

↑ Fungal fruiting structures, called pycnidia (black spots) exuding spores during wet weather on Scots pine foliage. cots pines (aka Scotch pines) have traditionally been the most popular and common Christmas Tree species grown in Kentucky. Scots pine production is seriously threatened by Diplodia tip blight disease, which has been increasing in severity in the region since 2000.

Symptoms of Diplodia tip blight, which is caused by the fungus *Diplodia pinea*, include needle and shoot blight, resinous cankers, branch dieback and "shepherd's crooks" of elongating shoots. Even low levels of these symptoms damage the aesthetics of the tree, negatively affecting its sale value. It is not clear why tip blight has recently become so damaging, but a regional drought that began in the late 1990s may be a contributing factor.

The primary recommendations for tip blight control are sanitation (removal of blighted shoots and branches and severely diseased trees) and avoiding shearing trees during rainy or humid weather, when the fungus is producing spores.

It was suggested that shearing diseased trees could increase dispersal of spores, and that shoot tips wounded by shearing could provide infection sites for the pathogen. Disinfecting shearing tools with alcohol, bleach dips or washes can reduce the possibility of spore transmission. However, this is inconvenient and can damage the tools, and it is rarely practiced on Kentucky Christmas Tree farms.

The goal of the study described here was to investigate tip blight disease progress in Scots pine Christmas Tree plantations in Kentucky, including an analysis of the role of shearing, in order to improve management recommendations.

Three Choose & Cut Christmas Tree farms used for our study had grown primarily Scots pines for at least 15 years, and none had a serious problem with tip blight disease before 2000. Disease severity (the number of diseased shoot tips as a percentage of the total) was estimated for each tree on each of the three farms approximately one month prior to shearing in 2005. The average disease severity on Farm 1 was 2.85% (n=751), on Farm 2 it was 1.32% (n=514) and on Farm 3 it was 0.38% (n=540). On Farm 1, the individual trees

ranged from 0 to 80% blighted tips, on Farm 2 the range was from 0 to 44%, and on Farm 3, it was from 0 to 40%.

Detailed observations of tip blight disease progress were made on Farm 1, located in Fayette County Ky., in 2006 and 2007. The first symptoms were observed in both years during the first week of May, four to five weeks after bud break. The earliest recognizable symptoms were water-soaked lesions at the needle bases of elongating shoots (Figure 1A). The lesions developed first on one side of the shoot, and then quickly expanded to form a girdling canker (Figure 1B-D), which continued to enlarge toward the shoot tip (Figure 1D). Shoots with girdling cankers rapidly became dry and brittle, reddish-brown to black above the canker, and green below. They remained stunted, and pitch often exuded from the canker at needle junctions.

Symptoms were usually visible before the needles had fully emerged from the needle sheaths (Figure 1E). The needles in a cankered region often stayed green for a few days before finally becoming straw colored and stunted. A characteristic symptom of tip blight on Scots pines was formation of shepherd's crooks (Figure 1F). The diseased shoots curled over at the site of the cankers. On a few shoots, canker development occurred at the base, causing the entire shoot to die.

Culturing experiments demonstrated that the fungus was present only in the canker area initially and could not be found in the blighted tips. New symptomatic tips were not found after the last week of May in 2006 or 2007. Symptoms that developed directly from the cut end of a sheared tip, which would be expected if sheared tips were serving as infection sites for D. pinea, were never observed. All new tip blight infections occurred on unsheared tips, apparently via needle bases.

D. pinea was shown in a previous study to infect Austrian pines by penetrating directly through the shoot epidermis at the bases of emerging needles, and it has been reported that infection of Scots pine shoots can also occur by this route. Blighted tips were often seen on secondary, unsheared shoots that had developed from the axial buds of sheared shoots. However, there was no statistically significant difference between the numbers of diseased shoots that arose from buds on sheared shoots versus on unsheared shoots.

Field studies to directly investigate the role of shearing in disease development were done on Farms 2 and 3. Our first objective was to determine whether D. pinea spores could be acquired on cutting tools during the shearing process. Following that, we wanted to know if shearing could transmit spores from diseased to nearby healthy trees, resulting in increased disease severity on those trees.

To collect a sample, a sterile adhesive tape was pressed against the shearing blade surface after it was used to shear a tree, and then the tape was peeled off together with adherent spores and transported back to the laboratory. Two samples were collected from opposite sides of each tool immediately after shearing a diseased tree, and also after subsequently shearing nearby healthier or healthy trees. Tapes were placed into culture dishes and covered with a layer of cooled agar media. D. pinea colonies that grew from the tapes were verified and counted.

We recovered D. pinea spores from shearing tools in 2005 and 2006 on both farms. On Farm 3, more spores were recovered after shearing trees with higher levels of disease. However, on Farm 2, there was no correlation. Numbers of spores recovered from the tools on Farm 3 were always higher than on Farm 2, even though there was

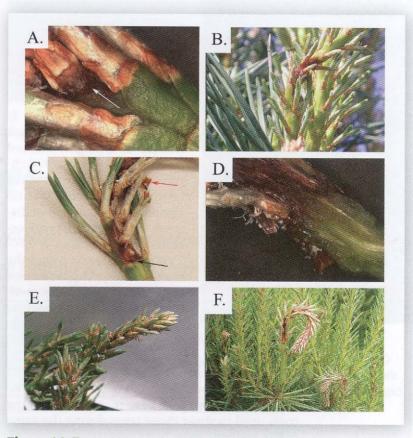


Figure 1A-F. Tip blight symptom development on Scots pine Christmas trees. A) Water-soaking and canker formation at a needle base (arrow). B) Canker formation along one side of the shoot causing the shoot to begin to crook over. C) Canker formation along only one side of the shoot. Black arrow is pointing to the base of the canker where it is limited by a needle junction. Red arrow is pointing to needles that are beginning to turn straw color. D) Dissection of the base of the necrotic lesion on a Scots pine tip infected with Diplodia pinea. Browning does not extend below needle-shoot junction. E) Brown to black discoloration of the shoot often occurs before needles emerge from their sheaths, and precedes needle discoloration. The canker develops at a needle base and progresses up to the tip while the shoot below the canker stays alive. F) Shepherd's crooking often occurs at the diseasedhealthy tissue junction but sometimes the bend occurs further up the shoot.

more disease on Farm 2.

Farm 2 used a rotary motorized shearer, whereas Farm 3 used handheld hedge pruners, suggesting that different types of shearing tools may become contaminated to differing degrees. Farm 3 sheared their trees later than Farm 2 (July versus June), and at least once during the multi-day process each year, trees on Farm 3 were sheared when they were wet, and spores could be seen exuding from the D. pinea fruiting structures, the pycnidia, on diseased foliage (see photo on page 12). Current recommendations advise against

shearing wet trees, and our data support the idea that shearing wet trees can increase the numbers of spores that adhere to the tools.

Even though D. pinea spores were found on the shearing tools, there was no evidence from our study that these spores were transmitted to nearby healthier trees, or that they caused tip blight disease on those trees. D. pinea aggressively infects through wounds and can cause shoot dieback within weeks (Figures 2 & 3). However, in our study, dieback was never found on sheared tips during three years of observations on any of the three farms. In fact, there was no significant association of diseased shoot tips with previously sheared tips at all.

We also observed that the number of spores recovered from tools did not decrease after shearing several healthy trees. It is possible that spores

became stuck in the pitch on the blades and didn't easily come off again during shearing. Finally, there was no correlation between the levels of disease that developed on sheared trees, and the number of spores that were recovered from the tools used to shear those trees during the same or the previous year.

The largest number of spores recovered from a single tape press sample from the field was 10, but there were typically many fewer. We asked whether the number of spores applied to the sheared tips affected tip blight disease occurrence and severity. Four shoot tips from the same whorl on 96 healthy Scots pine seedlings maintained in a greenhouse were selected for treatment. The top 2 to 4 cm of the shoot tips were cut off, and D. pinea spore suspensions containing 100,000, 5,000, 100 or 0 spores/ml, were applied

to the wounds immediately after cutting. The experiment was done twice.

The degree of dieback produced was greater as the number of spores applied increased (Figure 2). Furthermore, 8% of the shoots that were inoculated with only 100 spores did not subsequently develop tip blight symptoms, whereas 100% of the shoots inoculated with at least 5,000 spores became blighted. Thus, it appears that the amount of inoculum acquired on the shearing tools may have been too low for transmission to occur in our field studies.

Sheared shoots produce copious quantities of pitch that hardens and forms a cap over the wound. We asked whether this cap and other wound response processes eventually protect sheared shoots from D. pinea infection. To test this possibility, we used four fully elongated shoots in the same whorl on each of 50 Scots pine seedlings maintained in a greenhouse. The top 2 to 4 cm of each shoot was removed with a sterile scalpel, and 10,000 D. pinea spores in water were applied to the wound at 0, 6 and 24 hours after cutting. Plain water was applied to the remaining shoot tip in each whorl at 0 hours as a control. Symptom development was observed for three weeks.

Shoot dieback resulted when D. pinea spores were applied to the shoot tips after shearing, but no dieback occurred on the water controls. The amount of dieback was significantly reduced as the time elapsed since wounding increased from 0 to 24 hr (Figure 3). This suggests that there is a relatively short period of time, less than 6 hours, after shearing when shoot tips are most vulnerable to infection by D. pinea. As time goes on, more spores would need to be deposited to result in the same degree of disease.

Between 2005 and 2007, when our experiment ended, the average disease severity was statistically unchanged for Farms 2 and 3. On Farm 1, however, which had originally been the most seriously affected by the tip blight disease, the average decreased significantly, from 2.85% in 2005 to only 0.003% in 2007 (P<0.0001). Some of this improvement was due to the removal by the grower of 37% of his trees, comprising the most severely diseased specimens, between 2005 and 2006.

However, the surviving trees on Farm 1 were also significantly healthier at the end of the study than at the beginning (average decrease in disease severity of 2.5% per surviving tree, P<0.0001). This resulted from a reduction in the number of diseased shoots (achieved primarily by shearing), combined with a reduction in new shoot infections.

There was no change in the health of the surviving trees on the other two farms, suggesting that the removal of diseased shoots by shearing



Scots pine **Christmas Tree** disfigured by tip blight symptoms.

had been matched by new shoot infections. Coincidentally, it was later revealed that Farm 1 had been treated with Cleary's 3336™WP (thiophanate-methyl) fungicide (18oz package/25 gal water) for the first time during the spring of 2005, and repeated in 2006 and in 2007. The first spray was applied as the shoots were beginning to elongate (mid-April), and another application was made 7 to 10 days later. This coincided with the primary period of infection of Scots pine shoots, based on observations of Farm 1 in 2006 and 2007. The experience of Farm 1 suggests that temporary use of fungicides might be useful as a curative if combined with good sanitation, including shearing and removal of the most heavily diseased trees.

To summarize our findings, spores of the tip blight fungus D. pinea were found on tools after shearing diseased trees on two farms in 2005 and 2006. However, there was no evidence that disease transmission was occurring on the farms via shearing. In greenhouse studies, application of at least 5,000 D. pinea spores to sheared shoot tips was necessary for consistent production of symptomatic infections. Allowing sheared shoot tips to seal for at least six hours before applying inoculum reduced subsequent disease development.

The data presented here suggest that infection

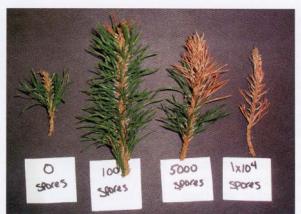


Figure 2. When sheared Scots pine shoots were inoculated with different numbers of D. pinea spores after shearing, there was a visible difference in the length of shoot necrosis that developed.



Figure 3. There was also a difference in the length of shoot necrosis that developed when sheared shoots were inoculated at different times after shearing.

of sheared tips is unlikely unless the number of spores transmitted to them is relatively high, and the tips themselves are freshly cut and haven't had an opportunity to seal. Current recommendations for disease control emphasize sanitation (pruning and removal of symptomatic pine tissue) to reduce inoculum.

Shearing, because it removes most of the newly infected shoots each year, is itself a form of sanitation, and thus may actually be more beneficial than harmful for disease management.

Shearing results in a reduction in disease severity on individual trees, as long as tips can be protected from subsequent infection. Results of this study suggest that disinfecting shearing tools may not always be necessary, and that shearing, especially when done during dry weather as early in the season as possible, before tips become significantly colonized and begin to produce large numbers of spores, can actually be beneficial as a form of sanitation in some cases for management of tip blight on Christmas Trees.

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About the Authors: Amy Bateman received her master's degree in Plant Pathology from the University of Kentucky in 2007. The work described in this article is one portion of her M.S. thesis research. She is currently employed by the USDA-ARS Small Grains and Potato Germplasm Research Unit in Aberdeen, Idaho. Dr. John Hartman is an emeritus Extension Professor of Plant Pathology and Dr. Lisa Vaillancourt is a Professor of Plant Pathology at the University of Kentucky. These researchers co-advised Ms. Bateman and directed the research described in this article.

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