Differential Wound Response in Southern California Avocado (*Persea americana* cv., Lauraceae) Plantations Comparing Three Distinct Injection Methodologies 2-Years Following Treatment

By Joseph J. Doccola, Peter M. Wild, Eric Bristol, and Joe Lojko.

Introduction

There is increased interest in the use of trunk injected phosphonates for plant health response in *Phytophthora*-susceptible Avocado (Darvis et al, Pegg et al). When roots are infected by disease, uptake of essential minerals becomes compromised.
Phosphorous (P), an essential mineral, tends to be abundant in soils, but of limited availability (low solubility). P is important in energy utilization—it forms high energy (triple) bonds, the result of kreb cycle metabolism (i.e., carbohydrates metabolized in the citric acid cycle, energy released converts ADP to ATP). P is an important component of amino acids, proteins, and enzymes and therefore critical to plant function. It is a macro-element required in significant quantities (percent vs ppms for micro-elements).
In this study, we are interested in trunk injected Phosphorous acid and tree wound response in Avocado. We compared Arborjet injection technology with methods and formulations in use in Avocado at the time. The results of the study demonstrate that the method of application (device) and formulation result in biologically significant outcomes. Trees were dissected two years after treatment.
Trees are autotrophic meaning energy utilization is the result of the photosynthetic process. Photosynthates are required for growth, metabolism, reproduction, storage and defense (Shigo,1991). Wound response, a defense mechanism requires carbohydrate stores. All trunk treatment applications require wounding the tree. However, the way the three methods wound trees vary significantly.

Three distinct injection methodologies were compared in this study of wound response in southern California Avocado plantations. The purpose, to compare differential wound response utilizing three distinct injection methods, these were:

1. drill and syringe, phosphorous acid
2. cambial injection, phosphorous acid
3. Arborjet Air/hydraulic injection, buffered phosphite

Wound response is based upon three key factors, which include:

1. tree health
2. application method
3. formulation

Tree Condition

Avocado treated in these plantations showed initial symptoms of *Phtyophthora spp*. infections. Visual assessment of canopy dieback was used as an indicator (figures 1-3). Mean canopy dieback was approximately 12.5%. 30 year-old plantation trees had a mean DBH of 16” Many of the trees were multi-stemmed typically with 4 leaders. . Phosphorous acid or buffered phosphite was applied at 5mL/DBH" rate in treated trees.

Application Method

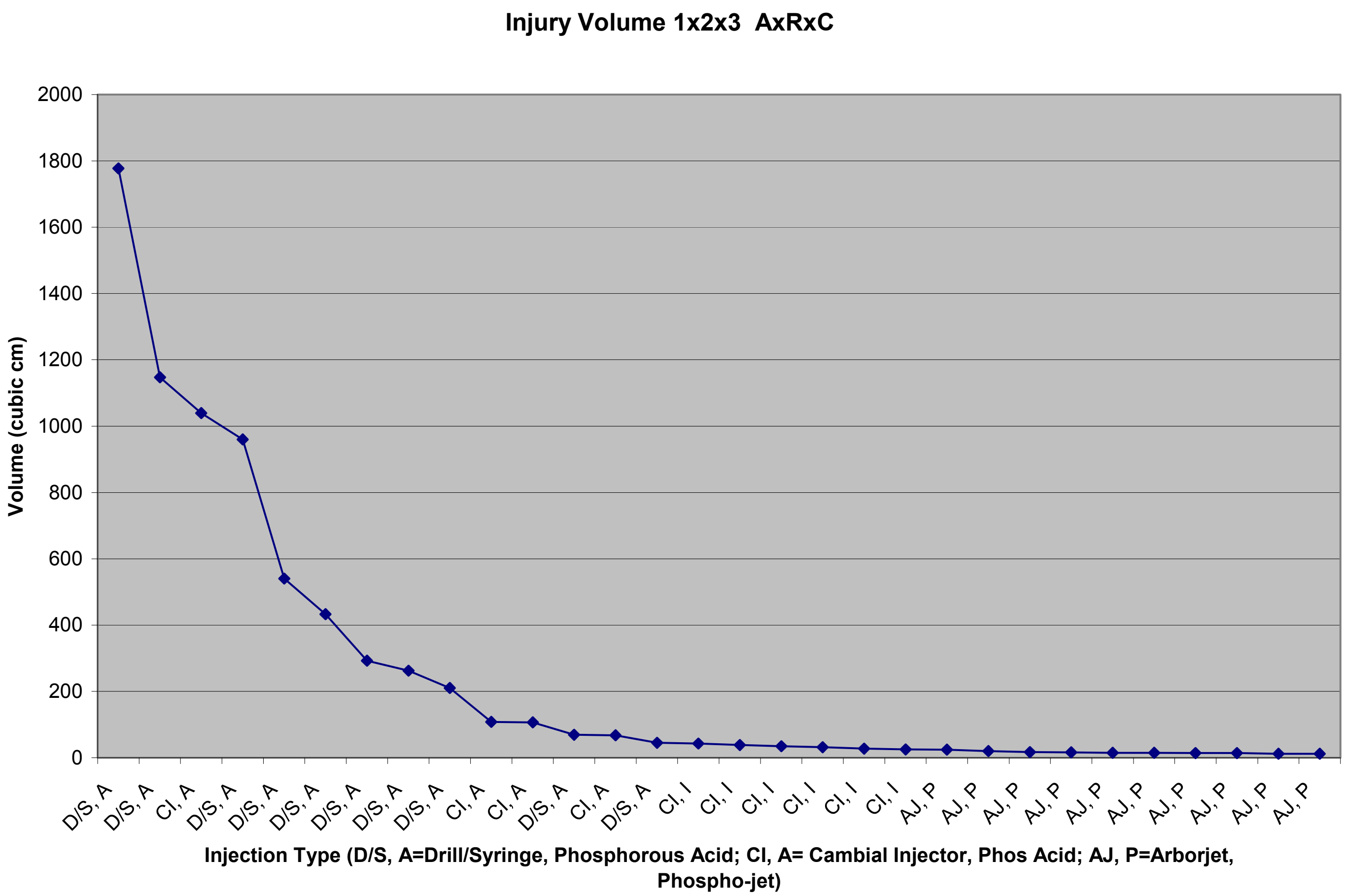
Methods 1 and 2 were the standard procedures used in the Avocado plantations. The first procedure utilized a 3/8" diameter x 1-2" deep (varies), angled to accept syringed phosphorous acid solution. No septum or plug was used. Method 1 is NOT a pressure application.
The second procedure utilized a cambial injector to apply phosphorous acid. No drilling was required. This second method relies on the development of high pressure to displace the inner bark to create a "pocket" for formulation application. ½ to 1mL per trigger squeeze and up to 5mL may be applied. To apply the specified dose, the application needed typically 16 (5mL/DBH" x 16DBH"=80mL per tree) injection sites around the trunk. The applied formulation is slowly absorbed through the side wall pits of the xylem elements for translocation within the tree.
Method 3 utilized the Arborjet Trunk injection system. This method requires drilling into the active sapwood. Depths are limited to 5/8" depth (1.5cm). A 9/32" (0.7cm) drill used to apply 4 sites around each tree. Input pressures limited to 200psi. A buffered (neutralized) proprietary phosphonate solution (Phospho-jet) was injected at the 5mL/DBH" rate. 10 trees per treatment were assessed.

Wound Analyses

Tree autopsies were conducted in Temecula, CA on Avocado Plantations between March 31 and April 1, 2004, two years following injection treatments. Analyses of reaction zones 1-3, and Barrier Zone, 4 (wall 1= axial progression, wall 2= radial, and wall 3 = circumferential; wall 4 = woundwood development) were performed at Arborjet, Inc. in Winchester, MA, and on site in Temecula, CA. Chart 1, below summarizes these results (volume in cubic centimeters). Thirty trees were sampled for wood volume lost to compartmentalization. According to the CODIT model as described by Shigo, the three reaction zones are ordered according increasing extent of reaction. Thus wall 1>wall2>wall3. The impacts of loss of wood volume to compartmentalization are numerous. Among them: the reduction of carbohydrate storage (thus affecting tree vitality), the compromise of conduction of vascular tissues, injury to the vascular cambium and the creation of an infection court at the wound site. Of the 30 analyses performed: 20 samples received unbuffered Phosphorous Acid with Methods 1 or 2, and 10 samples of Avocado injected using Arborjet Technology with the buffered Phosphite product. Regardless of method, the buffered phosphite (i.e., Phospho-jet) exhibited the lowest degree of phytotoxicity in trees as measured by the area of sapwood hypersensitivity (wall 1-3).
On the other hand, volume of compartmentalization was related to extent of the drill wound. Method 1 (i.e., drill/syringe) resulted in a mean drill hole volume of 4.2 cm^3 vs. Method 3 (i.e., Arborjet) resulted in a mean injury volume of 1.8 cm^3. Method 2, cambial injector does not require drilling into the sapwood, so drilled volume is negligible. Drilled volumes differed by a factor of 2x (methods 1 and 3 compared). This has biological significance, and standardization of the drilled sapwood reservoir is therefore important. Because axial and radial walls are weakest of the reaction zones, the hypersensitive reactions tended to be elongate. Arborjet technology standardizes the depth to 1.5cm into the active sapwood. Method 1 used a 3/8"D (0.9cm) drill bit that angled into the sapwood, creating a deeper than necessary injury in radial extent. The likelihood that deep injuries cause more sapwood to compartmentalize is clear in this study. The probability of compromising older barrier zones (wall 4) was evident (figures 7, 16 and 19).
The extent of wounding as recorded by injury volume tells a different story. This is the area of hypersensitive reaction <u>at the time of wounding</u>, and the result of injection formulation. When comparing the mean injury volume of Methods 1 and 3, Method 1 resulted in a 42.5x greater injury. Mean injury volume (cm^3) was 775 and 18.2 respectively.

Although the drilled volume for the cambial injector was negligible (damage to sapwood) the injury volume could be high (to 1039.5 cm^3). This is the result of cambial necroses due to the extent of exposure to the injected phosphorous acid. The injector requires the bark to be displaced from the sapwood in order for the formulation to be delivered. An inordinate and unnecessary amount of vascular cambium is exposed in the injection process. The product must slowly diffuse through the vascular sidewall pits to in order to be taking up in the vascular (transport) tissues and transpirational stream—a significantly slower process. Only small amounts may be applied via cambial injection, therefore more application sites (up to 16 vs. 4 of the other methods) are required. The requirement for additional injury increases the probability of tree girdling. Repeated circumferential applications using this simple tool is therefore not recommended.

CHART 1



Results

Methods 1 and 2 using unbuffered solutions resulted in significantly greater phyto-toxicity than Arborjet methodology defined as volume of hypersensitive reaction.

Phosphorous acid (unbuffered) proved to be phytotoxic to tissues at the injection site. The resulting necroses using the Drill-Syringe method resulted in dieback and infection (xylem necroses, phloem edema) at the injection site, with no wound closure (figures 5, 6, 10-12, 14, 17).

Using the Cambial Injector, greater circumferential injury and phyto-toxicity was observed. The greater exposure of the vascular cambium to concentrate is undesirable, and therefore not recommended.

The proprietary buffered phosphonate solution (Phospho-jet) resulted in little or no phytotoxicity at the injection site. Tree response to wound closure was typical. No infected tissues or dieback around injection sites was observed.

Of the three methods, the Arborjet Injections (method 3) with buffered phosphonate, resulted in the lowest wound profile and highest degree of wound closure (figures 26, 27).

Conclusions

Tree health, method of application, and formulation used affect wound response. Trees need sufficient carbohydrate stores to respond to an injection treatment. All trees in the treatment responded to phosphonate, however the degree of injury to the tree greatly depended upon method of application and formulation used. The least phyto-toxic was the buffered phosphonate. Utilization of a septum (such as the Arborplug) standardized and limited the extent of the injection wound, and cambial exposure to concentrated formulations. Without a septum, the wound to accept sufficient formulation needs to be angled downward and deeper than necessary. The deeper wounds are likely to compromise previously compartmnentalized wood (figures 2, 4). Moreover leaving an open wound where air-borne *Phytoohthora ramorans* is present is ill advised as this presents an open infection court. On the other hand, with the use of cambial injection, only a limited amount may be applied in any application site, requiring higher pressures for bark displacement, and greater circumferential exposure of the vascular cambium to concentrate, thus risking circumferential girdling. More (up to 4x) cambial injection sites were needed to apply the specified dose. It is counter-productive to expose the secondary vascular cambium to concentrated formulations—this tissue is crucial to secondary growth and to maintain leaf-root connectivity. The least invasive methodology in these studies proved to be a xylem injection standardized by Arborjet procedure using a buffered phosphonate solution.

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Figure 1 Canopy dieback in Phythophthora infected Avocado

Figure 2 Tree with severe dieback

Figure 3 Avocado with early dieback symptoms, upper right of canopy

Figure 4 Tree with healthy canopy

METHOD 1: DRILL & SYRINGE

Figures 5-19, below illustrate injections using a large, deep drill hole and unbuffered concentrated solution. This technique was employed in Avocado plantations prior to Arborjet technology. Note damage to sapwood and bark tissues. This method does not utilize a septum (plug) or require pressure.

Figure 5 Detail of branch canker, surrounding site of open injection wound, drilled and injected with un-buffered phosphorous acid. Note sunken bark indicative of dead vascular tissues.

Figure 6 Detail of stem canker, surrounding site of open injection wound

Figure 7 Cross sectional area affected by deep drilling wound; reaction zone is non-existent.

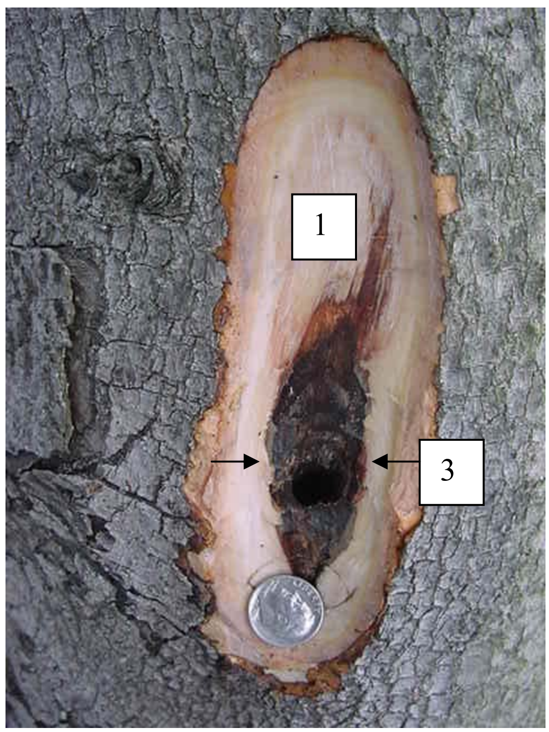

Figure 8 Close-up of autopsied Avocado stem tissue at site of wound following drill and syringe method. The injection site is shown at the time of wounding (reaction zone 1, axial and wall 3, circumferential are shown). The black tissue is hypersensitivity to the low pH solutions used in these injections.

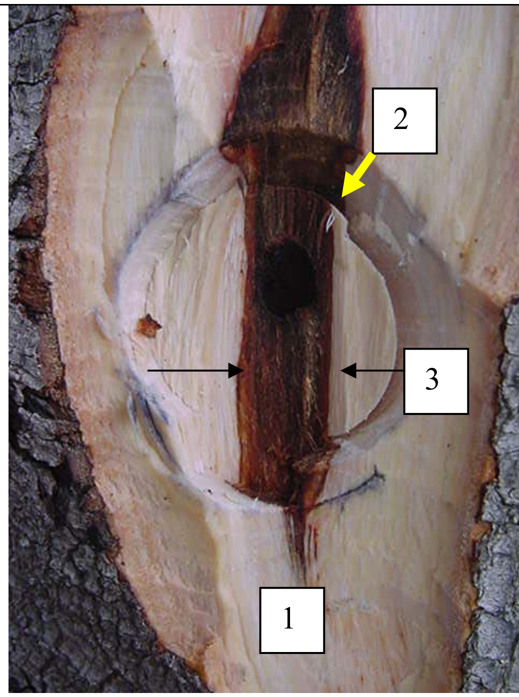

Figure 9 Note extensive injury in axial (1), radial (2, yellow arrow) and circumferential (3, black arrows) directions.

Figure 10 Detail of canker surrounding open wound in Avocado branch.

Figure 11 In this branch, 27% of the branch circumference is girdled.

Figure 12 Same branch as above, with dead bark removed to expose extensive tissue dieback. Sample with extensive dieback associated with deep injection. Note: no septum or pressure was used with this injection method.

Figure 14 Section of Avocado at injection site. Note reaction zone 2 is in the radial direction, that is toward the pith (yellow arrow).

Figure 15 Same sample, as above with bark removed. Illustrates hypersensitivity in axial (wall 1) and in circumference (wall 2).

Figure 16 Radial section of Avocado branch showing extensive drill wound and compartmentalization of tissues.

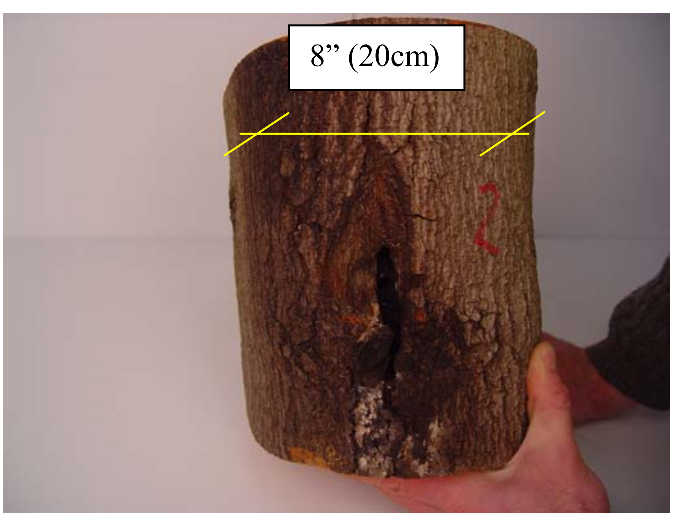

Figure 17. Bolt cut from an 8”DBH Avocado drilled and injected with phosphorous acid. Bark is water soaked, reddened, distinct (mal-) odorous, indicative of an active infection.

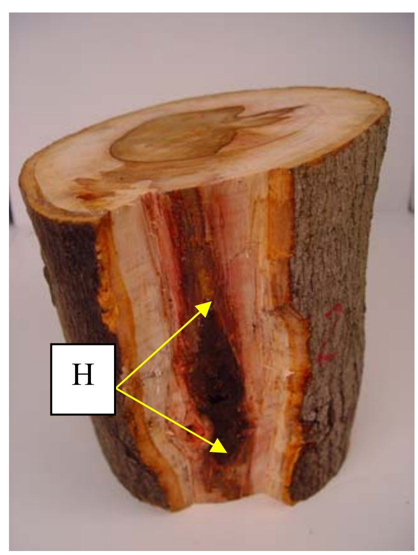

Figure 18. Same bolt with bark removed. Tangential view, note extensive hypersensitive reaction (blackened tissue, H) immediately surrounding the injection site (arrows).

Figure 19. Radial view. Note coalescence of discoloration with previous wound column (arrows).

METHOD 2: CAMBIAL INJECTOR

Figures 20-22 illustrate reactions in Avocado using a cambial injector that relies on bark separation, exposing a greater area of the vascular cambium to concentrated product. Because the cambial tissue is embryonic and nondifferentiated (i.e., secondary meristem), it is non-conductive. Movement of injected chemicals occurs in the transport tissues, primarily, the xylem (sapwood). Xylem is composed of vessels and tracheids. These transport tissues have sidewall pits (pores) that absorb injected chemicals. The absorption through the sidewall pits is slower than a direct injection into the sapwood.

Figure 20. Cambial Injection site 2 years following treatment in Avocado, prior to bark removal. No drill method.

Figure 21. Arrows illustrate the circumferential extent of reaction zone 3 in the cambial region. Note blackened tissues. Left arrow shows hypersensitive reaction occurring beyond the sample.

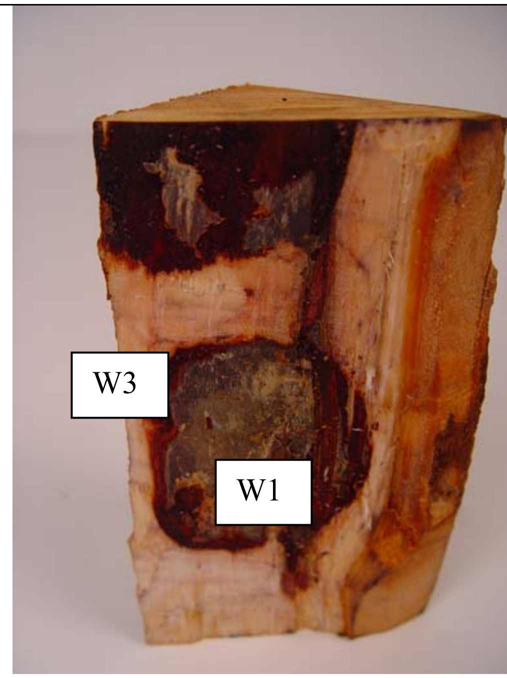

Figure 22. With bark removed sapwood with extensive necroses (dead tissues) in axial(W1) and circumferential (W3) directions. Given the manufacturer specification of one injection per 6" circumference, this method, though appearing benign is more likely to result in tree girdling than drilling directly into the sapwood.

METHOD 3: ARBORJET TECHNOLOGY	
Figures 23-30. The following dissections of injection sites in Avocado illustrate Arborjet technology. The Arborjet system uses the patent-pending Arborplug technology. Injections of PHOSPHO-*jet* a pH buffered solution.	

Figure 23. Tangential view with bark removed to show woundwood development (wall 4) 2 years following injection.

Figure 24. Sapwood removed to expose tissues at the time of injection (2 year old tissue). In tangential and radial views. Note limited reaction zone around Arborplug (arrows).

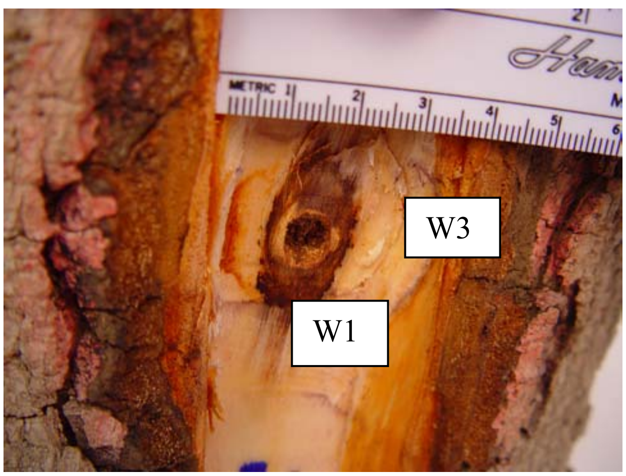

Figure 25. Close-up of injection site using Arborjet technology showing very limited reaction zone (walls 1 and 3). Wall 4 (new tissue) removed to expose reaction zone at time of injection..

Figure 26. Excised bark from Avocado injected using Arborjet technology. Applications were color coded with florescent paint to mark the application sites for the duration of the study. Note closure of injection site.

Figure 27. Excised Injection site with bark removed, tangential view, showing woundwood development and healthy sapwood tissue.

Figure 28. Excised tissue, radial view showing limited reaction zone (wall 1 & 3) and embedded Arborplug two years following injection.

Figure 29. Excised Arborjet Injection Site two years following treatment, radial view. Note embedded Arborplug.

Figure 30. Tangential view of injection site showing limited reaction zones associated with buffered Phospho-jet solution.

ARBORjet
A Revolutionary Plant Injection System