

# Systemic Tree Injection of Propizol (14.3% wt./wt. propiconazole ME) in Austrian Pine (*Pinus nigra*) for Control of Diplodia Tip Blight (*Diplodia pinea*)

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**Abstract.** Diplodia tip blight (*Diplodia pinea*) affects mature pines, including Austrian (*Pinus nigra*) and Ponderosa (*P. ponderosa*) pines. Infections spread from needle fascicles to branch and, if unchecked, to the entire tree. Efficacy studies of fungicide injections in conifers are limited. Minute vascular tissues and resin exudate, a response to drilling, present impediments to injection. The efficacy of Propizol (14.3% propiconazole) for control of Diplodia tip blight in Austrian pines was evaluated. We evaluated (1) time of year, (2) injection spacing, and (3) fungicide dilution with respect to injection efficiency. Late fall injections expedited uptake, which is consistent with the reduced monoterpene emission rates in autumn and winter reported by Kim et al. 2005 and Lim et al. 2008. The time required for the dose to be administered was recorded for close and wide spacing of injection sites. Close spacing had the greatest impact on reducing the application time, irrespective of time of year. Low volume injections required less time to apply compared to high volume. Regardless of the application method, we observed a significant decrease in disease incidence in Propizol-treated trees. Injections applied in late fall resulted in a mean reduction in infections of new candles in the next growing season. Injections in the following spring, however, did not result in improvement in candle condition until a year later. We believe that these differences are based on whether the fungicide was applied prior to or after infection. Based on these findings, we recommend Propizol prior to infection for optimal results.

**Keywords.** Austrian Pine; Diplodia Tip Blight; Propiconazole; Tree Injection.

## INTRODUCTION

Tip browning of pines is symptomatic of Diplodia tip blight (*Diplodia pinea*, *syn. Sphaeropsis sapinea*). Most affected are Austrian pine (*Pinus nigra*) and Ponderosa pine (*P. ponderosa*), and to a lesser degree, Scots pine (*P. sylvestris*) and Mugo pine (*P. mugo*) (Ziems 2008). Stanosz and Smith (2009) reported *D. pinea* on native pines, including loblolly (*P. taeda*) and longleaf (*P. palustris*) in the gulf coast region. Ong et al. (2007) reported *D. pinea* shoot blight of Afghan (*P. eldarica*) and Austrian pines in Texas. Diplodia tip blight primarily affects established landscape trees, generally 30 years or older (Ziems 2008). Untreated, repeat infections can spread from needle fascicles to the entire tree branch and ultimately result in tree death. It kills branches by causing cankers and infects the vascular tissue with bluestain (Flowers et al. 2003). Environmental stresses are factors that

elicit disease. In South Africa, in Monterey pine (*P. radiata*), it is associated with wet conditions and high temperatures during shoot expansion (Swart et al. 1985). Storm damage (wind, ice, hail) and moisture stress are factors that predispose the tree to infection. Moisture stress was shown to predispose red pine (*P. resinosa*) to infection and occurred in the driest year of study in Wisconsin (Blodgett et al. 1997). Austrian pine (*P. nigra* subsp. *nigra*) is widely planted and valued for its tolerance to drought, ice, wind, and salt. However, Sherwood et al. (2015) demonstrated that drought-stress increased Austrian pine susceptibility to *D. pinea*.

Hartman et al. (2009) considered injections of fungicides into the lower trunk and root flare using oxy-carboxin, debacarb, and tebuconazole against Diplodia tip blight in Kentucky with apparently little success, although the two latter fungicides were effective in

**Table 1. Austrian pine foliar rating scale**

Rating	Description	Percent of foliage affected
1	Green, healthy canopy	0% foliar symptoms
2	Green canopy, symptoms of stress	1-24% yellow to red foliage
3	Canopy stressed or infected	25-49% yellow to red foliage
4	Canopy in decline	50-74% yellow to red foliage
5	Tree in severe decline or dead	75-100% yellow to red foliage

vitro. In their experiments, fungicides were applied at label rates of 14 mg active ingredient per inch (2.5 cm) diameter. Mayfield et al. (2008) compared the efficacy of propiconazole and thiabendazole against laurel wilt (*Raffaelea* spp.) in Redbay trees (*Persea borbonia*) and found that 1 ppm propiconazole was fungitoxic, and 0.1 ppm was fungistatic, whereas activity of thiabendazole was observed on the order of 10 ppm. Docola et al. (2011) found that propiconazole injected into loblolly pine (*P. taeda*) was effective at limiting lesion development from *Ophiostoma minus* for two years. Our primary aim was to quantify the effectiveness of Propizol (14.3% wt./wt. propiconazole, Arborjet, Inc., Woburn, MA) (PPZ) against Diplodia tip blight in Austrian pine by tree injection. A secondary objective was to optimize the injection process by making late season applications and/or by altering injection site spacing.

## METHODS

Our study sites were located at Forest Akers West Golf Course (3535 Forest Road, Lansing, MI 48910) and at Forest Akers East Golf Course (2231 S. Harrison Road, East Lansing, MI 48823). The course was first built in 1958, and the east course was redesigned in 1997, therefore the pines range from 20 to 60 years

old (east and west, respectively). The golf course trees were planted as buffer trees or define the course and form (respective) even-aged stands. The within-stand size difference, we believe, is a function of competition among trees. Austrian pines were selected based on a foliar rating scale that we developed (Table 1). Only trees rated 1 through 3 were selected for study. The number of replicates per treatment was 10; trees were blocked (i.e., trees rated 1, 2, and 3 were equally represented among treatments) in a complete randomized block design.

The Austrian pines had a mean diameter at breast height of 49.3 cm  $\pm$  11.4 standard deviation, ranging from 30 to 80 cm dbh. The untreated checks had the greatest mean diameter, but this did not vary significantly from the PPZ infusion trees, which in turn were not significantly different from the other remaining treatments (Table 2).

The four treatments were applied to Austrian pine with symptoms of stressed or infected foliage. These were (A) Propizol (14.3% wt./wt. propiconazole, Arborjet, Inc., Woburn, MA) (PPZ) injection, (B) Propizol micro-infusion, (C) TBZ injection (16.7% wt. /wt. thiabendazole, experimental only) and TBZ infusion, and (D) untreated checks. The treatments were applied in late fall of 2015 (e.g., injection), with follow-up treatments applied in spring 2016 (injection or infusion). Late fall (winter) tree injections were delayed until soil temperatures dropped to  $\leq 35^\circ$  F ( $1.6^\circ$  C) to minimize resin flow. Tree injections were installed using the No. 4 Arborplug™ (Arborjet, Inc.), a backflow preventer which requires a 9 mm dia.  $\times$  15 mm deep drill-hole to install. Injections were applied neat every 4 inches (10 cm) of stem circumference at 8 milliliters per inject site (equivalent to 6 mL/2.5 cm dbh dose rate) using the QUIK-jet Air®, an air over hydraulic device. The number of injection sites was calculated using the formula: cm dbh  $\times$  0.30 (inch dbh  $\times$  0.75). The mean number of

**Table 2. Austrian pine cm diameter at breast height (dbh) across treatments. The untreated controls had the largest mean diameter, but these were not significantly different from the Propizol infusion trees, which in turn were not significantly different from the other treatments.**

Variable	Treatment	No.	Mean	Application time
cm dbh	A. Propizol injection	10	44.5b	December 2015
	B. Propizol infusion	10	49.8ab	May 2016
	C. TBZ injection/infusion	10	44.4b	December 2015/May 2016
	D. Untreated checks	10	58.4a	N/A

Means that do not share a letter are significantly different, Fisher LSD method and 95% confidence

injections was 14 per tree. Tree micro-infusion® applications were made using the Tree I.V. (Arborjet, Inc.) and diluted with de-ionized (DI) water. The number of micro-infusion sites consisted of eight injection points evenly spaced around the base of the tree. Propizol was diluted with 9 parts DI water and thiabendazole with 19 parts DI water (10% and 5% solutions, respectively). Propizol was infused at 120 mLs in 1080 mLs water (equivalent to 17,160 mg of active per tree) and TBZ was infused at 60 mLs in 1140 mLs water (equivalent to 10,020 mg of active per tree).

Nondestructive canopy assessments were made at the time of initial treatment (2015) and at 1 and 2 years after treatments. In April of 2018, a branch sample was taken from each pine from the north side at mid-canopy prior to budbreak. Candle growths from 2016 and 2017 were assessed. The assessments made included (1) the number of fascicles, (2) the number of lesions per fascicle, (3) the lengths and weights for each growth increment, and (4) color of the needles at the time of autopsy. Percent lesions were calculated by dividing the number of lesions observed over the number of fascicles per growth increment. Grams per centimeter were calculated for each growth increment. Statistical analyses were conducted in MINITAB v17 (State College, PA) using one-way ANOVA and Fisher LSD method for mean separation. ANCOVA (analyses of covariance) procedure using cm dbh as a covariate was used to evaluate the effects of tree diameter. Significance level was accepted at  $\alpha = 0.05$ .

## RESULTS

### Effects of Tree Cm DBH

The larger diameter trees would have greater capacity for carbohydrate synthesis and therefore have greater resistance to disease when predisposed to environmental stress. Therefore, given a large tree versus small tree (both untreated), we ought to see more disease incidence in the smaller trees. We did not see this. Although the untreated checks were significantly larger compared to two of the three other treatments (i.e., PPZ and TBZ), the effects of size on treatment outcome was not significant ( $P = 0.14$ ).

### Injection and Infusion Time

Mean time of Propizol injections was 6.8 minutes  $\pm$  1.4 standard deviation, with a minimum of 4.5 and

maximum of 9.8 minutes. In contrast, mean infusion time was 105.7 min  $\pm$  46.1 standard deviation; the minimum elapsed time was 60 and maximum was 180 minutes. The injections were on average 15.5 times more efficient to apply compared to the infusions.

### Canopy Condition

Except for the PPZ winter 2015 injections, we did not observe treatment differences in 2016, which proved to be a severe infection year. In 2017, we observed greater treatment differences, where trees that had received PPZ significantly improved in canopy condition (green, healthy) compared to the TBZ or untreated trees (up to 24% stressed canopy) (Figure 1).

### Environmental Conditions

In Table 3, the mean temperatures, precipitations, and growing degree days (GDD) for Lansing, MI for the 2016 and 2017 growing seasons are presented. In 2016, dry conditions prevailed in June, early in the growth cycle; the trees received only 17.1% of the monthly normal precipitation. There was a greater incidence of infection observed in that year. In contrast, in 2017, precipitation for the same period was above normal; trees received 108.7% of the monthly precipitation. In that year, we observed a decrease in plant infection.

### Evaluation of Excised Candles

Both the number (i.e., occurrence) and percentage of needle fascicles lesioned in 2016 from Diplodia did not differ significantly among treatments. The percent of lesioned needles in 2016 was  $68.8 \pm 5.5$  standard error compared to  $10.9 \pm 1.8$  standard error in 2017. Across treatments, the incidence of lesions was significantly different between the two consecutive growth increments ( $T$ -value = -9.94,  $P = 0.00$ ). Environmental conditions favoring disease were much higher in 2016 compared to 2017. Across treatments, we observed a net reduction in lesioned fascicles from 2016 to 2017 of  $-59.9\% \pm 5.7$  standard error. The greatest reduction in lesioned fascicles was observed in PPZ applied neat by injection (Treatment A) of  $-75.9\% \pm 9.9$  standard error, but this was not statistically different from the other treatments.

However, both the number of lesions and the percent of lesions observed differed significantly among treatments in 2017. Treatments that received PPZ injections or infusions were statistically different compared to either TBZ or the untreated checks in

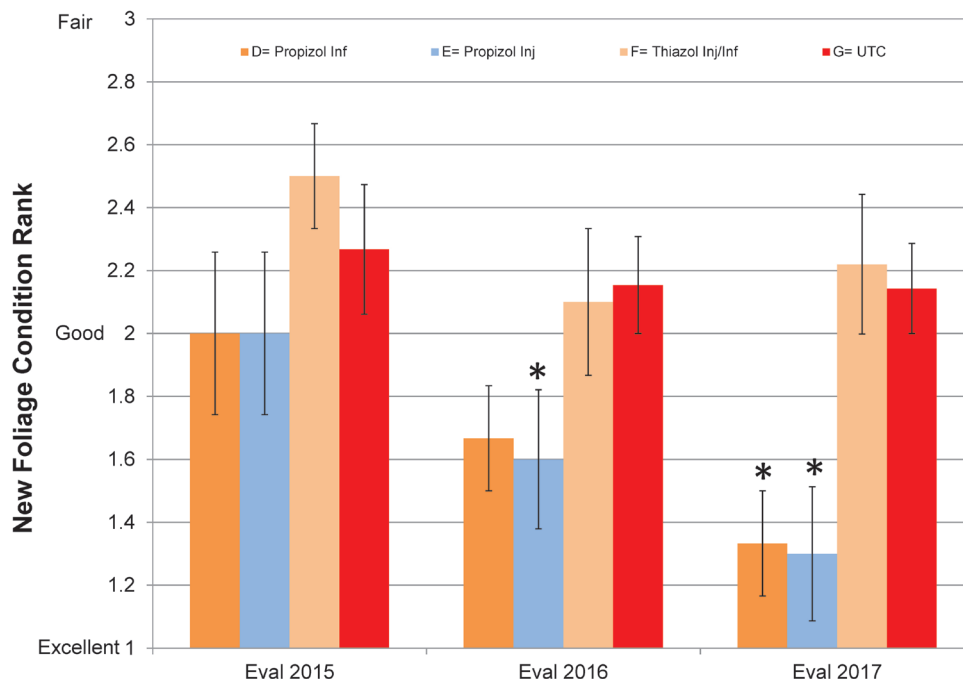


Figure 1. Evaluations of new candles in Austrian pine at the commencement of the study in 2015 through 2017. In 2016, only the winter 2015 injected Propizol was statistically different (\*) compared to all other treatments. In 2017, the propiconazole micro-infusion and injection treatments were statistically different (\*) compared to the thiabendazole and untreated controls at  $\alpha = 0.05$ .

both number and percent of lesioned needles in 2017 (Figure 2).

### CONCLUSION

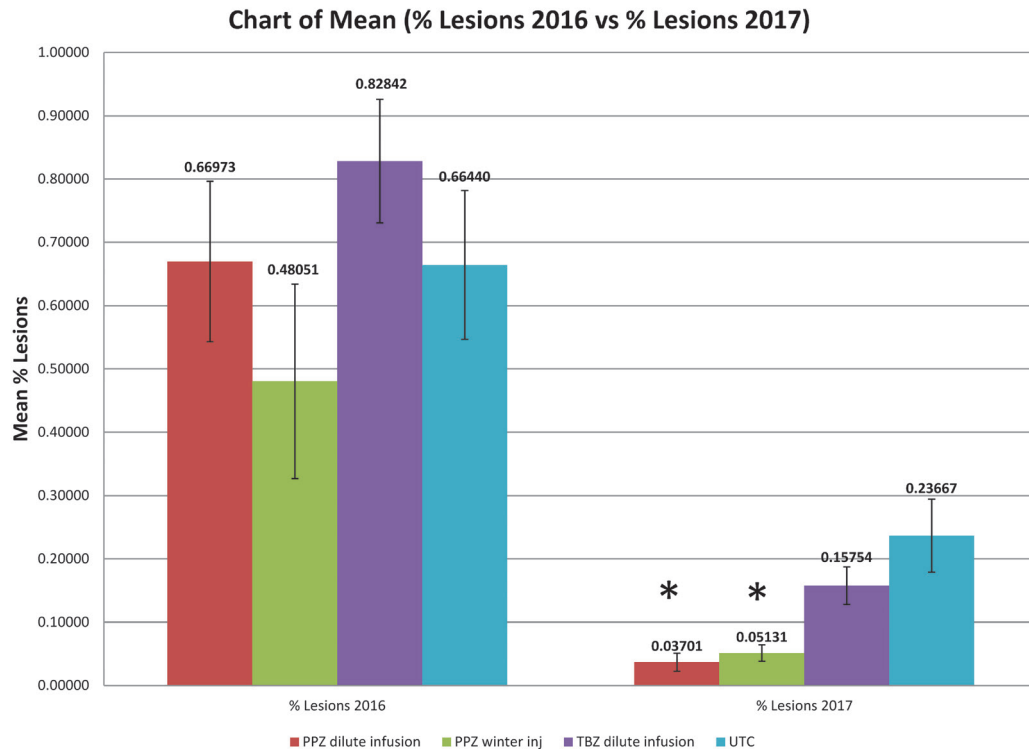
We evaluated (a) two systemic fungicides, (b) two application windows (fall and spring), (c) injection spacing, and (d) volume of liquid injected for the control of *Diplodia* tip blight. We found that Propizol was effective. Thiabendazole, however, was not. Further, greater activity was observed when Propizol was injected in the late fall prior to the spring infective

period. In-season applications, whether applied neat or dilute, were effective one year later. In our study we applied 537 to 903 mg per 2.5 cm dbh of TBZ and PPZ, respectively, compared to the Hartman et al. (2009) study that applied only 14 mg per 2.5 cm dbh of debacarb, carboxin, or tebuconazole, which may have accounted for the differences in efficacy. Both tebuconazole and propiconazole are triazoles; whereas thiabendazole and debacarb are benzimidazole fungicides.

Propiconazole is a broad spectrum fungicide that acts to inhibit the biosynthesis of sterols, which are

Table 3. Mean temperatures, precipitations, and growing degree days (GDD) in 2016 and 2017 for Lansing, Michigan for the 6 months of the growing season, where base temperatures are  $\geq 50^\circ\text{F}$  ( $10^\circ\text{C}$ ) (data from [weatherunderground.com/history/airport/KLAN/](http://weatherunderground.com/history/airport/KLAN/)).

Mo/YR	Mean temp (F)	mean GDD	sum GDD	cm precip	Mo/YR	Mean temp (F)	mean GDD	sum GDD	cm precip	Normal precip
Apr-16	45	2	66	6.5	Apr-17	53	5	137	11.6	7.58
May-16	59	9	292	7.48	May-17	58	9	267	6.23	8.4
Jun-16	69	19	567	1.48	Jun-17	71	21	634	9.38	8.63
Jul-16	74	24	730	8.38	Jul-17	73	23	718	6.75	7.1
Aug-16	74	24	744	16.6	Aug-17	68	18	557	4.35	8.08
Sep-16	66	16	482	7.48	Sep-17	66	16	468	2.35	8.75



**Figure 2. Comparison of percent Diplodia lesions on needle fascicles for 2016 and 2017 growth increments on excised candles by treatment. The greatest reduction in % lesions was observed in 2016 only in the winter Propizol injections (applied December 2015), though this was not statistically significant. However, the PPZ treatments (\*) were statistically different in 2017 compared to TBZ and UTC treatments at  $\alpha = 0.05$ .**

critical to cell wall formation in fungi (Extension Toxicology Network 1997). Propiconazole is considered a fungistat, either slowing infection or stopping fungal growth altogether. This mode of action may help to explain the differential activity observed; that is, we observed a greater reduction in pre-infection candles in fall (i.e., prior to infection) compared to the spring applications (applied post-infection). These observations help to guide recommendations to protect pines of a size and age that are susceptible to infection.

The introduction and uptake of liquid into resinous conifers is slow due to the restrictive architecture of the vascular tissues (i.e., tracheids) and due to resin exudate (from preformed resin ducts), a defense response to wounding. In order to expedite tree injections, several options were considered to address these challenges. We compared close and far circumferential spacing, 10 cm and 20 cm apart, respectively. The most efficient time of application was the closer injection spacing.

Infusion applications were applied in each case at 1200 mLs per tree. Dilution reduces formulation viscosity but increases the injection volume. Micro-injections were administered neat (undiluted), and on average 114 mLs were applied per tree. Injection into trees averaged 6.8 minutes, whereas infusions required 105.7 minutes. On a per tree basis, injection was approximately 16x faster to apply compared to infusion (105.7 vs. 6.8 minutes). The faster injection time may be of interest to the applicator wishing to inject trees efficiently.

The rate of injection is based on the trees capacity to move liquid upward, which is limited by tracheid diameter (Hagen-Poiseuille law), but also requires open stomates in the canopy. Additional resistance to injection is presented by resin exudate at the drilled site. Micro-infusions by Tree I.V. rely on a manifold of four injector needles, each placed in a different quadrant (circumferentially) of the tree bole. A common line is pressurized to a maximum of 4.76 atmospheres (70 PSI). The uptake into trees, however, is



less dependent upon input pressure than on evapotranspiration in the canopy, which may explain our observation of the individual lines emptying differentially (i.e., sequentially and not simultaneously). This variability also accounts for the extended time for infusion to be completed. The micro-injection applications, on the other hand, require less volume and are applied at higher pressure (5.44 atmospheres or 80 PSI). Each injection point gets a known and measured dose (in this study, 8 mLs). The sequential drilling, insertion of the Arborplug, and immediate injection reduces the effects of resin exudate. This approach in methodology (i.e., sequentially drilling, plugging, and injecting) is therefore recommended when making applications in conifers with resinous xylem.

Treatments applied in late fall (in Michigan, early December) when resin flow is reduced may aid in uptake. Applications within season, using the closer (10 cm) spacing alone, expedites uptake. Even so, each injection site ought to be drilled, plugged, and injected sequentially to minimize the impact of the resin response. The fall or late fall application has an added advantage in that it places product in the tree prior to the infection period and is therefore recommended when using the Propizol fungicide.

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### Conflicts of Interest:

Joseph J. Doccola reported being employed by Arborjet, Inc., the distributor of Propizol. The remaining authors made no disclosures.

**Résumé.** Le dépérissement des pousses du pin (*Diplodia pinea*) s'attaque aux pins matures, incluant le pin noir (*Pinus nigra*) et le pin ponderosa (*P. ponderosa*). Les infections s'étendent à partir des fascicules d'aiguilles jusqu'à la branche et, si non contrôlé, jusqu'à l'arbre tout entier. Les études d'efficacité des injections de fongicides chez les conifères sont rares. L'exsudat de résine et des minuscules tissus vasculaires, réagissant suite au perçage, constitue un obstacle à l'injection. L'efficacité du Propizol (14.3% propiconazole) pour le contrôle du dépérissement chez les pins noirs, fut évaluée. L'évaluation prit en considération (1) la période de l'année, (2) l'espacement des sites d'injection, et (3) la dilution du fongicide en ce qui concerne l'efficacité de l'injection. Des injections automnales tardives accélèrent l'assimilation, ce qui est cohérent avec les taux d'émission réduits de monoterpène à l'automne et à l'hiver tel que signalé par Kim et al. 2005 et Lim et al. 2008. La durée requise pour l'administration de la dose fut enregistrée selon que les espacements des sites d'injection étaient rapprochés ou éloignés. Les faibles espacements eurent le plus grand impact quant à la durée réduite pour l'administration de la dose, indépendamment de la période de l'année. Les injections à faible volume nécessitent moins de temps d'application en comparaison avec celles à haut volume. Sans considération de la méthode d'application, nous observâmes une diminution significative de l'incidence de la maladie chez les arbres traités au Propizol. Les injections effectuées tard à l'automne résultèrent en une baisse moyenne de l'infection des nouvelles pousses (chandelles) la saison suivante. Toutefois, les injections réalisées au printemps suivant n'apportèrent aucune amélioration chez les chandelles avant la prochaine année. Nous considérâmes que ces différences prenaient leur origine selon que le fongicide était appliqué avant ou après l'infection. Sur la foi de ces découvertes, nous recommandons le recours au Propizol préalablement à l'infection pour des résultats optimaux.

**Zusammenfassung.** Diplodia Spitzenmehltau (*Diplodia pinea*) beeinflusst ausgewachsene Kiefern, einschließlich Schwarzkiefer (*Pinus nigra*) und Ponderosa-Kiefer (*P. ponderosa*). Infektionen verbreiten sich von Nadelbüscheln zu den Ästen und wenn unentdeckt über den ganzen Baum. Studien über die Effizienz von Fungizid-Injektionen sind begrenzt. Sofortiges Austreten von vaskularem Gewebe und Harzen, eine Reaktion auf das Anbohren, stellen ein Hindernis bei der Injektion dar. Die Effizienz von Propizol (14.3% propiconazol) zur Kontrolle von Diplodia Spitzenmehltau in Schwarzkiefern wurde bewertet. Wir evaluieren (1) Zeit des Jahres, (2) die Abstände der Injektionen, und (3) die Fungizid-Dilution in Bezug auf die Injektionseffizienz. Injektionen im Spätherbst führten zu einer Aufnahme, welche vergleichbar war mit der von Kim et al. 2005 und Lim et al. 2008 berichteten reduzierten Monoterpenemissionsrate im Herbst und Winter. Die erforderliche Zeit für die Verabreichung der Dosis wurde für dichte und weite Abstände auf der Injektionsfläche aufgezeichnet. Weite Abstände hatten den größten Einfluss auf die Reduzierung der Applikationszeit, unabhängig von der Jahreszeit. Injektionen mit kleinem Volumen erforderten weniger Zeit zu Applikation als vergleichsweise hohe Dosen.

Unabhängig von der Applikationsmethode beobachteten wir bei Propizol-behandelten Bäumen einen signifikanten Rückgang von Krankheitsanzeichen. Spät im Herbst injizierte Applikationen führten zu einer mittleren Reduktion von der Infektion neuer Kerzen in der neuen Wachstumssaison. Trotzdem führten Injektionen im folgenden Frühling nicht zu einer Verbesserung der Kerzenkondition bis ein Jahr später. Wir glauben, dass diese Differenzen darauf basieren, ob die Fungizide vor oder nach der Infektion.

**Resumen.** El tizón de la diplodia (*Diplodia pinea*) afecta a los pinos maduros, incluidos los pinos austriacos (*Pinus nigra*) y Ponderosa (*P. ponderosa*). Las infecciones se propagan desde los fascículos de aguja a la rama y, si no se controla, a todo el árbol. Los estudios de eficacia de las inyecciones de fungicidas en coníferas son limitados. Los tejidos vasculares y el exudado de resina, una respuesta a la perforación, presentan impedimentos para la inyección. Se evaluó la eficacia de Propizol (14.3% de propiconazol) para el control del tizón de diplodia en pinos austriacos. Evaluamos (1) época del año, (2) espacio de inyección, y (3) dilución de fungicida con respecto a la eficiencia de la inyección. Las inyecciones tardías de otoño aceleraron la absorción, lo que es consistente con las tasas reducidas de emisión de monoterpene en otoño e invierno informadas por Kim et al. 2005 y Lim et al. 2008. Se registró el tiempo requerido para la administración de la dosis para una separación cercana y amplia de los sitios de inyección. El espacio reducido tuvo el mayor impacto en la reducción del tiempo de aplicación, independientemente de la época del año. Las inyecciones de bajo volumen requirieron menos tiempo de aplicación en comparación con el alto volumen. Independientemente del método de aplicación, observamos una disminución significativa en la incidencia de enfermedades en los árboles tratados con Propizol. Las inyecciones aplicadas a fines del otoño dieron como resultado una reducción media en las infecciones de velas nuevas en la próxima temporada de crecimiento. Sin embargo, las inyecciones en la primavera siguiente no mejoraron el estado de las velas hasta un año después. Creemos que estas diferencias se basan en si el fungicida se aplicó antes o después de la infección. En base a estos hallazgos, recomendamos Propizol antes de la infección para obtener resultados óptimos.