FEATURE ARTICLE

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Introduction

In some situations, injecting trees with fungicides is an effective treatment for the management of Dutch elm disease (DED). Several injection products are on the market, and various means of application are recommended. Each product and method has pros and cons. The "best" product depends on the individual tree— its current condition, the objectives of the treatment, and the resources available. The purpose of this article is to bring the options and documentation together into one package, so that you can make an informed decision on what you will recommend for injection.

Basic principles of why and when injection works

To understand why and when injection works, you need to understand how the DED fungus gets into and kills elms. The fungus infects the vascular tissue of elms, causing the vessels in the active, outer rings of xylem to become clogged. The fungus gets into an uninfected elm in one of two ways: either through roots grafted to diseased elms, or by elm bark beetles feeding in the branches or upper crown of the tree. When an elm becomes infected through root grafts, the fungus can spread very rapidly and extensively throughout the tree's vascular transport system. Injection of currently available fungicides is not effective in protecting trees from root graft infection, or in therapeutically treating trees that have become infected through root grafts. Injection can be effective in preventing or treating infection caused by bark beetle inoculation.

When a bark beetle that is contaminated with DED fungus spores feeds on a healthy elm, several factors determine whether the tree will become infected by the fungus, or if infected, die. These factors include the inoculum load and point of introduction (this can vary by beetle species), the aggressiveness of the pathogen (at least three species of *Ophiostoma* cause DED, and they differ in aggressiveness), the physiology of the tree (vitality, vessel structure, etc.), the suitability of the environment for fungal growth (temperature, moisture, chemistry, etc.), and the ability of the tree to compartmentalize the infection (may differ by elm species or cultivar, health of the tree, etc.). Injection of fungicide into trees can be effective by either making the infection court unsuitable, or by stopping fungal growth within the tree. The former is the basis of preventive fungicide injection; the latter is the basis of therapeutic injection. Kondo (1978a), Campana (1977) and Stipes (1988) addressed some of the many factors that limit the effectiveness of fungicide injection.

To be effective in preventing infection, a fungicide must inhibit or kill the DED fungus, and it must be present in adequate concentration at all potential points of infection. Even when injected at fairly high dosage, the quantity of chemical present at the points of potential introduction of

the DED fungus is quite low (Elliston and Walton, 1979). The chemical, dosage and means of application are critical to success.

For therapeutic treatment, the fungicide must be applied before the fungus has caused extensive damage to the vascular system of the tree, so early detection and timely treatment are critical to success. Sherald and Gregory (1980) and Lanier (1988), among many others, specifically addressed therapeutic injection.

Chemicals for DED control have been researched since the 1940's (Zentmeyer et al., 1946; Dimond et al., 1949). There are extensive bodies of literature on the subject, including major portions of symposia proceedings (Kielbaso, 1978; Kondo et al., 1981; Miller, 1991). We will get more into specific chemicals and modes of action later in this article.

A bit more about methods of injection

There are two common ways of injecting the available fungicides into the vascular system of elms. Microinjection is forceful injection of a low volume of concentrated chemical into holes drilled into the stem or base of the tree. Macroinjection is the injection (under pressure) or infusion (without pressure) of large volumes of dilute chemical solutions into holes drilled in the stem or base of the tree (Stipes et al., 1999[in press]).

Kondo (1978b) refined the macroinjection system for injection into the excavated root flare of trees. He found that if the root flare was excavated the circumference of the stem was greater and more injection holes could be well-spaced around the stem, resulting in better chemical distribution in the crown of the tree. He also observed that the wood tissue in this stem-root transitional area seemed functionally different from stem tissue, and drill wounds in this area closed more rapidly with less wetwood problems than wounds higher on the stem. The holes are drilled with a 7/32 to ¼ inch drill bit, and plastic tees are inserted into the holes. The tees are all connected by tubing to a pressurized container (10-20 p.s.i.) of the fungicide solution. The amount of chemical and volume of solution to inject are based upon the diameter of the tree. In small diameter trees, there is proportionately less vascular tissue in the tree per unit of diameter, so care must be taken not to overdose small trees. This method has been demonstrated to thoroughly distribute chemical in the crown (Stennes and French, 1987). However, there are some drawbacks to macroinjection: injection wounds, if repeatedly inflicted, may eventually result in significant discoloration and decay (Shigo and Campana, 1977). The chemicals may also damage the cambium around the injection site. The chemicals may also cause foliar phytotoxity, especially on smaller diameter trees.

Microinjection for DED treatment is accomplished with pre-packaged canisters of chemical. Generally, the tips of the injection canisters are placed into holes drilled into the trunk or root flare of the tree and then are pressurized by the squeezing of a built-in plunger. The products are self-contained and require no extra water, which provides an advantage of convenience. Our literature search did not reveal any documentation that microinjection provides adequate distribution and effective concentrations of the chemical to consistently prevent or arrest DED infections. Microinjection has the same disadvantages as macroinjection in regards to phytotoxicity and injection wounds.

There is an art and a science to properly injecting chemicals. The procedure should be done by a certified arborist or skilled tree care specialist who has been specifically trained in the procedure.

Currently available chemicals

Six chemicals (in various formulations) are currently registered in the USA for injection to manage DED: three benzimidazole compounds (carbendazim phosphate, thiabendazole hypophosphite, and Debacarb), two triazole compounds (propiconazole and tebuconazole), and a patented formulation of copper sulphate pentahydrate. Another benzimidizole product, with carbendazim hydrochloride, is currently pending registration.

As a group, benzimidazoles are systemic and are active at low concentrations. Benzimidazole compounds affect fungal growth by interfering with mitotic cell division (Campana, 1977; Stipes Personal Communication). In low concentrations, benzimidazoles are fungistatic (prevent growth, but don't outright kill) toward the DED fungus (Grieg, 1986; Janatulo and Stipes, 1976). At higher concentrations, they are fungicidal (kill the fungus) (Janatulo and Stipes, 1976). Their acid salts are water soluble, and thus can be adapted for use in injection systems. The benzimidazole salts vary in their effectiveness against the DED fungus (Schreiber and Gregory, 1981) and in their chemical behavior in the tree. The effectiveness of these compounds depends on how well they distribute in the crown, the rate at which the chemical is applied (and thus concentration in the plant tissues), and how well they persist in the tree. Four different formulations of benzimidazoles are (or soon will be) available for DED management.

Carbendazim is a breakdown product of benomyl, which is insoluble in water. Acid salts of carbendazim are water soluble, and many have been tested for usefulness in DED injection; two have been available as commercial products. Carbendazim hydrochloride (originally available as Lignasan®) and carbendazim phosphate (originally available as Lignasan BLP®) were both shown to be effective against the DED fungus without high phytotoxicity to elm plant tissue (Kondo et al., 1973; Smalley et al., 1973; Gibbs and Dickinson, 1975; Schreiber et al., 1978; Schreiber and Gregory, 1981; and others). Carbendazim phosphate readily moves throughout the tree in the transpiration stream, but unfortunately does not move into new wood as it is produced, even when applied at high dosage rates, so preventive treatments using this chemical must be applied annually (Stennes and French, 1987; Nishijima and Smalley, 1978). Clifford et al. (1977) found little difference in persistence between carbendazim phosphate and carbendazim hydrochloride; both compounds declined rapidly between 2-3 months after injection.

Carbendazim phosphate is no longer available as Lignasan BLP®, but can be acquired as Elm Fungicide®. Proper root-flare injection at rates ranging from 0.98 to 3.2 g of carbendazim phosphate per cm of tree DBH results in levels of carbendazim in the twigs during the season of injection at levels inhibitory to the DED fungus (Nishijima and Smalley, 1978; Elliston and Walton, 1979; Stennes and French, 1987). The current label rate for Elm Fungicide® is 0.16 to 0.35 g carbendazim phosphate per cm tree DBH, which is far below the documented effective rate.

The original carbendazim hydrochloride formulation (Lignasan®) has not been commercially available in the USA for many years, but a new product called Eertavas® is pending registration. This product contains 4.7% carbendazim hydrochloride, which is a much higher concentration of

carbendazim than the currently available carbendazim phosphate product. At the time of this writing, we have no information on the label dosage rate for Eertavas®.

Thiabendazole hypophosphite (available as Arbotect 20S®) has also been shown to be effective against the DED fungus (Stennes and French, 1987; Greig, 1986). When injected at 5.6 g active ingredient per cm tree DBH, thiabendazole continues to appear in new wood in concentrations high enough to be detected by bioassay through three growing seasons (Stennes and French 1987). Protection of mature trees from artificial inoculation with the DED pathogen has been shown to last for two seasons in northern climates (Stennes, 1981). It is worth noting that in southern climates, with longer growing seasons, the effective period of the chemical may be shorter (Bruce Fraedrich, Personal Communication). Many arborists have successfully used the highest label dosage of Arbortect 20S® on a 2-3 year rotation to protect high value elms from DED for 15 years. Thiabendazole also has demonstrated effectiveness for therapeutic injection (Lanier, 1988; Stennes, 1999 [In press]). Unfortunately, however, the injection solution can be very damaging to the cambium as well as to parenchyma cells in a column of wood surrounding the injection site (Lanier, 1987; Andrews, Blanchette and French, 1982). Foliar phytotoxicity has also been reported (Lanier, 1987).

Debacarb is another benzimidazole fungicide available for injection. It is available in combination with carbendazim in microinjection canisters, as Fungisol®. The label does not indicate whether the carbendazim is formulated as carbendazim hydrochloride, carbendazim phosphate or a different salt. Lanier (1987) reported that Fungisol® did not significantly prevent infection of artificially inoculated branches, but that there did appear to be some effect on symptom progression within the tree. Lanier (1987) also tested this product as a therapeutic treatment and was not able to demonstrate effectiveness. Our literature search revealed no published documentation of the distribution or concentration of the active ingredients in elms trees treated with Fungisol®. The current label rate for this fungicide is approximately 0.016 g active ingredient per cm tree DBH. A study on carbendazim salts in other formulations determined that the minimum application level at which carbendazim phosphate could be detected in elm shoots by bioassay is 0.98 g active ingredient per cm tree DBH (Stennes and French, 1987). Elliston and Walton (1979) found that carbendazim applied at low levels (0.16 g active ingredient per cm tree DBH) resulted in low or undetectable recovery of the chemical in twigs. The documented effective dosage rate for carbendazim is 60 times higher than the current label rate for Fungisol®, which brings the efficacy of this product into question.

The triazole fungicide propiconazole is effective in management of oak wilt disease (Appel and Kurdyla, 1992), and it is also labeled for management of DED. It is a highly systemic sterol inhibitor that prevents fungal growth by interfering with cell wall formation. The commercially available formulation of propiconazole (Alamo®) is microencapsulated to make it soluble in any clean water near neutral pH. Stipes (1994; 1999[in press]) has demonstrated propiconazole to be effective in preventing DED infection following challenge inoculations by the DED fungus. The rates of propiconazole used in Stipes' (1999[in press]) studies number 6, 9, 10, and 11 varied from 1.1 to 3.6 grams of active ingredient per cm tree DBH. The current highest label rate for Alamo® is equivalent to 1.1 gram active ingredient per cm of tree DBH. Propiconazole at the highest label rate may provide protection for multiple seasons, as two mature elms which were challenge inoculated multiple times at multiple points were protected against DED

infection for an equivalent length of time as trees treated with thiabendazole hypophosphite (Stennes, unpublished data). However, according to recent findings by Stipes (1999[in press]), residual activity of propiconazole is considerably shorter than that of thiabendazole hypophosphite. Therapeutic treatment of 24 mature symptomatic American elms in 1996 and 1997 with the highest label rate of Alamo® resulted in 79% survival by the end of the 1998 growing season, so propiconazole has demonstrated therapeutic value (Stennes, 1999 [in press]). Foliar toxicity of propiconazole is low, even at rates of up to 3.8 grams per cm tree DBH (Stipes, 1999[in press]), though severe phytotoxicity may occur with high dosage rates on small diameter trees when treated early in the growing season (Bruce Fraedrich, personal communication). Propiconazole does not require high dilution rates with water so treatment is considerably faster than with thiabendazole hypophosphite, and there is less tissue injury at the injection site (Stennes, 1999 [in press]).

Another triazole fungicide, tebuconazole, has very recently been registered for use against DED. Mauget's Tebuject® is a microinjection product containing this fungicide. There are no published research data on the use of tebuconazole to manage DED.

The activity of copper sulphate pentahydrate (available as Phyton 27®) is based on the fungicidal effect of metallic copper. Knutson (1991) reported a 22% higher level of copper in the leaves of a Phyton 27® treated elm compared to leaves of untreated elms at 15 months after treatment. The distributors of Phyton 27® claim protective effect against DED for at least 36 months, but there are no published data on this treatment. Leaf abscission is common following Phyton 27® injection, but is usually followed by refoliation. Lanier (1987) reported severe vascular tissue discoloration and damage at Phyton 27® injection sites and only seemingly marginal fungicidal effect within the tree.

An injected non-fungicide protective treatment has recently been developed. It is a suspension of live spores of the fungus *Verticillium dahliae,* which is injected into the tree with a specially developed "gouge pistol"(Elgersma et al., 1993; Sutherland et al., 1995; Unidentified, 1998). Treatment reportedly protects against new infections by the DED fungus during the year of treatment by inducing resistance in the tree. This product, marketed as Dutch Trig®, is currently available in U.S. under test exemption and is being tested primarily by Bartlett Tree Research Laboratories. The product was developed by ARCADIS Heidemij in the Netherlands and is distributed in the U.S. by Innovative Tree Services L. L. C., Tampa, FL.

There are pros and cons to this new "Dutch Trig®" treatment. The injection holes are small and seal quickly. The application procedure is relatively rapid; A 75 cm DBH tree can be treated in approximately 10 minutes. Single treatment cost may be considerably less than fungicide (price in US is still to be finalized), but the treatment must be performed every year. Early results of tests on American elm in the U.S. have shown a protective, but not therapeutic effect. Another potential drawback is that the fungus *Verticillium dahliae* is a plant pathogen, and the isolate used in this product is of European origin. This isolate cannot be recovered from trees one season after inoculation. APHIS did consider the risk of introducing an exotic strain of a plant pathogen prior to allowing experimental use of this product in the U.S. We also do not yet know the cumulative effect of annual treatments on the health of the tree: treatment causes discoloration of the annual ring, so apparently the physiology of the host tree is affected.

Table 1. A summary comparison of chemicals available for injection to manage DED.

Common name	Chemical name	Commercial products (Trade name, source and % active ingredient in formulation)	Current Label Dosage Rate for commercial product	Comments (see text for full discussion)
Carbendazim phosphate (MBC•PO_4)	Methyl-2-benzimidazole carbamate phosphate	Elm Fungicide® (formerly Lignasan BLP) Elm Research Institute, Harrisville, NH (0.7% Carbendazim phosphate)	Approximately 126 ml per inch of diameter Note: 126 ml/inch DBH = 0.35 g active ingr. per cm DBH	Benzimidazole fungicide. Low phytotoxicity. Does not persist in newly formed wood; treatments must be applied annually. Effectiveness against DED fungus documented in scientific literature, but at a rate higher than current label.
Carbendazim Hydrochloride (MBC•HCl)	Methyl-2-benzimidazole carbamate hydrochloride	Eertavas® St. James Tree Service Winnipeg, Manitoba, Canada (4.7% carbendazim hydrochloride)	Not yet known.	Benzimidazole fungicide. Low phytotoxity. Low persistence.
Thiabendazole hypophosphite (TBZ•H_2PO_2)	2-(4-thiazolyl) benzimidazole hypophosphite	Arbotect 20-S® Novartis Corp., Greensboro, NC. (20% Thiabendazole)	"3 yr rate" is 12 oz. (72 ml) per inch of diameter Note: 12 oz/inch DBH = 8.1 g active ingr. per cm DBH	Benzimidazole fungicide. May cause phytotoxicity at injection site and in crown. Effective for up to 3 seasons (in North) at highest label rate. Preventive and therapeutic effectiveness against DED fungus documented in scientific literature.
Debacarb (DEBC)	2-(2-Ethoxyethoxy) ethyl 2-benzimidizole carbamate	Fungisol®, Abasol®, and Imisol® microinjectors J.J. Mauget Company, Burbank, CA (1.7% Debacarb and 0.3% Carbendazim)	2.0 ml per inch of diameter Note: 2.0 ml/inch DBH = 0.016 g active ingr. per cm DBH	Both active ingredients are benzimidazole fungicides. Very limited published data indicates some effectiveness for preventive of progression of symptoms. Label dosage rates are far below rates proven effective for similar chemicals.
Propiconazole	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole	Alamo® Novartis Corp., Greensboro, NC (14.3% encapsulated Propiconazole)	Microinjection: One 10 ml unit per inch DBH. Macroinjection: 6 to 10 ml per inch DBH (preventive); 20 ml per inch DBH (preventive and therapeutic) Note: 20 ml/Inch DBH = 1.13 g active ingr. per cm DBH	Triazole fungicide. Available in both microinjectors and as liquid for macroinjection. Low phytotoxity. May be effective for more than one season at highest label rate, though persistence of chemical in elm tissue is low. Preventive and therapeutic effectiveness against the DED fungus has been documented in scientific literature.
Tebuconazole	alpha-[2-(4-chlorophenyl)-ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	Tebuject®microinjectors J.J. Mauget Company, Burbank, CA (% actuve ingredient unknown)	Unknown	Brand new product. Triazole fungicide. No information available on dosage or effectiveness at time of press.
Copper sulphate pentahydrate	Copper sulphate pentahydrate	Phyton 27® by Source Technology Biologicals, Inc. of Edina, MN (21.36% copper sulphate pentahydrate)	Approx 4.0 ml per inch of diameter (actual rate is based on a table of diameter classes) Note: 4.0 ml/inch DBH = 0.34 g active ingr. per cm DBH	Fungicidal activity based on metallic copper. Effectiveness of this chemical to prevent or arrest DED is not documented in independent scientific literature. Manufacturer will provide data upon request.

Fitting it all together into a management strategy

Injection is only one part of an overall management strategy for Dutch elm disease, but it does provide some options for protecting or saving high value individual trees. The recent USDA Forest Service publication "How to Identify and Manage Dutch elm Disease" (Haugen, 1998) provides information on how various management activities can be used to interrupt the DED disease cycle. A copy of this publication is enclosed with this issue of PDQ. It is also available on the WWW at http://willow.ncfes.umn.edu/ht_ded/ht_ded.htm

Injection is only for high value trees. With the exception of DDT sprays to prevent smaller European elm bark beetle feeding, all of the available evidence indicates that every historically successful Dutch elm disease management program has depended almost exclusively on sanitation to reduce bark beetle populations and the available inoculum of the DED fungus. Even with injection of selected trees, no program will succeed without the sanitation necessary to minimize disease pressure. Conversely, individual owners of high value elms may not be able to rely on sanitation alone for protection if their city does not enforce mandatory removal of diseased landscape or wild elms. Subsequently, where stringent sanitation practices cannot be followed, fungicide treatments play an important role.

Therapeutic treatment is only an option for early stages of infection, but it is a potentially powerful tool when added to successful sanitation programs that pivot around thorough inspections and prompt removals. It is not always effective, but a success rate as low as 50% may be more than enough to justify the cost of the effort. The cost of tree removal is high, and the value of large stately elms is even greater.

There are risks to tree health in injecting trees. A long-term preventive injection program may cause significant stem damage to a valuable elm. Consider whether early detection (and thus opportunity for therapeutic treatment) is likely for a high value elm. Consider whether bark beetle and DED fungal populations are high in the surrounding area. As with any resource management decision, it is important to weigh the risks against the benefits.

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