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GLITCHES AND GAPS IN THE SCIENCE AND TECHNOLOGY OF TREE INJECTION[1]

by R. Jay Stipes

"To inject or not to inject; that is the question," to paraphrase a famous English playwright might explain in part the rationale for this second symposium on "Systemic Chemical Treatments in Tree Culture."

Tree injection or infusion is elected for use when: 1) there is no other way to introduce nutrients or pesticides, 2) environmental contamination hazards by spraying techniques are objectionable, 3) it is economically more beneficial and/or more effective to inject than to spray, and for assorted other reasons. Tree injection is jargon used often to embrace loosely any method for introducing liquids into woody stems; this is erroneous and therefore needs clarification. *Injection* is the forceful propulsion of liquids into woody stems using pressurized cylinders of compressed gases (nitrogen, carbon dioxide, air). In human or animal medicine, administration of medicine via a hypodermic syringe would be an example. *Infusion,* on the other hand, is the introduction of liquids that relies solely on atmospheric pressure and the tree's own uptake/translocation capability (transpirational pull, root pressure, etc.); examples of the latter would be the "gravity flow reservoirs," Medicaps and other trunk implantation devices and similar systems. In human medicine, an intravenous "drip" would be a comparable example.

Some amongst us are "conscientious injectors," others are absolutely not, while still a residue of us are in the middle ground of doubt. Why is this? Is it because we are not convinced of the absolute value and efficacy of tree injection? As with most human endeavors, principles and philosophies, ill-defined borders abound. Unlike the exact sciences where, for example, two chemical components are placed in the test tube and the expected compound results from the reaction under a set of rigidly controlled conditions, in the biology of the tree world, the water is markedly murky.

Where now is tree injection as a science and an art (the technological component)? For this, we might envision a horizontal line with tick marks along it; at one end is total ignorance and uselessness, and at the other end perfect enlightenment and total practicality. My question is, "Where now are we on this continuum?" My guess would be in the middle somewhere, in the medieval ages, with some bright rays of a renaissance on the horizon. Many of you have provided some awfully exciting fragments of information or pieces of the injection puzzle, but there are many missing pieces, or what I choose to call "gaps and glitches." Before we consider a few of them, let me emphasize that this paper is more of a brief philosophical overview than an exhaustive review of the corpus of literature extant on the topic which indeed is growing although not burgeoning. I hope, therefore, that my colleagues will not be offended at various deletions. As in everything I offer by written and spoken word, this constitutes one person's opinions, open for evaluation and challenging. The various gaps and glitches are not mentioned in any particular ranking order of importance. Any one of them, if deficient, is a weak link that can render the chain

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weak, ineffectual or entirely useless. In addition, in the consideration of these points, we shall use Dutch elm disease (DED) and its causal pathogen *(Ophiostoma ulmi = Ceratocystis ulmi)* as the model for consideration. The same principles, however, are applicable to almost any other systemic treatment of trees for management of growth or various pests and diseases.

Tailoring of fungicide molecules for activity, mobility and persistence. Most fungicide (or other pesticide) molecules have not been deliberately synthesized or tailored to thwart a particular pest, to have a particular mobility or to persist a certain length of time. That is, pesticide molecules are synthesized *de novo* by industrial chemists, then tested on a standard set of diseases or insect pests; and if one of them is acceptably active, then it is processed through a large battery of other tests to quantify pesticidal activity, animal toxicology, residual activity and other phenomena. In some cases, after a fungicide has been discovered, then the developer will modify the molecule by changing substituents on the molecule (adding or deleting atoms, lengthening carbon chains, etc.), and thereby dramatically changing its activity. In some cases, only one atom change will convert the pesticide from activity on one target (say a fungus) to another (a weed). This structure/activity relationship is therefore crucial. It might prove greatly helpful if, for example, the producer of thiabendazole hypophosphite (Arbotect 20S) fungicide used for DED control would modify its molecular structure to determine whether it might become more fungitoxic, more systemic, more residual and less phytotoxic. Most companies, however, find that it is not cost effective to do this, especially for products that have a very limited market such as that for DED.

Injection port. The site of injection has been problematic since the inception of tree injection activity. Even today, after a number of refinements, many unknowns abound. Since most pesticides applied to trees do not move inward following application to intact leaves, bark or roots, or if they do and pose an environmental contamination risk during application, injection into the sapstream is often elected. The first historical records indicate that holes were bored into the trunk, and reservoirs of the liquid to be infused or injected attached to the injection port (May, 1941). In these earlier trials, deep injection holes were drilled into several growth rings into the bole; in recent work (Zimmermann and Brown, 1971; Ellmore and Ewers, 1986) we have learned that sap flow or transport of liquids in the xylem of ring-porous trees, and specifically elm, occurs in the outermost ring or two. This was an important gap to have bridged in our understanding of injection. Also earlier, large diameter injection holes, some an inch or more, were made in the trunk; research in recent years has indicated that uptake of fungicide is as good in much smaller diameter holes, and subsequent wound closure of them is much better (Neely, 1979).

Possibly because of convenience and to avoid the unaesthetic, fluxing holes on the trunk, some individuals decided to inject root flares (flare roots), and it was found that uptake and translocation were much improved over trunk injection. Kondo (1972) pioneered the injection of excised lateral roots, and demonstrated that movement of benzimidazole fungicides and dyes could be obtained by this method; distribution was remarkably uniform, and possibly the best that has ever been achieved by any injection or infusion method. Very recently, Phair and Ellmore (1984), using in part the information from Zimmermann and Brown (1971) and Ellmore and Ewers (1986), injected American elm with Arbotect 20S into the newly synthesized outer growth ring (springwood) at chest level with success. Stipes et al.(1987) found that Arbotect 20S translocated as well or better using this "shallow pit" method as using single point injection sites with the flare root method.

Spacing frequency of injection sites has been a question also, and trees such as elm or oak occurs essentially upward "in streaks" following deep-hole trunk injection or infusion (Stipes and others, unpublished research). Therefore, it is essential that we know how many or few injection holes are needed to achieve adequate fungicide translocation. At this time, spacing of injection ports is empirical and whimsical, depending on the subjective judgement of the injector. Kondo (1979), does not recommend placing injection holes in the "valley" areas (spaces between flare

root insertions) when the flare root region is injected.

Physiological effects of sequential injections. Little research has been done to determine adverse effects of injection wounds in trees. The introduction of any xenobiotic or foreign compound into a living organism is often deleterious, and "subclinical" (insidious, often invisible) "side effects" of fungicides in trees are virtually unknown (Campana, 1979). Andrews et al. (1982) reported that some fungicides elicited more discoloration than others in the wood surrounding injection wounds, while water caused little. In addition, they reported the alteration in tylose formation and the sequence of microorganism colonization. A number of others have reported bacterial and yeast infections and therefore fluxing injection sites. If the wetwood core is greatly enlarged and in close proximity to the outer few growth rings where most current injections are made, anatomical connections ("communications") are established that permit wetwood infection to occur in the few sapwood rings critical to survival of the tree. Repeated (sequential) injections therefore might prove catastrophic to the tree (Stipes and Campana, 1981, Color plates 100, 102, 103 and 108). Futhermore, energy is required to repair the injured tree, and this is a drain on the resources.

Sorption of fungicides to tree tissues. It is well known that, due to the molecular species that consequently dictates charge and other properties, certain molecules move freely within the woody tissues, while others are "trapped" and adhere closely to lignin or cellulose in the tracheary elements. This accounts, in part, for the better mobility of some compounds over others. G.E. Ellmore (personal communication, 1987) reported that some dyes used to study translocation patterns are more mobile than others. If molecules are designed specifically for tree injection then objectionable features can be avoided and the desired systemicity can be chosen.

Barak et al. (1983) found that lignin adsorbed fungicides and herbicides differently; the more lipophilic fungicides were adsorbed to the greatest extent. Carbendazim, the parent molecule of Lignasan BLP used in DED control, was found to be adsorbed more than expected due to its partial protonation at pH 5.0, whereas the other pesticides they assayed were nonionized. Stennes and French (1987) found that Lignasan BLP moved much more quickly in the injected elm than Arbotect, but had a much shorter residual life.

Dosage standardization. Due to our past ignorance of the depth of functional sapwood (tree rings) in elm until recently divulged (Ellmore and Ewers, 1986), and the volume of sapwood to be protected, dosages have been empirically established. To further complicate this, American elms are markedly diverse in their morphology or shoot configuration, ranging from slender tall boles with short terminal crowns to multi-trunked trees that branch prolifically near the base so that standard dosages for trees of comparable DBH (diameter at breast height) have been essentially meaningless. To establish dosages, experimenters would use a shotgun approach to determine what concentration would be phytotoxic to a number of replicated trees in a treatment, then use a sub-phytotoxic dosage based on the average response in the test. Dosages are commonly based on DBH or circumference. Lanier (1987) has substantially honed this dosage system by using the bark surface technique employed by foresters to determine wood volumes; the curve that fits a straight DBH/dosage regimen can vary significantly with his bark surface/dosage one. Much more needs to be done to refine this schedule, but this has been a significant breakthrough.

Further, it was earlier believed that, once the dosage had been established, larger volumes of water as a diluent would provide better translocation than using the same dosage in a small volume of water. Research by Stipes and associates (Kolpak et al., 1978) showed that the reverse is quite true with Arbotect 20S; the explanation for this is yet to be divulged. Whether using more concentrated fungicide solutions would work for all compounds is unknown. In this, I emphasize that there is a tremendous volume of springwood vessels in the crown to be protected, and when a fungitoxic level is required in the crown, a substantially greater-than-fungitoxic level must be administered at the injection ports. Dilution is phenomenally greater than one suspects as the

fungicide traverses upward.

When one compares dosages of, for example, the benzimidazole fungicides which have comparable fungitoxicities to the DED fungus, there are hundred fold differences in recommended dosages suggested by the manufacturers; this points up the dramatic dearth of comprehension of dosage as it relates to movement, residual life and amount of vascular tissue to be infused or injected and protected.

Uniformity and rapidity of translocation. Uniformity in translocation has already been addressed briefly and will be subsequently, but the phenomenon is extremely important in controlling vascular pathogens. The scolytid bark beetle vectors of the DED pathogen attack preferentially 2-to 4-year old twig crotches in the elm crown. Therefore, it is essential that the injected fungicide reach that area and remain there during the susceptibility period. Stennes and French (1987) have found that marked differences are exhibited by rapidity of translocation by two benzimidazole fungicides (Lignasan BLP and Arbotect 20S) in Minnesota; Lignasan moves very rapidly to the crown (several hours) following injection, while Arbotect requires approximately a month to achieve optimum translocation. Lignasan, however, is very short-lived, while relatively high levels of Arbotect 20S residues were detected at least 24 months following injection. These researchers also found that Arbotect that is injected one year moves into the newly synthesized sapwood the following year.

Meterological/temporal effects on fungicide distribution. Over a series of injection trials, using a number different methods, individuals have reported vast differences in uptake. Trees of the same species size, age and in the same geographic area vary greatly in the reception and distribution of a number of compounds. Also, the time of day has a great impact on uptake by infusion the optimum time occurring between 11 a.m. and 2 p.m. In addition, weather conditions exert significant impacts on uptake and distribution; most individuals have reported that a sunshiny day following a period or day of heavy rainfall is generally the best time to inject for maximum and rapid uptake and not during a drought as human reason might suggest.

Dilution gradient effect on the target fungus. As mentioned earlier, the fungicide must be administered as concentrated as possible at the port of injection, because great dilution occurs as the material is being transported upward. No one has really determined the volume of springwood vessels in an elm of a prescribed size, but it must be enormous, especially in large specimens. By the time the fungicide reaches the infection court area (twig crotches) where the scolytid beetles deposit the fungus as they feed, the concentration must be adequate to either kill (be fungicidal) or inhibit (be fungistatic); for most fungicides, this is in the 1-10 ppm range or else the candidate fungicide would not be suitable for use. At the lower concentrations, say at 1 or less ppm, the fungicide may be fungistatic in which case disease control in a therapeutic sense may not be achieved, while at the higher concentrations, the fungicide could kill any living propagules of the fungus.

Detection of fungicide (residue analysis). Mobility is an essential requirement of fungitoxicants injected into trees, but this mobility must be evaluated. One of the deficiencies of injection systems is their failure to translocate the fungicide into the infection courts (places where infection starts) in a uniform manner (Truax and Stipes, 1981). Various tests have been devised to evaluate this. The first, of course, is disease control. Some individuals have performed experiments in areas where disease incidence is either lacking or spotty, and therefore concluded that their injections were effective because disease did not occur in their treated trees! Others have completely avoided the use of control (uninjected) trees, deeming them to be unnecessary; such evaluations are unscientific and unethical, providing nothing of scientific worth.

Certain laboratory tests have been conducted to detect fungicide residues in tissues from treated trees. Stem sections ("whips") are commonly removed from the crown and assays performed with sections ("cookies" or "disks") from them;' this is done by overspraying the sections with a fungicide-sensitive fungus or by observing for a clearing reaction when the disk is placed on a petri plate with agar on which a "lawn" of a sensitive fungus has been placed. These tests do not

yield an accurately quantitative analysis, but at best a general range or index, ranging from a "strong" to a "weak" reaction (Stennes and French, 1987). The Merck Company has developed a sophisticated but complicated wood analysis that provides a ppm residue profile (Shriver et al., 1979); it is extremely time-consuming and expensive and is not commonly used. Therefore a rapid and accurate residue determination analysis needs to be developed, and can be done with adequate research.

Physiological effects of chronic exposures to xenobiotics. Little or no consideration has ever been given this topic. Living plants have evolved over the millenia to free themselves of or become tolerant to toxic chemicals. Any compound, therefore, that is not a natural metabolite or constituent of a tree likely will be injurious to its metabolism. Moreover, damage can be done that is not visible, and we term this type of injury "subclinical" or "physiogenic." Good physicians will not prescribe drugs to patients on a casual basis if a non-drug treatment exists because of the potential unknown physiological effects that drugs might effect. Various phytotoxic effects have been observed and catalogued for a number of fungicides and insecticides injected into elm trees; however, subtle damage likely occurs in treated trees where no visible damage is noted. A gap, therefore, that requires bridging is the careful observation of growth and general health parameters over long periods of time of trees injected with fungicides.

Resistance/tolerance phenomena. Many pesticides elicit a resistance or tolerance response in or on treated plants to pests and pathogens. These responses are more common when systemic compounds, especially systemic fungicides, are used because they exhibit a single mode of fungitoxic action. Although this has not been reported in treated trees, it does occur *in vitro,* that is in laboratory tests using the fungus in fungicide-amended agar (Schreiber and Townsend, 1976).

Total/partial eradication of the pathogen. The cardinal principle in the management of plant diseases is to eliminate the pathogens that cause them or to keep their inoculum densities (spores, mycelia, etc.) as low as possible; DED, therefore, is best managed by a comprehensive sanitation program (Sinclair and Campana, 1978).

When the DED pathogen makes ingress in the elm, it proliferates relatively rapidly and ramifies into many tissues; and the internal vascular lesion almost always far exceeds that indicated by symptomatic leaves. The author, during a consulting visit to Colonial Williamsburg, observed only three or four yellow leaves on an American elm; and upon further observation, he found the infection had advanced into the trunk to the root/shoot interface! If infection is initiated in the crown, as most of them are, the fungus traverses from the crown branches down the main trunk(s) into the root system. When chemotherapy is effected on a tree with minimal crown involvement, one is interested in eradicating the fungus by integrating chemotherapy and radical surgery using Campana's guidelines (Sinclair and Campana, 1978). No one has adequately investigated the ability of fungicides to course through infected xylem vessels which would be necessary to eradicate residual fungal infection. Gaps therefore exist in determining how the pathogen can be eradicated from minimally infected elm trees, and in fine tuning the integrated radical surgery-chemotherapy technique.

It is well known that DED infections that originate via root grafts are impossible to manage, and the tree quickly dies; all efforts to inject fungicides to curtail these infections have failed. The root/shoot interface, or that region at the ground level appears to be critical in the proliferation of the fungus, and the anatomy of this region is poorly understood, another gap in our data bank.

Persistence/disappearance phenomena: treatment frequency. Another gap in our understanding of tree injection is how long materials last following injection. This, in part, depends on what and how much material we inject, how concentrated it is, and where it goes. Since dosage levels and schedules are relatively poorly understood, then persistence or residual profiles follow suit. Very few studies address this topic. The best recent information we have are the data of Stennes and French (1987). They found that Lignasan disappears relatively quickly, whereas the 3-year-rate of Arbotect provides pro-

tection up to 24 or more months. In a recent study, Stipes and associates (1987) found that residues of thiabendazole hypophosphite from the Arbotect 3-year rate dropped off precipitously in a study conducted in Roanoke, VA; he attributed the difference between their and the Stennes/French findings to the warmer climate in his study area that allow faster degradation of the fungicide.

Injection versus infusion. As discussed earlier, infusion is technically allowing the tree to take up the fungicide, using its own uptake potential, accorded to "transpirational pull," capillarity and root pressure; and this technique has often been called the "gravity flow" method, and with this no external pressure (compressed air, nitrogen, etc.) is used to introduce the chemical. Injection, on the other hand, is the forcible propulsion of a chemical into the tree, using compressed air, nitrogen, carbon dioxide or other propellent. The proponents and users of each technique attribute better distribution via their method, but the data are lacking. This information gap is important to bridge, because labor costs are higher when injecting, since the injector must "babysit" the project, whereas those who infuse the fungicide can set up a large number of units in sequence, and return at intervals to unhook their apparatus; vandalism and inquisitive persons may be problematic around unattended equipment.

Fungicides, tree growth regulators, or both. Elm tree growth regulation has been attempted with success in the control of DED, but the research has not been pursued to a practical endpoint. If spring vessel development can be deferred until the spring/early summer susceptibility period has passed or vessels occluded to prevent the transport of the fungus passed, then DED can be controlled (Beckman, 1958; Smalley, 1962). Pioneering work only has been done in this intriguing area. With the advent of new plant growth regulating compounds, the possibilities are many.

No one has previously attempted to integrate the use of fungicides and tree growth regulators, and this might prove to be highly effective in the control of DED. The injection of fungicides is a direct attack on the pathogen, either prior to or after infection has been initiated. The integration of an injection procedure utilizing both tree growth regulators and fungicides might prove to be the "silver bullet." The integration of several procedures in the control of DED should be investigated further, and this is another gap or glitch in the management of this disease.

Economics and practicality. In order for injection to be profitable to the practicing arborist, economics must be the "bottom line." Those who infuse claim higher profits because they can tend to many more trees per day than those who inject. The lateral root injection procedure championed by Kondo (1972) is undoubtedly the best way to achieve maximum and uniform distribution, but excavating lateral roots in many landscape situations is simply impractical; where possible and used, however, one can be assured of thorough distribution of the fungicide. Recently, work has been done to develop high pressure "shots" for trees, in which tree growth regulators have been injected; if these should work for fungicides, then such a method would be an extremely fast and economic way to protect elms and to inject many other trees.

Integration of control methods. As mentioned in an earlier section, the integrated approach is the best way to manage DED and in fact many or most other tree and other plant diseases. Several investigators have monitored combinations of methods, such as sanitation plus insecticide application, or sanitation plus use of resistance, but great gaps exist in evaluating a complete package of integrated disease management procedures. To date, it is impossible to rely on fungicide injection alone and expect to achieve complete or maximum control as some would report. When feasible, integration of all methods is the best means of controlling DED.

Case history studies. Any physician who does not keep case history records on his patients is not worth consulting. The most effective arborists are those who keep accurate records on trees they attend over the years, and because of this better value judgments on options in treatment can be chosen. Researchers are in a better position to keep records because they must. Many arborists indicate that they cannot afford to keep detailed records, but with the advent of computers that have software packages to process these kinds of data, the case histories of many

trees will be much easier to record and study.

Epilogue

So then how are these many gaps and glitches bridged? By research and careful observations made by practitioners. And this costs money. Millions of dollars are poured into various projects in the plant sciences, not to mention the billions allocated for research in human and animal medicine. The DED market is limited to begin with because it is a "specialty disease," and those earmarking funds for its study for decades have contributed pittance. Most of the technology developed for the control of DED has been done by researchers and practitioners working on "shoestrings." However, the love of investigation at any cost has propelled the few dedicated individuals in their efforts. Very few of this group have spent their lifetime in research, but several have been involved for shorter periods. "Hope springs eternal" in the elm lover's heart, and so surely as long as we have elms and DED, someone will be testing some new method or material to attempt to save this most handsome, useful and durable species that has adorned our landscape for centuries.

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AN URBAN FOREST INTEGRATED PEST MANAGEMENT PROGRAM FOR GYSPY MOTH: AN EXAMPLE

by Mark Ticehurst and Stuart Finley[1]

Abstract. An integrated pest management program for gypsy moth was designed, implemented, and evaluated in the urban forested community of Lake Barcroft, Falls Church, VA. The objectives of the program were to reduce or prevent defoliation, tree mortality, and nuisance associated with dense populations of gypsy moth. Intensive surveys of larvae, pupae, adult males, and egg masses were evaluated in 100 sites. Further evaluations were made of eggs per mass, egg viability, parasitism of eggs, larvae, and pupae, sex ratio of pupae, and tree susceptibility to infestation and defoliation. *Bacillus thuringiensis* and Luretape® were selectively applied. The larval parasites, *Cotesia melanoscelus* and *Glyptapanteles flavicoxis* were released throughout the Program area. The objectives were achieved. The cost was approximately $20. per residential lot per year.

Key words: Gypsy moth, integrated pest management, urban forest, implementation, evaluation.

The gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) is considered to be a forest pest throughout much of the world. The vast majority of the 1.3 million acres defoliated by the gypsy moth in the United States in 1987 (1) occurred on uninhabited forest lands. The economic impact of this pest is primarily recognized in terms of tree mortality. Tree mortality associated with gypsy moth defoliation during a three-year period on 690,000 acres in Pennsylvania was $104.2 million (4).

Currently the gypsy moth is invading the urban forests in the megapolis surrounding Washington, DC. The impact of this defoliator in this area is like-

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ting liquid fungicides in elm trees for the control of Dutch elm disease and other disorders. **In** Methods for Evaluating Pesticides for Control of Plant Pathogens, K.D. Hickey, Ed., American Phytopathological Society, St. Paul, MN, pp. 261-265.

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AN URBAN FOREST INTEGRATED PEST MANAGEMENT PROGRAM FOR GYSPY MOTH: AN EXAMPLE

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Abstract. An integrated pest management program for gypsy moth was designed, implemented, and evaluated in the urban forested community of Lake Barcroft, Falls Church, VA. The objectives of the program were to reduce or prevent defoliation, tree mortality, and nuisance associated with dense populations of gypsy moth. Intensive surveys of larvae, pupae, adult males, and egg masses were evaluated in 100 sites. Further evaluations were made of eggs per mass, egg viability, parasitism of eggs, larvae, and pupae, sex ratio of pupae, and tree susceptibility to infestation and defoliation. *Bacillus thuringiensis* and Luretape® were selectively applied. The larval parasites, *Cotesia melanoscelus* and *Glyptapanteles flavicoxis* were released throughout the Program area. The objectives were achieved. The cost was approximately $20. per residential lot per year.

Key words: Gypsy moth, integrated pest management, urban forest, implementation, evaluation.

The gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) is considered to be a forest pest throughout much of the world. The vast majority of the 1.3 million acres defoliated by the gypsy moth in the United States in 1987 (1) occurred on uninhabited forest lands. The economic impact of this pest is primarily recognized in terms of tree mortality. Tree mortality associated with gypsy moth defoliation during a three-year period on 690,000 acres in Pennsylvania was $104.2 million (4).

Currently the gypsy moth is invading the urban forests in the megapolis surrounding Washington, DC. The impact of this defoliator in this area is like-

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