *Leucostoma spp.* are initiated at pruning cuts. Unfortunately, their location on the tree is such that these infections often result in the loss of major limbs. Other types of wounds, such as leaf scars and winter and insect injuries, are also important, although cankers on the younger wood can be easily pruned from the trees in the spring. At present, there are no effective controls for this disease.

We began research on Cytospora canker of peach in 1983. Our long term goal was to incorporate disease resistance into new peach varieties. However, new information was needed to assist growers in managing this disease in the short term; the best way to make progress in both areas was to study the tree's responses to wounding.

What Happens in Bark After Wounding?

When tree bark is wounded, a series of complex biochemical events is initiated which, under ideal conditions, leads to the generation of new bark (12). In 1984, Dr. George Hudler, of Cornell University, described the major changes that take place in tree bark following wounding (16). Since then, we identified in the bark of many tree species two important roles for a wax-like biochemical called suberin. In 1984, suberin was identified as contributing to the impermeability of one of the new tissue layers formed after wounding from bark cells that were present when the wound occurred (1-4, 15). Until recently, this tissue had been called nonsuberized impervious

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tissue (NIT), although this is no longer appropriate given our new findings. Because the walls of cells in this tissue also are heavily lignified, it has been termed primary ligno-suberized tissue (17). The primary ligno-suberized layer is important because it is thought to provide the internal conditions necessary for the formation of the new bark or periderm. The walls of cells in the new bark also are composed of lignin and suberin. The vast majority of suberin in a wound occurs in the cell walls of the new bark. Disease resistance via periderm formation traditionally has been thought to occur because the periderm formed a structural barrier to pathogen ingress.

Is Wound Response Related to Disease Resistance?

When during the wound healing process in bark does the wound become resistant to certain types of infections? We attempted to answer this question by inoculating wounds in various stages of healing with the Cytospora canker fungus. We found that when the tree had generated a new periderm with at least three suberized phellem cells around the entire wound, this wound was no longer susceptible to the fungus (8). Wounds at all earlier stages were susceptible, although the frequency and severity of infection diminished with time after wounding. This finding suggested that the disease could be controlled with fungicides given that the wound was susceptible to inoculation for only 14 days. However, when we examined the rate of wound healing in the orchard, we discovered that in some tests wound healing did occur in 14 days and in other situations it took much longer (5, 7).

How Does Environment Influence Wound Response?

When we wounded trees every three weeks beginning in early May and ending in August, we found that the wound healing rate depended more upon the average temperature after wounding than upon the number of days after wounding or the time of year. For example, for the perimeter of the wounds to become completely lignified our observations determined that 256 degree-day units must be accumulated. For wounds to become completely suberized, 411 degree-day units must be accumulated. This means that a grower who pruned on March 15 would have to worry about wounds being susceptible for 62 days compared to only 21 days for the grower who pruned on June 15 (Table 1). Since most fungicides used in orchards have residual activities of only 7 to 10 days, it is evident why most fungicide controls for this disease have failed.

Soil moisture and plant water stress also influence wound healing in peach bark. In irrigation experiments, some peach cultivars exhibited delayed periderm formation in the nonirrigated plots, whereas in other cultivars wound responses were not visibily affected. Levels of water stress that are not severe enough to cause visible symptoms can result in delayed wound healing responses.

How Do Host Genetics Influence Wound Response?

In the experiments described above, we also examined the responses of sweet cherry *(P. avium)* and apple *(Malus domestica)* bark to wounding. In comparison to peach, the generation of new bark proceeds significantly faster in sweet cherry, which is only mildly susceptible to *Leucostoma spp.* Wound healing in apple, which is highly resistant to *Leucostoma spp.* in Ontario, proceeds at a rate faster than that of sweet cherry. Although these observations do no explain completely the observed relative susceptiblilty of

Table 1. Estimates for numbers of days for wound responses of peach bark to occur following hypothetical injuries made in March, April, May, and June in Vineland, Ontario.[z]

	Number of days	
Injury date	*Lignin formation*	*Suberin formation*
March 15	49	62
March 31	36	49
April 15	27	39
April 30	22	32
May 15	18	27
May 31	15	23
June 15	13	21

[z] Based on averaged annual degree-day accumulations (base = O C) in Vineland Station, Ontario, from 1935-1985. Estimated accumulated degree-days required for complete lignification and complete suberization are 256 and 411, respectively.

these species, a peach that has more rapid wound healing processes may be useful as a source of partial resistance to *Leucostoma spp.* In our screening of peach breeding selections at the Horticultural Research Institute of Ontario (HRIO), we "rediscovered" a clone named Hui Hun Tao, originally from China, that appears to possess this type of partial resistance. Breeding crosses are being evaluated to determine the heritability of this character and preliminary results indicate that it is transmissible via the female parent.

We have examined bark and xylem for nearly 20 eastern hardwood and softwood species and, for all the species examined, defense responses in the bark are basically similar with respect to the tissue changes described above (4, 9). Notable differences exist, however, in the relative ability of tree species to form barriers and generate new tissues. Indeed, as described below, notable differences occur in wound healing characters even within a species.

Genetic Variation Within Peach

In cooperation with Dr. Neil Miles, plant breeder at HRIO, we examined the wound healing responses of a range of peach varieties that varied from highly susceptible to moderately resistant in their field performance to peach canker. In a blind study, the various cultivars were wounded and examined for their rates of suberin accumulation using histochemical and microfluorometric techniques developed in our laboratory. The rate of suberin accumulation in the various cultivars was significantly different among cultivars during the months of May and June and these differences were correlated with the known field performance of the cultivars (10, 13). This was further evidence, although indirect, for the role of suberin in disease resistance. Furthermore, we had a method that could be used to test the relative susceptibility to disease of unknown genotypes from a breeding program. In a greenhouse study, when the resistance to inoculation of wounds of various ages on different cultivars was examined, we found that the cultivars with faster suberin accumulation in the field also became resistant to the fungus earlier (14).

These genetic differences in suberin accumulation represent varying levels of infection risk in the field. Partial resistance mechanisms in tree bark could operate in the following manner. Given that none of the peach cultivars is immune to the fungus, when spores land on the wound immediately after pruning and environmental conditions are favorable for infection, all cultivars are equally susceptible in terms of the frequency of infection (100% in all cultivars). Although the severity of infection (the size of the initial canker) may vary, as wound healing proceeds, the infection risk declines with time at different rates for the different cultivars. Those cultivars with lower infection risk exhibit less disease in the field.

In the above experiments, the various peach cultivars were examined for differences in the thickness and numbers of suberized cells in the new periderm. Our data showed that, in general, peach cultivars generated new periderm at similar rates. This result suggests that disease resistance via periderm formation may have a biochemical component, due to quantitative differences in suberin accumulation, as well as a physical component as a structural barrier to pathogens.

One further point to illustrate the complexity of disease resistance in trees is the apparent lack of association between wound healing in the bark and compartmentalization responses in the wood. In peach, a cultivar that exhibited rapid bark suberization exhibited relatively weak formation of xylem barriers, particularly ray parenchyma suberization (6). The cultivar with weaker bark responses possessed stronger xylem responses.

Can Wound Response Be Stimulated by Chemical Applications?

It seemed reasonable to speculate that if we could find a way to accelerate the bark wound response, either through the stimulation of anatomical or biochemical changes, then we could decrease the likelihood of infections. Scientists have been debating for years the use and abuse of wound protectant materials. Since peach is one of the most well understood bark systems for investigating wounds, we elected to test a variety of biochemicals and treatments for their ability to stimulate the formation of lignin and suberin and the effect of chemical applications on susceptibility of treated wounds to Leucostoma spp. Two of the materials we tested, fungal cell wall extract from *L. persoonii* and the small sugar cellobiose, were successful in stimulating lignin in the wounds (Table 2). In other plant systems, these materials also have stimulated defense reactions and it was not suprising to see that they worked in peach bark in the field. Several other materials either had no apparent effect or were deleterious to lignification.

The situation with suberin was different. None of the materials tested stimulated suberin and, to the contrary, several materials inhibited it, including Lac Balsam and white latex paint (Table 3). This finding suggests that these materials may not be beneficial in situations where rapid suberization is desired.

Our most interesting observation pertained to the effect of calcium on disease resistance in peach. Although calcium did not stimulate lignin or suberin in the wounds, wounds treated with calcium were less susceptible to infection and, when infections did occur, they were less severe than the other treatments (Table 4). Calcium is thought to influence disease processes by forming "bridges" between the polysaccharide subunits that comprise pectin, the "cement" that holds plant cells together. It is likely that this effect of calcium delayed the infection process sufficiently so that the plant's own wound response

Table 2. Lignin-thioglycolic acid (LTGA) extracted from bark of peach pruning wounds 14 days after treatment with various substances.

Treatment	*LTGA (mg/g)*[y]	*LTGA (Percent of control)*
Fungal wall extract (FWE)	31.0 a[z]	+ 81.3
Cellobiose (0.3 M)	29.2 a	+ 70.8
Plant wall extract (PWE) + FWE	23.2 ab	+ 35.6
Ca^{2+} (15 M)	22.8 ab	+ 33.4
Glutathione (3.25 mM)	20.6 ab	+ 20.7
PWE	20.3 ab	+ 18.7
Chitosan + Ca^{2+} (15 M)	20.3 ab	+ 18.6
Ca^{2+} (150 M)	18.8 ab	+ 9.7
Control (wrapped)	17.1 bc	0.0
Ethrel (7.0 mM)	15.1 bcd	− 11.8
Chitosan (20 g/ml)	15.1 bcd	− 11.8
GA_3 + IAA (100 M + 100 M)	14.9 bcd	− 12.5
Abscissic acid (100 M)	14.8 bcd	− 13.3
Lac Balsam	11.0 cd	− 35.7
White latex paint	8.6 d	− 49.6
Control (nonwrapped)	3.2 d	− 81.2

[y] Each value is the mean of nine observations except for control (treated with water and wrapped with parafilm (n = 48) and control (nonwrapped, exposed to the environment) (n = 15).
[z] Letters denote significant differences determined with single degree-of-freedom orthogonal comparisons ($P < 0.05$).

Table 3. Suberin autofluorescence (mV) from bark of peach pruning wounds 14 days after treatment with various substances.

Treatment	*Suberin autofluorescence*[y]	*Percent of control*
Control (nonwrapped)	7.4 a[z]	+ 33.4
Chitosan + Ca^{2+} (15 M)	7.4 a	+ 33.1
Fungal wall extract (FWE)	7.3 a	+ 32.2
Ethrel (7.0 mM)	6.9 a	+ 25.0
Abscissic acid (100 M)	6.8 ab	+ 23.6
Glutathione (3.25 mM)	5.8 abc	+ 4.3
Plant wall extract (PWE)	5.6 abc	+ 1.0
Control (wrapped)	5.5 abc	0.0
Chitosan (20 g/ml)	5.3 abc	− 3.8
Cellobiose (0.3 M)	5.0 abc	− 9.8
GA_3 + IAA (100 M + 100 M)	4.2 bcd	− 24.4
Ca^{2+} (150 M)	4.2 bcd	− 24.9
White latex paint	4.0 cd	− 26.8
PWE + FWE	3.9 cd	− 29.6
Ca^{2+}(15 M)	3.7 cd	− 33.7
Lac balsam	2.0 d	− 64.2

[y] Each value is the mean of six observations except for control (treated with water and wrapped with parafilm) (n = 38) and control (nonwrapped, exposed to the environment (n = 37).
[z] Letter denote significant difference determined with single degree-of-freedom orthogonal comparison ($P < 0.05$).

Table 4. Percent infection and canker length 7 days postinoculation from twigs pre-treated with various substances[y]

Treatment group	*Canker length (mm)*	*Percent infection*
Control	23.4 a[z]	66.7 b
BA_3 + IAA	16.4 b	82.5 a
LPWE	12.9 bc	48.5 c
LCWE	11.2 cd	74.7 ab
Cellobiose	10.8 cd	47.4 c
Calcium	6.6 d	21.0 d

[y] Values in both columns are the means of 50 observations except for control (n = 10) and GA3 + IAA (n = 40). LPWE and LCWE are cell wall extracts from *Leucostoma persoonii* and *L. cincta*, respectively.
[z] Letters denote significant differences determined with Duncan's multiple range test ($P < 0.05$).

was adequate to prevent establishment of the fungus. More work is needed to explore the potential of calcium treatments in the management of tree diseases.

Summary

Although many secrets have been revealed about how tree bark responds when injured, many more secrets are waiting to be discovered. For example, phytoalexins, antimicrobial substances that are produced by the plant after infection, are important in many plant diseases yet they have only begun to be investigated in woody plants. It is possible that these compounds are produced in wounded tissue and act as one of the first lines of defense to potential pathogens. In the field, the temperature models for wound healing need to be tested in combination with fungicide programs to determine whether they can be integrated into a control program for canker diseases.

Literature Cited

1. Biggs, A.R. 1984. *Intracellular suberin: occurrence and detection in tree bark.* Int. Assoc. Wood Anat. Bull. (n.s.) 5:243-248.
2. Biggs, A.R. 1984. *Boundary zone formation in peach bark in response to wounds and Cytospora leucostoma infection.* Can. J. Bot. 62:2814-2821.
3. Biggs, A.R. 1985. *Detection of impervious tissue in tree bark with selective histochemistry and fluorescence microscopy.* Stain Technol. 60:299-304.
4. Biggs, A.R. 1985. *Suberized boundary zones and the chronology of wound response in tree bark.* Phytopathology 75:1191-1195.
5. Biggs, A.R. 1986. *Phellogen regeneration in injured peach tree bark.* Ann. Bot. (London) 57:463-470.
6. Biggs, A.R. 1986. *Comparative anatomy and host response to two peach cultivars inoculated with Leucostoma cincta and L. persoonii.* Phytopathology 76:905-912.
7. Biggs, A.R. 1986. *Prediction of lignin and suberin deposition in boundary zone tissue of wounded tree bark using accumulated degree days.* J. Am. Soc. Hortic. Sci. 111:757-760.
8. Biggs, A.R. 1986. *Wound age and infection of peach bark by Cytospora leucostoma.* Can. J. Bot. 64:2319-2321.
9. Biggs, A.R. 1987. *Occurrence and location of suberin in wound reaction zones in xylem of seventeen tree species.* Phytopathology 77:718-725.
10. Biggs, A.R. 1989. *Temporal changes in the infection court following wounding and their association with cultivar variation in infection by Leucostoma persoonii.* Phytopathology 89:627-630.
11. Biggs, A.R., and Cline, R.A. 1986. *Influence of irrigation on wound response in peach bark.* Can. J. Plant Pathol. 8:405-408.
12. Biggs, A.R., Merrill, W., and Davis, D.D. 1984. *Discussion: Response of bark tissues to injury and infection.* Can. J. For. For. Res. 14:351-356.
13. Biggs, A.R., and Miles, N.W. 1985. *Suberin deposition as a measure of wound response in peach bark.* HortScience 20:902-905.
14. Biggs, A.R., and Miles, N.W. 1988. *Association of suberin formation in uninoculated wounds with susceptibility to Leucostoma cincta and L. persoonii in various peach cultivars.* Phytopathology 78:1070-1074.
15. Biggs, A.R., and Stobbs, L.W. 1986. *Fine structure of the suberized cell walls in the boundary zone and necrophylactic periderm in wound peach bark.* Can. J. Bot. 64:1606-1610.
16. Hudler, G.W. 1984. *Wound healing in bark of woody plants.* J. Arboric. 10:241-245.
17. Hudler, G.W., and Banik, M.T. 1986. *Wound repair and disease resistance in stems of Pachysandra terminalis.* Can. J. Bot. 64:2406-2410.

West Virginia University
University Experiment Farm
P.O. Box 609
Kearneysville, WV 25430