

PROPICONAZOLE AS A TREATMENT FOR OAK WILT IN QUERCUS RUBRA AND Q. ELLIPSOIDALIS

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Abstract. In Minnesota, propiconazole was injected into mature *Quercus rubra* and *Q. ellipsoidalis* at high risk to natural infection with *Ceratocystis fagacearum*, the oak wilt fungus. The trees were treated preventively in 1989 and 1990 at a constant rate of 0.168 g a.i./cm dbh using a root flare injection technique. Control trees were left untreated. Since 1989, 15 of 49 trees treated in 1989 have wilted in comparison to 18 of 50 control trees. Nine of 88 treated trees in 1990 have wilted since 1990, whereas 42 of 80 control trees have wilted. Propiconazole was effective in 1990 as a preventive treatment for oak wilt in *Q. rubra* and *Q. ellipsoidalis*. Propiconazole was detected in the vascular tissue of a treated tree 12 months after injection using a thin layer chromatography (TLC) assay procedure; propiconazole was not detected 20 months after injection. Results from the assay procedure imply treatment with the fungicide would be required approximately once every 2 years.

Key words: *Ceratocystis fagacearum*, *Quercus rubra*, *Q. ellipsoidalis*, oak wilt, propiconazole.

Oak wilt, caused by *Ceratocystis fagacearum*, is a vascular wilt disease that attacks all *Quercus* spp. (5). The red oaks (subgenus *Erythrobalanus*) are most susceptible to wilt, with trees dying within 2 to 8 weeks of becoming symptomatic, whereas the white oaks (subgenus *Lepidobalanus*) are more resistant to wilt with trees often taking several years to succumb.

Oak wilt is transmitted in two ways; overland via Nitidulid beetles (5,7,8,13), and underground via root grafts (2,5). In Minnesota, root graft transmission of oak wilt is believed to account for 90 to 95% of disease spread (5). Because of the prevalence of root grafting among oaks, oak wilt has the potential of being a serious problem in Minnesota, where homogeneous oak stands are frequent. Many of the control methods currently available are directed toward disrupting root graft transmission.

Mechanical disruption of roots is the most successful control method currently available (11). With this method a vibrating plow blade is drawn through the soil to sever any existing grafts. Two plow lines are often put in; a primary and a secondary plow line. The secondary plow line is placed immediately outside of the infection center. A primary plow line is placed outside of the row of healthy-appearing trees adjacent to the infection center. This control method is up to 93% effective in preventing further spread of the disease (11); however, most of the trees within the primary plow line die.

In Texas, Appel (1) recently reported the sterol-inhibiting fungicide, propiconazole, as an effective preventive treatment for oak wilt in live oaks (*Q. fusiformis* and *Q. virginiana*, subgenus *Lepidobalanus*). Propiconazole inhibited mycelial growth of the fungus *in vitro*. The fungicide also suppressed wilt development 100% in containerized seedlings, and was 97 to 100% effective in preventing live oaks from becoming infected with oak wilt in the field.

In Minnesota, propiconazole was evaluated as a possible preventive treatment for *Q. ellipsoidalis* and *Q. rubra* (subgenus *Erythrobalanus*) at high risk to natural infection with oak wilt. Trees at high risk are those within 15 m (root grafting distance) of an active infection center.

Materials and Methods

Site Location: Sites 1 through 5 which contained control and treated trees from 1989 will be referred to as Experiment A. Sites 1, 2, and 3 were located in Anoka County, site 4 in Ramsey County, and site 5 in Dakota County. Sites containing control

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and treated trees from 1990 will be referred to as Experiment B. These sites include site 6 (Anoka County), site 4 (Ramsey County), and site 7 (Dakota County). Additional information for each site is in Table 1.

Root Flare Injection: Propiconazole was injected into trees at a constant rate of 0.168 g a.i./cm dbh (10), using a root flare injection technique (12). Injection pressure was kept as constant as possible between 1.4 to 2.1 kg/cm². Trees were connected to the injection apparatus a maximum of 24 hr, with 2 hr being the average time for injection. Concentration of the solutions injected into the trees varied from 0.21 g a.i./L (trees injected in 1989 and 1990) to 0.42 g a.i./L (trees injected in 1990). No phytotoxicity was observed in trees injected at either concentration. Trees were treated from 1 June through 15 September in each year. Control trees were left untreated.

In 1989, deionized water was used to dilute the fungicide. In 1990 and 1991, well water available at the injection sites was used. The solubility of the chemical in deionized and in well water was approximately equal.

Injected trees and control trees were observed the summer of injection and the following summers for symptoms of oak wilt. A completely wilted tree was regarded as a dead tree. Trees with oak wilt symptoms were sampled for the presence of

C. fagacearum.

Data collected from all experimental sites were analyzed using a two-tailed Student t test. The number of treated trees wilting (p_1) was compared with the number of control trees wilting (p_2) using the hypothesis; H_0 : p_1 equals p_2 versus H_a : p_1 does not equal p_2 .

Assay procedure: An assay procedure developed by CIBA-GEIGY consisted of a thin layer chromatography (TLC) technique to detect the presence of propiconazole in the vascular tissue of an injected tree. A sample of vascular tissue from an injected tree was coarsely ground into pulp then suspended in ethyl acetate for extraction. The liquid portion remaining from the extraction was flash evaporated to dryness and the precipitate suspended in acetone. The sample solution was then spotted on a silica gel TLC plate and allowed to dry. A solution of propiconazole in acetone (0.0675 g a.i./L acetone) was also spotted onto the plate to serve as a fungicide standard. The plate was placed in a developing tank filled with 100 mL of a toluene:chloroform:acetone solution (40:25:35, v:v:v) for 20 to 25 minutes. After development, the plate was dried in an oven at 100° C for 10 minutes. Once cooled, the plate was sprayed with a spore suspension of *Cladosporium caryigenum* in 2% malt extract (6,9). It was then incubated in a moist chamber in the dark for 72 to

Table 1: The data presented provides descriptions of the experiment sites including average diameter of the trees on each site, average number of injection ports used per tree, average time for injection per tree, and soil type found on each site.

Site	No. of Trees	Mean dbh (cm){SD}	Mean No. injection ports {median}	Mean time for injection (min) {median}	Soil type	Year site used {experiment}
1	15	30.9 {9.3}	35.2 {32.0}	122.5 {115.0}	sand	1989 {A}
2	50	26.8 {6.4}	0	0	sand	1989 {A}
3	22	31.9 {20.0}	39.7 {38.0}	122.3 {105.0}	sand	1989 {A}
4	4	40.2 {10.3}	43.5 {45.5}	115.0 {102.5}	silt/loam	1989 {A}
4	5	61.2 {33.2}	40.6 {40.0}	286.0 {245.0}	silt/loam	1990 {B}
5	8	44.2 {16.5}	43.5 {45.5}	115.0 {102.5}	silt/loam	1989 {A}
6	76	10.9 {9.4}	45.9 {13.0}	1971.0 {277.5}	sand	1990 {B}
6	80	13.2 {9.6}	0	0	sand	1990 {B}
7	7	63.2 {15.4}	34.1 {36.0}	165.7 {135.0}	silt/loam	1990 {B}

96 hours. Bands of clear zones in the fungicide standard lane and the sample lanes were compared.

Samples for determining longevity of the fungicide within the vascular tissue of a treated tree were taken at dbh (1.4 m). To determine fungicide distribution within a treated tree, samples were taken at ground level, dbh, 3.0 m, and 3.8 m. Samples from control trees were taken at dbh. Trees were sampled at 1, 2, 8, 12, 20, and 23 months after injection.

Results

Experiment A: There were five experimental sites in 1989. Trees on sites 1, 3, 4, and 5 were treated; those on site 2 were left untreated to serve as controls. A summary of the data collected from all Experiment A sites is in Table 2.

Fifteen of the 49 trees from Experiment A wilted. By comparison, 18 of the 50 control trees wilted. The number of treated trees wilting and the number of control trees wilting were not significantly different from each other ($P = 0.57$). The treated sites located on silt/loam soil did much more poorly than the treated sites located on sandy soil (10 of 12 trees wilting and 5 of 37, respectively). Mean diameter of the treated trees wilting was 38.7 cm (SD = 14.7), while the mean diameter of control trees wilting was 26.6 cm (SD = 7.4).

On site 1, three of 15 treated trees wilted. Of the three wilted trees, one was injected when oak wilt symptoms were present. This tree did not leaf out

in 1990. The other two trees wilted approximately 22 months after injection. Site 2 contained the 50 control trees. No trees wilted in 1989. However, two trees wilted in 1990, followed by 16 more in 1991. Twenty-two trees were injected on site 3, with two of these trees wilting in 1991. In 1989, four trees were treated on site 4. Two of those four trees were also injected in 1990 at a lower injection rate (0.084 g A.I./cm dbh); five more were injected at the original rate (see "Experiment B"). Of the four trees treated in 1989, three wilted in 1991. One of the trees receiving two treatments wilted and both of the trees receiving a single treatment wilted. On site 5, eight trees were injected. Three of the trees wilted in 1990; four more wilted in 1991.

Experiment B: Three sites were used in Experiment B (1990 injection sites). One, site 6, contained both treated and control trees. The other sites, sites 4 and 7, contained treated trees. The data collected from these sites are summarized in Table 3.

Eighty-eight trees were injected, while 80 trees were left untreated (controls). Nine of the 88 treated trees wilted compared to 42 of the 80 control trees. There is a statistically significant difference between the number of treated trees wilting and the number of control trees wilting ($P < 0.001$). Again, treated trees located on sites with silt/loam soil did not fare as well as those located on the site with sandy soil (three out of 12 wilting compared to six out of 76, respectively). The mean diameter of the treated trees wilting equaled

Table 2: Data collected from Experiment A. Trees were treated in 1989.

Site	Treatment ^a	No. of trees	No. wilted in 1989	No. wilted in 1990	No. wilted in 1991	Total No. wilted
1	0.21 g/L	15	1	0	2	3
2	0	50	0	2	16	18
3	0.21 g/L	2	0	0	2	2
4	0.21 g/L	4	0	0	3	3
5	0.21 g/L	8	0	3	4	7
Totals	Treated	49	1	3	11	15
Totals	Control	50	0	2	16	18

^aTreatment = concentration of the solution (fungicide in water) used.

Table 3: Data collected from Experiment B. Trees were treated in 1990.

Site	Treatment ^a	No. of trees	No. wilted in 1990	No. wilted in 1991	Total No. wilted
6	0.21-0.42 g/L	76	3	3	6
6	0	80	37	5	42
4	0.21 g/L	5	1	1	2
7	0.21 g/L	7	0	1	1
Totals	Treated	88	4	5	9
Totals	Control	80	37	5	42

^aTreatment = concentration of the solution (fungicide in water) used.

30.6 cm (SD = 21.2) in comparison to a mean diameter of 16.5 cm (SD = 10.5) for control trees wilting.

Seventy-six trees were treated on site 6. Of the 76 trees, 3 wilted in 1990 with 3 more wilting in 1991. An additional 80 trees located on this site were left untreated to serve as controls. Thirty-seven of the 80 control trees wilted in 1990; five wilted the following year.

In addition to the four trees originally treated, five trees were injected on site 4. One of the trees had oak wilt symptoms when injected. This tree leafed out the following summer but eventually died of oak wilt. Another tree wilted in 1991. Site 7 contained seven trees. None of the treated trees wilted in 1990, but one wilted in 1991.

Results From Chromatography Assay: Propiconazole was detected in the vascular tissue of treated trees 1, 2, 8, and 12 months after injection. No fungicide was detected in the samples taken from trees treated 20 and 23 months after injection. No fungitoxic compounds were detected in samples taken from control trees.

In another study, samples were taken from a tree (32.1 cm dbh) 8 months after injection at heights varying from ground level to 3.8 m. Another tree (33.5 cm dbh) was sampled similarly 20 months after injection. The fungicide was detected in the 32.1 cm dbh tree up to the height of 3.0 m. The fungicide was not detected in the 33.5 cm dbh tree at any height.

Several trees that wilted after injection were sampled for the presence of propiconazole. Two trees that wilted within 1 month after injection were

sampled at ground level. The fungicide was detected in the vascular tissue of both trees. A tree that wilted within 12 months of injection was sampled at dbh. Again, the fungicide was detected using the TLC assay procedure. Propiconazole was not detected in two trees that wilted 23 months after injection.

During the assay procedure, two bands were often detected in the sample lanes on the silica gel plates. Both bands matched the one formed in the lane with the fungicide standard. When a standard solution made from an older supply of propiconazole (16-18 months) was used, again, two bands were usually detected.

Discussion

The results obtained from Experiment B suggest propiconazole is effective as a preventive treatment for oak wilt in *Q. rubra* and *Q. ellipsoidalis* at high risk to natural infection by *C. fagacearum*. The total number of treated trees that wilted was significantly different from the total number of control trees that wilted ($P < 0.001$). In 1990, the year of injection, this disparity was most obvious with four wilted trees of the 88 treated with propiconazole compared with 37 wilted trees of the 80 control trees. However, in 1991, five of the remaining treated trees wilted, as did five of the remaining control trees. One possible reason for this may be the irregular habit with which *C. fagacearum* spreads through root grafts. One section of the infection center may have been enlarging faster than in the other sections.

Although the results from Experiment B sug-

gest propiconazole is effective as a preventive treatment, the pooled results from Experiment A do not indicate the fungicide is an effective control. In Experiment A, there was no significant difference between the number of treated trees wilting and the number of control trees wilting in 1989, 1990, and 1991. It should be noted the majority of treated trees wilting were those on sites that did not have sandy soil.

A trend seen on sites 1, 3, 4, 5, 6, and 7 is the number of treated trees that wilted increased as time after injection increased. On sites 1, 3, 4, and 5 (Experiment A), the number of treated trees wilting increased steadily from 1989 to 1991 (from one to three to 11). While the pattern is less striking on sites 6 and 7 (Experiment B), it is still present; four trees wilted in 1990, with this number increasing to five in 1991.

Another trend seen on sites 1, 3, 4, 5, 6, and 7 is that trees growing on sites with sandy soil fared better with the treatment than trees on sites with other soil types. The reasons for this are unknown; possible explanations for this phenomenon may be obtained in future research.

In Michigan, it has been shown that spread of oak wilt is related to soil type, tree diameter, and distance between adjacent trees (3). In Minnesota, tree diameter did not play as large a role as in Michigan (personal observation). On sites that contained treated trees, tree diameter did not appear to be a major factor as both large and small diameter trees died; no patterns were observed in disease spread. The sites containing control trees also exhibited no apparent patterns. Distances between wilted trees and adjacent healthy trees on treated sites versus on control sites were approximately equal (data not shown).

No mycelial mats (4) were observed forming on treated trees that wilted, while mats were present on control trees. Treatment with the fungicide may have an effect on overland transmission, as well as root graft transmission. Another observation made during field research was that the longer the time of injection, the more likely the tree would wilt. Once symptoms of oak wilt appeared, complete wilt occurred within 2 to 8 weeks for most treated and control trees. One treated tree retained the lower 30% of its canopy as green foliage for

approximately 10 weeks. This tree was removed by the cooperators at that time; no further observations were possible.

The results from the TLC assay procedure showed that propiconazole was present in treated trees up to 12 months after injection. Propiconazole was not detected in samples taken 20 or 23 months after treatment. This information helps explain the trend seen in the field. If the fungicide is not present in the vascular tissue after a certain period of time, or loses effectiveness over time, it follows that the number of treated trees wilting in the field would increase as time after injection increased.

The fungicide evidently breaks down over time, as well. On the silica gel plates, two bands were seen in the fungicide standard lane when the standard solution made from an old supply of propiconazole was used. Two bands were also seen in the sample lanes. These results support the hypothesis that the chemical does degrade, or lose effectiveness, over time.

The results mentioned above suggest that *Q. rubra* and *Q. ellipsoidalis* at high risk to infection with oak wilt be treated with propiconazole every 18 to 20 months (once every 2 years). Time between treatments may need to be adjusted depending on the site.

Using the TLC assay procedure, a rough estimate of the distribution of the fungicide within the vascular tissue of a treated tree was determined. The chemical was detected in the vascular tissue of a treated tree up to a height of 3.0 m, 8 months after injection. Distribution throughout the root system was not determined. Ideally, propiconazole should be distributed throughout the entire tree. The results of the assay procedure suggest that little or no fungicide was translocated to the canopy of the treated trees. The majority of the propiconazole may have been translocated to the root system, but as the root system was not sampled, this statement is conjecture. As the goal of the treatments was to prevent root graft transmission of *C. fagacearum*, translocating most of the fungicide to the root system would be preferable to translocating it to the canopy. More research needs to be done to discover the approximate distribution of the fungicide throughout an

entire treated tree. The amount of fungicide needed to ensure complete distribution throughout the tree also needs to be determined.

The results from this research suggest that propiconazole is an effective preventive treatment for oak wilt in *Q. rubra* and *Q. ellipsoidalis* at high risk to root graft transmission of oak wilt. The cost of the fungicide and the injection process, however, will limit the use of this control measure. Vibratory plowing is still the best control method available in a large stand. Injection with propiconazole is more suited to an urban setting, where valuable shade trees can be protected successfully.

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Résumé. Du propiconazole fut injecté à des *Quercus rubra* et des *Q. ellipsoidalis* à maturité du Minnesota qui étaient à risque élevé d'infection naturelle par le *Ceratocystis fagacearum*, c'est-à-dire le champignon de la flétrissure du chêne. Les arbres furent traités préventivement, en 1989 et 1990, à un taux de 0.168 g par cm de D.H.P. au moyen d'une technique d'injection racinaire. Des arbres-contrôle furent laissés sans traitement. Depuis 1989, 15 des 49 arbres traités en 1989 ont présenté de la flétrissure en comparaison avec 18 des 50 arbres-contrôle. Neuf des 88 arbres traités en 1990 ont présenté de la flétrissure dans la même année, alors que 42 des 80 arbres-contrôle ont subi le même sort. Le propiconazole fut effectif en 1990 comme traitement préventif de la flétrissure du chêne pour le *Q. rubra* et le *Q. ellipsoidalis*. Le propiconazole fut détecté, au moyen de la procédure d'essai sur couche chromatographique mince, dans le tissu vasculaire d'un arbre 12 mois après l'injection; mais il ne fut pas 20 mois après. Les résultats de la procédure d'essai indiquent que le traitement avec le fongicide pourrait être requis une fois par deux ans approximativement.

Zusammenfassung. In Minnesota wurde das Fungizid 'Propiconazole' in ältere *Quercus rubra* und *Q. ellipsoidalis* injiziert bei hoher Gefahr der natürlichen Ansteckung mit *Ceratocystis fagacearum*, dem Eichenwelkepilz. Die Bäume wurden 1989 und 1990 vorbeugend mit einer "root flare" Injektions-Technik behandelt bei einer konstanten Rate von 0,168 g je Zentimeter Stammdurchmesser. Kontrollbäume bleiben unbehandelt. Seit 1989 sind 15 der 49 Bäume, die 1989 behandelt wurden befallen, im Vergleich zu 18 der 50 Kontrollbäume. Neun von 88 Bäumen, die 1990 behandelt wurden, sind seit 1990 befallen, dagegen 42 der 80 Kontrollbäume. 1990 war 'Propiconazole' als eine vorbeugende Behandlung gegen Eichenwelke in *Q. rubra* und *Q. ellipsoidalis* wirksam. Das Fungizid wurde 12 Monate nach Injektion im Gefäßsystem eines behandelten Baumes mittels Dünnschichtchromatographie entdeckt; 20 Monate nach der Injektion wurde jedoch nichts mehr gefunden. Die Ergebnisse der Versuche lassen darauf schließen, daß eine Behandlung mit dem Fungizid ungefähr alle 2 Jahre einmal notwendig ist.