Abstract

Austrian pine (Pinus nigra) is widely planted in landscapes throughout the central and eastern United States. The tree is valued for its ability to withstand urban growing conditions while providing a pleasing appearance in the landscape. Diplodia tip blight, also known as pine tip blight or Sphaeropsis tip blight is a serious disease of conifers worldwide and is especially devastating to two-needed pines such as Austrian pine. The causal fungus is Diplodia pinea (syn. Sphaeropsis sapinea). Culture plating and PCR were used to show that D. pinea frequently causes latent infections in asymptomatic Austrian pine. Cytological studies revealed that the latent infections are mostly located in shoot epidermal and cortical tissues at the base of the needles and that the spread of infection may be blocked by tree defenses. Diplodia tip blight disease killed 84% of 449 Austrian pines growing on the University of Kentucky campus over a 15-year period and the pines developed increasing levels of disease as they aged. Landscape maintenance professionals are reluctant to spray fungicides in public settings and have difficulty obtaining good results with foliar sprays in any case. A block of mature, diseased Austrian pines and another block of healthy, maturing pines were treated with the fungicides oxycarboxin, debacarb, tebuconazole, or water (control)injected into the lower trunk and root flare of individual trees for four years. The fungicide treatments did not significantly affect disease levels. The causal fungus could be isolated readily from diseased and symptomless shoots regardless of the treatment. D. pinea was very sensitive to tebuconazole and debacarb based on in vitro fungicide tests. Basal drenches with paclobutrazol affected shoot growth but did not significantly reduce tip blight disease levels or tree mortality. Under mid-south USA climate conditions, Diplodia tip blight of Austrian pine is destructive and difficult to manage.

Introduction

Austrian pine (Pinus nigra), introduced from Europe almost 250 years ago, is widely planted in eastern and central U.S. Of the 3,000-4,000 trees growing on the University of Kentucky (U.K.) campus in recent decades, at least 10% have been Austrian pines. Diplodia tip blight (pine tip blight, Sphaeropsis tip blight) is a serious disease of conifers worldwide. The causal agent, Diplodia pinea (syn. Sphaeropsis sapinea) causes tip or shoot blight which is especially devastating to two-needed pines such as Austrian pine (8).

Recent research in our laboratory revealed that on the U. K. campus almost 40% of symptomless Austrian pine shoots harbored latent D. pinea infections (4). Microscopic examination showed that these latent infections are likely kept in check by tree defenses (3), but infections may become active in stressed trees. A polymerase chain reaction (PCR) test was developed to detect D. pinea in pine tissues (2) to eliminate the requirement for destructive sampling needed to grow the pathogen in culture.

In previous studies, Diplodia tip blight disease was reduced with Bordeaux mixture applied early in the growing season (7). Early applications while candles are elongating is generally indicated on the labels for fungicides containing thiophanate methyl. However, grounds managers reported that foliar application of fungicides was not working on the U.K. campus (5), possibly due to inadequate spray coverage for large trees, or imprecise timing due to having to spray large numbers of individual trees over a large landscape during a narrow application window. Furthermore, application of foliar fungicides is complicated by concerns about human and environmental exposure on institutional landscapes such as the U. K. campus.
To minimize chemical exposure concerns, systemic fungicides can be delivered into trees by injecting chemicals directly into small, shallow holes in the outer xylem of the root flare (9). Oxycarboxin, (Carboject™), debacarb (Fungisol™), and tebuconazole (Tebuject™) (Mauget, Inc.) are examples of fungicides that can be injected into trees.

Paclobutrazol (Cambistat™) (Rainbow Tree Care Scientific Advancements) is a plant growth regulator which inhibits gibberellin synthesis in plants. The triazole-based chemical also has fungicidal activity (6). Application of paclobutrazol may improve tree health and reduce the effects of plant disease for up to three years after application (1).

Our research objectives were to document the decline of a population of Austrian pines over a 15-year period due to Diplodia tip blight and to test other chemical approaches as an alternative to foliar fungicide application for reduction of disease levels and/or latent infections.

Materials and Methods
Austrian Pine Tip Blight Survey

Austrian pines growing in landscape groupings on the U.K. campus (38.0 deg. north, 84.5 deg. west), located in Lexington, KY, USA were surveyed for tip blight disease during the month of July eight times over fifteen years from 1992 to 2007. For each tree, the age was estimated by counting the number of whorls of branches along the main trunk, and an estimate of whole-tree percent tip blight [number of dead shoots vs. number of total (dead plus live) shoots] was made. In cases where lower branches had been removed due to tip blight, age and disease estimates were adjusted accordingly.

Fungicide Injection for Tip Blight Management

In vitro test of injected fungicides. Oxycarboxin, debacarb, and tebuconazole, were tested for fungicidal activity against D. pinea and compared to water controls. Fungicides were incorporated into acidified potato dextrose agar (APDA) in Petri plates at rates ranging from 1ppm to 1,000 ppm. Plugs (4mm diameter) of mycelium from actively growing cultures of D. pinea were transferred to the plates. Radial growth from the original plug was measured after 4 days.

Injection experiment 1. Sixteen mature (20-22 years old) diseased (1-50% tip blight) pines located in a landscape on the U.K. campus received one of four treatments. Treatments, arranged in a randomized complete block design and replicated on 4 individual trees, consisted of injections at labeled rates of a) oxycarboxin, b) debacarb, c) tebuconazole and d) water used as a control. Trees were assigned to groups so that each treatment was applied to trees with a similar range of symptoms. Injections were started when pine shoot candles were partly elongated on 8 May 1999, 6-9 May 2000, 5 May 2001, and 29-30 May 2002; capsules were removed 4-12 days after injection.

Injection experiment 2. Forty maturing (13-14 years old) disease-free pines comprising a screen planting on the U.K. campus were injected four years consecutively in 1999, 2000, 2001 and 2002 as described in Experiment 1. The experiment was designed as a randomized complete block experiment with 10 replicates. For both experiments disease symptoms were evaluated in mid-to late summer each year by estimating the percent of diseased shoot tips per tree.

Recovery of D. pinea from shoots of treated trees. In July of 2000, 2001 and 2002, shoot, bud, and needle samples (two each) from asymptomatic and diseased shoots were collected from each treated tree, and the pathogen was cultured on (APDA) using standard microbiological techniques. The fungal cultures were identified and confirmed microscopically using the method described by Flowers et al. (4).

Basal Drench for Tip Blight Management

Suspensions of paclobutrazol were applied at label rates to the bases of 20 mature (17-19 years old) Austrian pines growing in a screen planting on the U.K. campus in July, 2003. A shallow encircling trench was carved into the soil adjacent to the trunk and buttress roots of each tree and the suspension was distributed uniformly into the trench. The experiment, replicated five times (four trees per replicate) was established in a randomized complete block arrangement. Experimental controls were treated with water. Pines were evaluated in July of 2004, 2005, and 2006 for levels of tip blight disease, detection of latent infections using PCR assays (2), tree survival, and treatment effects on tree growth.
Results and Discussion

Austrian Pine Tip Blight Survey

In 1992, the survey identified 449 Austrian pines on the U.K. campus to be monitored over a 15-year period, with an average age of 15.1 years and distributed among 47 landscape groupings. From the original 449 trees, 377 (84%) died or were removed due to excessive tip blight disease, until only 72 remained in 2007 (Figure 1). Mature pine cones with *D. pinea* pycnidia embedded in the cone scales were usually observed on trees that were 13 years old or older. Tip blight symptoms in the lower branches typically occurred in subsequent years. Pine tip blight incidence increased as trees aged (Figure 2).

During the 15 years of the U.K. campus survey, Austrian pines were so disfigured or killed by Diplodia tip blight that they generally needed removal by about age 25-30. During the 15-year interval, frequent summer dry periods and drought (1994, 1999, 2002, 2005, and 2007) could have contributed to increased disease susceptibility of landscape Austrian pines.

Fungicide Injection for Tip Blight Management

*In vitro* test of injected fungicides. Tebuconazole completely inhibited fungal growth at the rate of only 1 ppm, and debacarb stopped fungal growth at 6 ppm, whereas for oxycarboxin, inhibition didn’t occur until fungicide concentrations were greater than 1,000 ppm. The *in vitro* tests of the fungicides used for injection against *Diplodia pinea* suggest that two of the fungicides, debacarb and tebuconazole might have sufficient activity to affect Diplodia tip blight.

Injection experiment 1. After four years of fungicide injections, trees continued to die until 2002, when no new trees died. Tip blight disease in these mature trees in 2002 increased 121-245 percent over levels in 1999. The increase in disease was slowest in debacarb-treated trees, where 2002 disease levels were 121% of 1999 disease levels. Disease severities for other treatments in 2002 were about double the levels seen in 1999. Due to high variability of disease between trees, treatment means were not statistically different (Waller-Duncan K-ratio t-test, P=0.05).

Injection experiment 2. In the injected younger, less-diseased trees, tip blight levels gradually increased. In 1999, there was no noticeable disease in this plot; by 2002, disease ratings ranged from 13-23 percent, but showed no noticeable treatment effect.

Isolation of *D. pinea* from shoots of treated trees. *D. pinea* recovery from both symptomless and diseased shoots increased over the three-year period paralleling the increase in disease over the same time period.

Fungicide injections neither “cured” pines of Diplodia tip blight disease, nor prevented symptomless trees from developing tip blight. In the latter case, it is possible that latent infections were already prevalent and that the fungicides were not distributed to these latent infection sites because of blocking by necrophylactic periderm (3). Injection with debacarb appeared to slow the progress of tip blight in diseased trees, but this trend was not statistically significant due to highly
variable disease levels between trees and due to loss of some trees to tip blight before the experiment was completed.

During the injection process, some of the capsules quickly filled with pitch from the tree. Thus, it is difficult to know whether or not all capsules were actually emptied into the injection sites. Our results are not encouraging for arborists who would like to use fungicide injections for Diplodia tip blight management in Austrian pines.

**Basal Drench for Tip Blight Management**

Most of the pines in the test groups continued to deteriorate following treatments in 2003, whether treated with paclobutrazol or not, and by 2006 several had to be removed. Average tip blight levels in the remaining trees were high and were not significantly different between paclobutrazol and water treated trees. The causal fungus was very commonly detected in symptomless tissues for both treatments. By the end of the experiment, nearly half of all the trees in the experiment had died from tip blight or had been removed due to excessive disease.

Although it appeared that Diplodia tip blight development slowed in paclobutrazol-treated pines at the end of our experiment, these trends were not statistically significant. The current study did not provide definitive evidence that paclobutrazol root-flare basal treatments will cure pines of tip blight disease, or stop disease progress. Paclobutrazol-treated Austrian pines grew more slowly than water-treated pines which is an expected effect of this growth regulator chemical.

**Conclusions**

In some instances, Austrian pines may not be the best choice of tree to plant because of their extreme susceptibility to tip blight disease. Their useful lifetime in the landscape may be less than 25 years, and after age 13, they are likely to show disease symptoms. Foliar fungicides are often ineffective and are difficult to apply to large trees, especially when there are many trees to treat. Based on research reported here, current alternative fungicide treatments are not likely to be more effective. Management of Diplodia tip blight in mid-south USA Austrian pines is difficult.

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**References**