

Control of anthracnose with fungicides on ornamental lupines

Author

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Significance

Lupines (*Lupinus* spp.), particularly Russell hybrids, are grown as perennial ornamentals in North America. Anthracnose has been observed from about 1997 in Russell hybrid lupines in several Michigan nurseries. Anthracnose of lupine is seed-borne and occurs in most regions where forage lupines are grown. Seedlings are very vulnerable and show wilting, leaf and stem necrosis leading to death. More mature plants exhibit stem bending and leaf spots. In severe cases, the crowns rot and foliage is generally discolored (red to yellow). Isolation from affected tissues consistently yields a fungus that has been identified as *Colletotrichum gloeosporioides*. Growers in Michigan have noted that fungicides registered for use on lupines were not effective for control of anthracnose, especially when the disease occurred early in the season. Fungicides are normally most effective when applied to plant tissues before infection. However, applications may have been made too late to affect disease development.

Objectives

The objectives of this study were: 1) to determine the effectiveness and application timing for a range of fungicides available to the floriculture industry to control anthracnose on ornamental lupines, 2) to evaluate the rate of decline of tray-planted lupines and 3) to determine whether any fungicides effective against other anthracnose diseases on legumes could control anthracnose in lupine. In addition to conventional synthetic fungicides, several products thought to stimulate the plant's natural plant defense systems were tested. These products act in different ways to activate natural defenses such as physical or mechanical barriers within the host plant to protect the plant from a range of pathogens. To accomplish these objectives, two experiments were carried out.

Developing an anthracnose index for lupines

Materials and methods

The first experiment was conducted to determine the mortality and rate of decline of lupine seedlings affected by anthracnose. Temperature-controlled (65°F) environment chambers were used for all trials. Plastic trays (50-wells per tray) were filled with

potting compost. One lupine seed was planted into each well. Each treatment was replicated ten times. Seedling trays were placed on plastic trays and watered every four days from below to ensure adequate soil moisture. Seedling death that occurred between planting and emergence was determined by counting the number of emerged plants ten days after planting. Plants were rated on a scale of 0 to 4 based on the percentage of plant tissue with lesions.

Table 1. Rating system used to determine symptom class based on % of plant tissue with lesions.

Class	Percent of plant tissue with lesions
0	No visible symptoms
1	1-2%
2	3-10%
3	11-50%
4	51-100%

The symptom classes were used to calculate an anthracnose index for each 50-plant sample. Symptoms of lupine foliar anthracnose were evaluated 10, 15, 20 and 25 days after planting, to obtain a rate of index development on immature lupine foliage.

Results

On average nearly 87 percent of seeds planted emerged. Anthracnose symptoms appeared after about 15 days and 25 days after planting; many plants were severely affected. There was a significant increase in the number of plants with greater than 50 percent foliar tissue affected by anthracnose 25 days after planting compared with 10, 15 and 20 days after planting.

Evaluation of foliar fungicide treatments for seedling lupine anthracnose control

Materials and methods

In a second set of experiments, we evaluated the effectiveness of fungicides against early symptoms of seed-borne foliar anthracnose. The experiments were replicated three times. Seeds were planted in peat mix in a 50-seedling tray-bed. Plants with two fully expanded leaves (about 20 days after planting) were inoculated with a spore suspension of *C. gloeosporioides*. Foliar fungicides were applied once, 24 hours after inoculation. An anthracnose index was used to categorize the data. Plants were evaluated 35 days after planting.

Results

Anthrachnose developed quickly from seed-borne infection, but the rate of anthrachnose development was enhanced by the supplementary inoculation. The untreated, non-inoculated control (index of 20.2) was significantly lower than the all other treatments (Table 2). The inoculated control reached an index of 100 fifteen days after inoculation. Products with an index between 40 and 50 (in the range of 3 to 10 percent foliar symptoms) were: fluazinam 5SC 0.42 pt /A, (experimental product from Syngenta), Cygnus 50WP 0.4 lb/A and Daconil WS 6SC 1.5 pt/A.

Discussion and future research direction

Seed-borne *C. gloeosporioides* significantly reduced the establishment and survival of ornamental lupine seedlings. Plant survival declined considerably


within 25 days after planting, suggesting that fungicide treatment is necessary at an early stage in seedling development. Although several treatments were statistically better than the untreated control, the percentage of foliage with symptoms was still too high to be considered acceptable. The most effective treatments were fluazinam, Cygnus and Daconil. The experimental SAR (systemic acquired resistance) product CJ-1 also appeared to be somewhat effective; however, all treatments were applied to plants already affected by anthrachnose, after artificial inoculation. Further experiments are currently underway to determine the time period before inoculation when fungicides should be applied to achieve better control of lupine anthrachnose. Cygnus will be used as the model fungicide for these experiments. 

Table 2. Effectiveness of foliar fungicides and plant defense system stimulators (SARs) against seed-borne anthrachnose of lupines as rated by an anthrachnose index. The lower the index number, the more effective the treatment.

Treatment and rate/acre	Lupine anthrachnose index (maximum = 100) ¹	
Untreated non-inoculated (negative control)	20.2	e ²
Fluazinam 5SC 0.42 pt	40.5	d
Cygnus 50WP 0.4 lb	46.8	d
Daconil WS 6SC 1.5 pt	47.2	d
Cleary's 3336 50WP 0.75 lb	60.7	c
SAR Experimental CJ-1	64.0	c
Heritage 2SC 0.8 pt	64.7	c
Terraguard 50WP 0.5 lb	81.3	b
Planthelper Biological Trichoderma 4SC 1.2 pt	90.5	ab
SAR Experimental Encore 3.6EC 0.25 pt	91.7	ab
Tilt 3.6EC 0.25 pt	91.7	ab
Manzate 75DF 2.0 lb	93.2	ab
Kocide 4.5FL 2.67 pt	93.5	ab
Medallion 5SC 0.42 pt	95.8	ab
Messenger 3WDG 0.42 lb	100.0	a
Untreated, inoculated (positive control)	100.0	a

¹ The anthrachnose index reflects both symptom class of individual plants (as described in Table 1) and the number of plants within each class. An anthrachnose index of 0-25 corresponds to 0-2% plant tissue with symptoms, an index of 26-50 corresponds to 3-10% symptoms, an index of 50-75 corresponds to 11-50% symptoms, and greater than 75 corresponds to 51-100% symptoms.

² There is no significant difference between treatments followed by the same letter. (p= 0.05)

Monitoring the spread of dogwood anthracnose in Michigan

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Funding

Michigan Department of Natural Resources Forest Management, Michigan Agricultural Experiment Station

Introduction

The native flowering dogwood tree, *Cornus florida*, is an important understory species in older growth forests, providing a food source for mammals and birds, and enhancing soil fertility. The flowering dogwood is also a prized ornamental tree and quite significant in the nursery industry.

Dogwood Anthracnose has been swiftly destroying the native flowering dogwood tree, *Cornus florida*, throughout the Appalachians since its introduction into the United States in the 1970's. The disease entered Michigan near Kalamazoo in 1990 on ornamental plants. More recently, other areas of the state have become infected. The cause of this disease is the exotic fungal pathogen *Discula destructiva*.

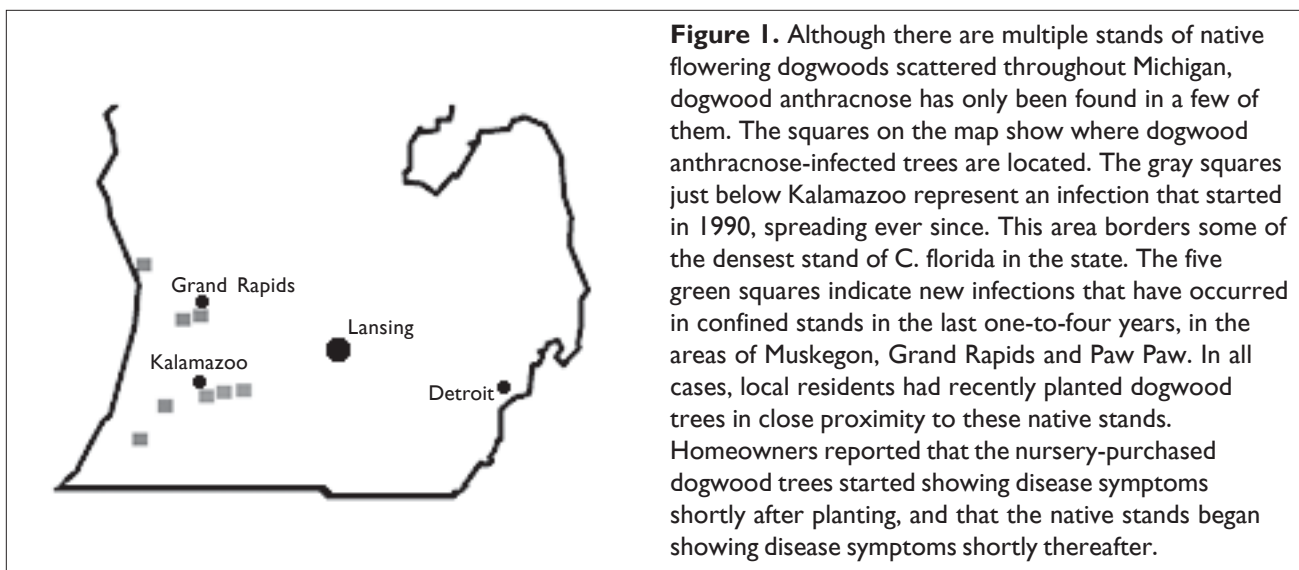
The state of Michigan provides a unique and valuable opportunity to study this disease because spread of the disease does not appear to be through natural means. Due to land use patterns, native stands

of flowering dogwoods in Michigan occur in disjointed scattered woodlots, most of which are currently uninfected. Methods for understanding and controlling the spread of this disease are urgently needed to protect the uninfected native stands. The focus of this research has been to track and monitor the spread of *D. destructiva*, to develop methods for easier isolation of the pathogen from field samples, and to identify the pathogen on arriving nursery stock. It is hoped that what is learned from this research will help to protect flowering dogwoods through the development of measures to prevent the spread of the pathogen in Michigan nurseries, and to halt the spread of the disease caused by planting infected nursery stock.

Symptoms and Signs of *Discula destructiva*

The most recognizable symptoms of Dogwood Anthracnose are tan necrotic leaf spots, which upon closer inspection reveal the small black fruiting bodies. These fruiting bodies, known as acervuli, produce large quantities of infectious conidial spores. Once the pathogen invades the terminal twigs, large numbers of acervuli form on the twigs. See color insert for photos of symptoms and signs on page A-3.

As the tree becomes weakened by the pathogen, it produces epicormic shoots. In the final stage of the disease, the main stem becomes infected, often times through the epicormic



shoots. The result is the formation of large stem cankers, which will rapidly kill the tree by girdling it.

Monitoring the Spread of Dogwood Anthracnose

Dogwood Anthracnose has been spreading most quickly in areas with high concentrations of *C. florida*, such as the Appalachian Mountain Range. Moving west, the concentration of *C. florida* is broken by farmlands and inopportune environments. Yet the disease has been spreading to these isolated stands, we believe in large part due to infected nursery stock. Our objective is to track the disease as it moves through Michigan.

Since 1997, fifty 100m transects have been established using GPS (Global Positioning System, with Geo Explorer program) to mark ten trees per plot. These plots allow for the monitoring of individual trees (usually in the densest parts of stands) for possible infection by *D. destructiva*. The map shown in Figure 1 indicates the location of plots and areas of infected native stands of dogwood.

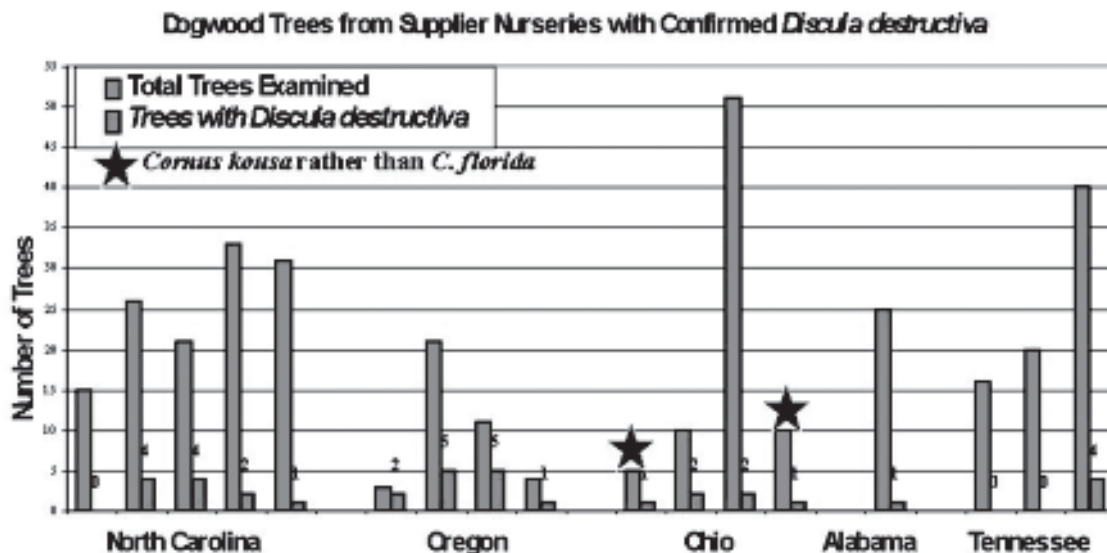
Nursery Inspection for Dogwood Anthracnose

Our observation of newly infected forest stands of *C. florida* in Michigan being directly infected by diseased nursery stock has focused our attention on

determining the source(s) of these diseased dogwood trees. Currently, state nursery inspectors are not trained to observe the inconspicuous fruiting bodies found on small dead twigs on the incoming dogwood trees. These small dead twigs, loaded with potential inoculum, end up infecting healthy dogwoods in several ways. The twigs, when moistened by nursery irrigation systems, produce inoculum that infects other dogwoods in the nursery. In addition, these twigs can fall off the tree into the temporary mulch planting beds where they can provide a source of inoculum for future years stock. Either trees with infected twigs or those recently infected inside the nursery are then sold and transplanted throughout Michigan. Our objective then was to inspect Michigan nurseries as their shipments of dogwood trees arrived from other states in late April of 2001 in order to assess the magnitude of diseased trees arriving in the state (see Figure 2).

Five Michigan nurseries were inspected in late April of 2001 as soon as they received their dogwood shipments. These shipments came from ten different nurseries located in five states, which included Ohio, Tennessee, North Carolina, Alabama and Oregon. Fifteen different samples of *Cornus florida* were collected along with two samples of *Cornus kousa*, each sample containing 3 to 52 individual trees.

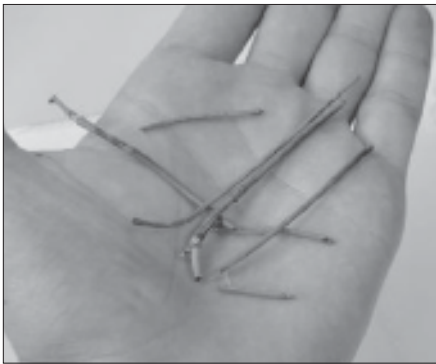
Figure 2. All five Michigan nurseries were found to be receiving infected dogwood trees from out-of-state nurseries. Eight of the ten out-of-state nurseries were found to be exporting infected trees to Michigan, from all five states mentioned earlier. Of the 17 total sample sets collected, 14 were found to have at least one tree with *D. destructiva*. Both samples of *C. kousa* were also found to have at least one infected tree (Figure 2). This is significant since *C. kousa* is known to be partially resistant to Dogwood Anthracnose, but has now been proven to be a vector for bringing the disease into Michigan.



Individual trees were inspected by hand for dead twigs, which were collected, numbered, and brought back to the lab for closer analysis. Fruiting bodies from collected twigs were sectioned and identified as *Discula destructiva* based on conidiophores and spore morphology and size.

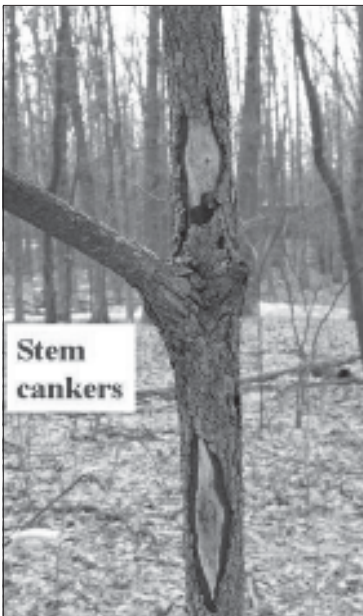
Development of Discula Selective Media

Based on the results of our nursery survey, it became apparent that a better method for the detection of *Discula destructiva* was needed. Our objective was to produce a selective media that would allow the early and heavy sporulation of *D. destructiva* from field samples, which is necessary for identification. By producing a selectively exclusive media, not only samples with fruiting bodies but with other suspicious symptoms such as leaf spots, can be easily tested for *D. destructiva*. This will allow for more accurate reports of how many diseased dogwood trees are coming into Michigan nurseries.



Left: The fruiting bodies of the fungus causing dogwood anthracnose are present on small, dead twigs, making the disease difficult to detect.

Below: Stem cankers caused by *Discula destructiva* on cormus florida.



Below: Imported nursery stock that was inspected for presence of dogwood anthracnose.



Below: Epicormic shoots produced by dogwoods weakened by *D. destructiva*.



Summary

We have found Dogwood Anthracnose established in several confined stands in Michigan and will continue to monitor both infected and uninfected stands for spread of the disease.

Our nursery surveys have proven that *Discula destructiva* is indeed coming into Michigan on infected trees that infect other dogwood trees in the nursery or native stands when planted in close proximity.

The development of a selective media will allow us to quickly diagnose field samples for Dogwood Anthracnose. Currently, trials are underway based on the results discussed above to prove the effectiveness of the new media on field samples.

By our proving that the spread of *D. destructiva* is indeed due to infected nursery stock, we hope to help the Michigan Department of Agriculture (MDA) establish guidelines for safe handling and planting of imported flowering dogwoods. An extensive program of education and outreach will help us save this highly visible and important forest tree from eradication from our forests. ✍

A new disease of ash causing economic losses in Michigan nurseries

Author

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Cooperators

Melodie Putnam, Diagnostician, Plant Disease Clinic Oregon State University; Priam Sood, Biotechnologist, Michigan Department of Agriculture (MDA)

Industry support

Ray Weigand's Nursery, Inc., The Cottage Gardens Inc.

Significance to industry

A new disease of ash known as "coin canker" has caused significant economic losses to several Michigan nurseries in 2001. The U.S.D.A. and the U.S. Forest Service are quite concerned about this disease because it appears to be an exotic organism newly introduced into the United States.

Background

The disease is called "coin canker" because the symptoms are circular, depressed areas on the smooth bark of the tree trunk, and they are distinctly colored reddish, somewhat resembling a half-penny. The cankers usually occur at a lenticel in the smooth bark and grow to the size of a quarter, however, several cankers can coalesce to form reddish, depressed patches. Severely affected trees have many dozens of cankers over the stem. Please see color insert on page A-3.


Cynthia Ash originally "coined" the name for the disease when samples arrived at the University of Minnesota plant disease diagnostic clinic in the 1990s. She was unable to isolate a causal organism. The disease was not seen again until this year in Michigan, and, therefore, the disease was largely forgotten.

Thanks in part to Dr. Priam Sood who provided

many samples of diseased ash trees, I was able to isolate, culture and identify the causal organism. The causal organism fruited at the centers of the coin cankers in late summer, and the fungus has been identified as *Gloeosporidiella turgida*. Little information is known about the fungus that has been reported on ash from the United Kingdom. The fungus is new to the Americas and has likely arrived from Europe in recent years.

Preliminary results

Thanks to donations of trees from two Michigan nurseries (Ray Weigand's Nursery Inc. and The Cottage Gardens Inc.), I am currently inoculating a number of white and green ash nursery trees (In cooperation with Dr. Sood and Melodie Putnam, the diagnostician at the clinic at Oregon State University.), to fulfill Koch's Postulates, the formal scientific proof that *G. turgida* caused the disease (**Editor's note:** Fulfilling Koch's Postulates is a step in the research process that must be completed before any other research can proceed.). Melodie has also isolated the causal fungus from Michigan stock and has been in constant communication and cooperation with us. Although the nursery stock originated in Oregon nurseries two years prior to symptoms appearing in Michigan, it is unlikely that the organism came from Oregon.

At this point in the investigations, it is assumed that unusual winter weather in the North Central States was a necessary pre-disposing factor to the infection by this fungus. Therefore, we are assuming the fungus is now in the state. There is no literature on this fungus other than reports from Europe, and no mention is made of disease symptoms, frequency of occurrence, damage or epidemiology of the disease. Research is necessary for us to understand the disease cycle and to estimate the potential of the disease to damage future nursery stock and forest trees and forest health in Michigan. However, funding will be needed to continue the research. 

Forensic DNA: The modern crime lab comes to your nursery!

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Cooperator

Gary Chastagner, Department of Plant Pathology,
Washington State University

Funding

The Nation of Turkey and the U.S. Forest Service
McIntire-Stennis program

Significance

Individual growers in Michigan have lost hundreds of thousands of dollars a year to needlecasts in the 1990s despite the use of fungicides. Effective treatment of needlecast diseases relies on correct identification of the pathogens involved. Many plant pathogens cannot be cultured on any known nutrients. In our lab, detection systems have been developed, tested and proven effective for twenty needlecast pathogens.

Background

Who done it?(sic) This is the old detective's phrase when no evidence can be found at the crime scene. Today, through new biotechnology such as PCR (polymerase chain reaction) one can find the faintest of DNA traces and clinch the case. So it is in our plant pathology lab at MSU. Five healthy looking pine needles with no trace of disease symptoms are all that is needed to identify the killer. It is now possible to determine when a conifer seedling, landscape or Christmas tree will suffer from a needlecast pathogen a year before the plant has any visible symptoms of disease.

Needlecasts are caused by an unusual group of fungi that cause unique, slow diseases. A fungal spore lands on the wet foliage usually during the flush of new needle and shoot growth. The spore penetrates but grows into only a few cells, then ceases growth. Over the next 12 to 24 months the fungus slowly colonizes the cells of the leaf. After about 9 to 12 months, the needle finally becomes a bit off color. When they become strikingly off color and dried 12 to 24 months later, then needles are cast.

Each needlecast disease on each conifer species varies somewhat in the progression of the symptoms

and the disease. Fundamentally, the fungi that infect conifers must be slow in damaging the plants because most conifers cannot produce a second flush of new leaves in one year to replace diseased leaves like deciduous trees can. The one spring flush of new needles is crucial to survival for the conifer.

The fungi that attack conifer foliage have changed over time by natural selection to grow particularly slowly and cause needles to cast in the second year after infection rather than the same year. Because of selection, it also is difficult or impossible to isolate and culture a needlecast fungus from an infected needle onto agar medium. The fungi either grow extremely slowly in agar medium or not at all. *Rhabdocline* needlecasts of Douglas fir, which Michigan trees often suffer, are caused by pathogens that cannot be cultured on any known nutrients. But the slow growth of needlecast pathogens still is capable of producing damaging, even devastating, disease. Despite the use of fungicides, Michigan growers have lost hundreds of thousands of dollars a year to needlecast diseases.

Progress report

Healthy green conifer needles contain living fungi inside that cause no diseases but simply wait for natural senescence to colonize the tissues. Often the non-pathogenic fungi in a healthy needle are the closest living relatives to the pathogenic species. When we began searching for DNA of a pathogen in a typical pine needle, not only is there DNA from all the organelles in a typical leaf, there is also DNA of several non-pathogenic fungi, which may be very similar to the unique DNA of the pathogen.

Because of the numerous sources of DNA contained in conifer needles, it took about five years of research to perfect a detection system comparable to that of the crime lab. Elsewhere throughout the world, scientists developed systems for detecting DNA of the fungi that cause Swiss needlecast of Douglas fir, and *Scleroderris* canker. Both diseases are common in Michigan, the latter on many conifers in the Upper Peninsula. Unlike other labs around the world, our lab began work with the philosophy that a detection system was needed for every needlecast disease that infects or might infect the many conifers grown in the nurseries of Michigan.

Pathogens of several pine species, spruce, Douglas fir, true firs, juniper, Thuja and arborvitae are included in the research. Systems that have been developed for detecting the pathogens are fast, sensitive and accurate. DNA can be extracted and purified from as few as five needles. The process takes two hours for one sample or 40 samples. The DNA is then examined with special detection systems using several machines and a few more hours of labor (see Figures 1 and 2). Complete testing of 40 samples or more can be finished in a workday. The detection systems are so extraordinarily sensitive that infection by a pathogen is detectable within one month of fungal penetration (spore germination and infection) of the leaf with just five needles per tree in natural field conditions. See sidebar on page 31 for

explanation of how PCR works.

In the last year, many different seed-sources of Douglasfir have been studied. The DNA detection systems have been employed to detect, identify to species and subspecies, and monitor the growth of the pathogens while disease progresses. The seed-sources are at the Washington State Research and Extension Center in a special disease nursery. Four different subspecies of *Rhabdocline* needlecast pathogens, the Swiss needlecast pathogen, endophytic non-pathogenic *Rhabdocline* species, and other endophytic fungi have been examined in symptomless needles. This study is a careful examination of differences in disease resistance in the Douglas fir seed-sources. Invisible disease was monitored from the month of needle flushing in spring, again in fall,

Table 1. Needlecast fungi and related species evaluated in the DNA probe research project. Not all of the fungi are pathogens; some are saprophytic endophytes. Multiple isolates of each fungus are tested and compared.

Host	Fungus	Common name (not all fungi have common names)
Pine	<i>Lophodermium seeditiosum</i>	Lophodermium needlecast
	<i>Lophodermium pinastri</i>	
	<i>Lophodermium conigenum</i>	
	<i>Cyclaneusma minus</i>	Cyclaneusma needlecast
	<i>Cyclaneusma niveus</i>	
	<i>Dothistroma pini</i>	Dothistroma needle blight
	<i>Hormonema dematioides</i>	
	<i>Aureobasidium pullalans</i>	
	<i>Scleroderris lagebergii</i>	Scleroderris canker
	<i>Sirococcus conigenus</i>	Sirococcus tip blight
Juniper	<i>Scirrhia acicola</i>	Brown spot needle blight
	<i>Kabatina juniperi</i>	Kabatina blight
	<i>Kabatina thujae</i>	Kabatina blight
	<i>Sclerophoma pithyophyla</i>	Sclerophoma blight
	<i>Phomopsis juniperovora</i>	Phomopsis blight
Spruce	<i>Rhizosphaera kalkhoffii</i>	Rhizosphaera needlecast
	<i>R. macrospora</i>	
	<i>R. kobayashi</i>	
	<i>R. audemansii</i>	
	<i>R. pini</i>	
Douglasfir	<i>Lirula macrospora</i>	
	<i>Rhabdocline parkeri</i>	Rhabdocline needlecast
	<i>R. pseudotsugae epiphyla</i>	
	<i>R. weiri weiri</i>	
	<i>R. weiri</i> f.sp. <i>obovata</i>	
	<i>R. weiri</i> f.sp. <i>oblonga</i>	
	<i>Phaeocryptopus gaeumannii</i>	Swiss needlecast

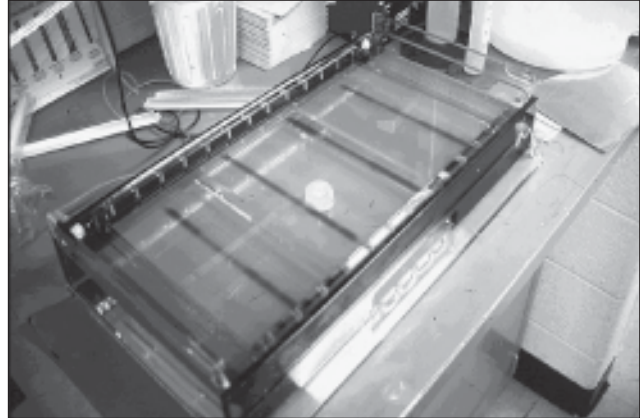
Plant Diseases and Disease Management

and again in the following spring. Once disease became damaging to the trees, the disease levels were rated visually for damage and the damage levels compared to the DNA detection results. The detection systems were remarkably accurate in identifying the subspecies of the pathogen responsible, as well as determining the level of

disease in each tree and seed-source. Additionally, they could detect the increasing amount of growth of the pathogen throughout a 12-month progression of the disease. So, no longer can the killers avoid detection, avoid identification or hide the severity of their crimes! ☹

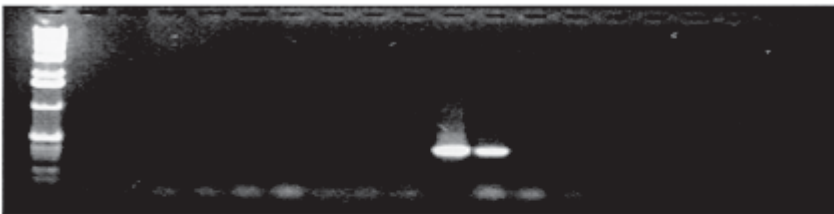
A laboratory process called PCR (polymerase chain reaction) is used to produce billions of copies of a specific short sequence of DNA in several hours. A solution containing the extracted DNA, a special enzyme, nucleotides (the building blocks of DNA) and additional short sequences of DNA called primers are placed in a PCR machine and alternately heated and cooled. The process allows for the selective replication of a particular region of DNA. The primers used create a “probe” that is designed to attach to a fragment of DNA that is species specific. Once this process is complete, small amounts of the resulting DNA fragments are placed in tiny individual wells on a plate of a clear gel. An electric current is run through the gel, and the DNA fragments move along the gel and separate into bands representing specific lengths. The bands fluoresce under ultraviolet light. If the species-specific fragment is present, it shows up as a bright band on the plate, at a position that corresponds to a sequence of DNA of a specific length.

Figure. 1 Gel box used with electric current to separate DNA fragments into bands used for identifying the needlecast fungi



Lophodermium seditiosum probes LS-1 and LS-4 amplification

HD AP1 AP2 SP RK RKO DP CN CM LS LP LC LSP LJ



Lophodermium pinastri probes LP-1 and LP-4 amplification

HD AP1 AP2 SP RK RKO DP CN CM LS LP LC LSP LJ



Figure. 2 Once the gels have been run, they are stained with a dye that fluoresces under ultraviolet light, and photographed. Results of two probes tested to determine whether or not they are species specific. Abbreviations above the gel lanes represent different fungi found in needles.