Alternative Technologies for Management of Soil-borne Diseases in Bareroot Forest Nurseries in the United States[1]

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Abstract—Many forest nurseries producing bareroot seedlings in the United States rely on soil fumigation with methyl bromide and other chemicals to control soil-borne diseases. Methyl bromide production and importation will be phased out by the U.S. Environmental Protection Agency by the year 2000. Growers need alternative production technologies to grow high quality seedlings without fumigation because substitute fumigants are undesirable due to environmental and human health hazards. The Forest Pest Management branch of the USDA Forest Service recently instituted field trials at several U. S. nurseries to evaluate alternative cropping regimes and determine their effects on soil-borne diseases. Various organic amendments, fallow periods, cultivation regimes, and cover crops will be compared to methyl bromide fumigation. Additional studies will evaluate beneficial microorganisms and suppressive soils, develop accurate sampling techniques for differentiating pathogenic F. *oxysporum* populations, karyotype *Fusarium* isolates, and develop ELISA assays for detection and quantification of plant pathogenic *Cylindrocladium* and *Macrophomina* spp.

INTRODUCTION

Most U.S. bareroot nurseries rely on chemical fumigation to reduce levels of soil-borne pathogenic fungi, nematodes, weeds, and insects. Soils are routinely fumigated every 2-3 years at approximately 90% of U.S. bareroot nurseries (Fraedrich 1993). In the northern and western portions of the United States, 80% of the nurseries fumigate soil, whereas fumigation occurs at 96% of the southern nurseries. Many nurseries fumigate soil before each seedling crop, except in the southern United States where soils are often fumigated before every other or every third crop. However, each seedling crop produced in the southern United States usually requires only one year, whereas crops in the western and northern states takes 2-3 years.

Methyl bromide, applied with chloropicrin, is used by almost all nurseries that fumigate their soil (Fraedrich 1993; James 1989). Although other chemical fumigants are available (table 1), most growers believe existing alternatives are not as effective as methyl bromide formulations. Dazomet (Basamid®) fumigant has been tested at half of the nurseries that fumigate their soil, but results show it is often less effective than methyl bromide (Fraedrich 1993).

Methyl bromide is widely used as a soil fumigant to produce strawberries, peppers, tomatoes, tobacco, citrus and ornamentals (NAPIAP 1993). It is also used for post harvest treatment of fruits and vegetables, quarantine treatments, and structural fumigation. The 1991 Montreal Protocol Assessment alleged that methyl bromide was in a category of chemicals that depletes stratospheric ozone (NAPIAP 1993). In response to this assessment, the United States Environmental Protection Agency (EPA) under the Clean Air Act, called for phase out of production and importation of methyl bromide by the year 2000. Although there are many uncertainties regarding methyl bromide's relationship to ozone depletion, the phaseout is progressing. Annual monetary losses resulting from a total ban of methyl bromide use in U.S. agriculture are estimated at $1.3 to 1.5 billion (NAPIAP 1993). The share of these losses attributed to forest tree nurseries amounts to $35 million/year.

Many nursery growers desire alternatives to chemical fumigation because of high costs of fumigation, health risks to nursery workers, visitors, neighbors and wildlife, and a desire to reduce environmental contamination, especially ground water supplies. Growers want an integrated pest management (IPM) approach for dealing with soil-borne diseases, weeds, and insect pests (Campbell 1991; James and others 1990; Raupp and Cornell 1988). Implicit in IPM is a formal decision-making process that considers pest populations and their impacts on hosts, and weighs the effectiveness of con-

trol methods against their impacts on economics, human health, and the environment. This approach requires much more information on biological interactions of soil microorganisms and a reliable system for disease prediction and monitoring of pathogen populations. Because fumigation with non-selective biocides kills all soil organisms, knowledge of the dynamics and interactions of soil micro-organisms have not been critical and little is currently known about them.

To prepare for the loss of methyl bromide, the Forest Pest Management branch of the U. S. Forest Service initiated alternatives to fumigation trials in four western states (California, Oregon, Washington, Idaho), Minnesota and Florida. This paper outlines the project's background, methods and expected outcomes.

RESEARCH BASIS

Although most U.S. forest nurseries routinely fumigate their soil, several western nurseries produce high-quality seedlings without fumigation. Methyl bromide fumigation is prohibited in Canada, so bareroot nurseries there have never fumigated. Yet they still produce high-quality seedlings. These precedents were examined to provide the basis for conversion from a reliance on fumigation to cultural and biological pest management methods.

Effects of *Brassica* cover crops, sawdust amendments, and fallowing with periodic cultivation on production of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings have been evaluated in several nurseries in Oregon and Washington (Stone 1993). This research into alternative cropping regimes indicates that quality conifer seedlings can be grown without soil fumigation by using bare fallow and cultivation. In this trial and others, cover crops stimulated increases in populations of potentially pathogenic organisms and may be detrimental to seedling health (Patrick and Toussoun 1970; Stone 1993).

Research in agricultural systems shows that suppressive soils (those which may contain pathogens, but within which no disease occurs) may be induced by cultural manipulations including addition of organic amendments to soil (Toussoun 1975,; Sinclair and others 1975; Schisler and Linderman 1989). In China, Japan, and other parts of the Orient, organic amendments have been used in agriculture with beneficial effects for many years (Hoitink and Fahy 1986; Huang and Kuhlman 1991; Kelman and Cook 1977). Through cover crop incorporation, and/or organic soil amendment, microorganisms antagonistic toward or competitive with soil-borne pathogens may be stimulated, resulting in lower disease levels (Baker and Cook 1974; Ramirez-Villapudua and Munnecke 1988). These soil treatments create a "biological balance" where pathogenic microorganism populations are kept low while beneficial competitors and antagonists are increased (Danielson and Davey 1969; James 1989; Vaartaja 1967). It may take several cropping cycles to achieve the desired "biological balance" of microorganisms, but once established, such communities are quite stable and should remain so indefinitely unless extensive disturbances occur, such as fumigation (James 1989).

Biological control has reduced soil-borne pathogens in agricultural systems and has great

Table 1 – Non-methyl bromide chemical fumigants for use in forest tree nurseries and their characteristic target organisms (from Andersen and Lee-Bapty 1992).

Fumigant Formulation	Trade Name	Target Organisms
dazomet	Basamid ®	Soil-borne pathogens, weeds, and insects
metam -sodium	Vapam® Busan ®	Soil-borne pathogens, nematodes, weeds, and insects
77.9% 1, 3 dicholopropene 16.5% chloropicrin	Telone C-17®	Soil-borne pathogens and nematodes
1, 3 dichloropropene	Telone II ®	Soil-borne nematodes
20% methyl iso-thiocyanate 40% dichloropropenes and dichloropropanes 10% inert	Vorlex ®	Soil-borne pathogens, weeds. insects and nematodes
Chloropicrin	None	Soil-borne pathogens and nematodes

potential in forest tree nurseries (Baker and Cook 1974; Campbell 1989; Papavizas 1985; Van Wyk and others 1988). Antagonistic and competitive fungi and bacteria provide control of many soil-borne pathogens (Baker and Cook 1974; Elad and Baker 1985; Sivan and Chet 1989). Since commercial biocontrol agents have been developed for agricultural crops other than forest tree seedlings, these agents need to be critically evaluated for efficacy in forest nurseries and integrated with other practices designed to enhance soil suppressiveness. Screening of potential biocontrol agents obtained from forest nursery soils is also needed to determine if nursery specific or region-wide biocontrol agents may be found.

Improvements in monitoring and detecting soil-borne pathogens are needed. Using current assay procedures, *Fusarium* and *Pythium* population levels have not been useful for predicting seedling disease development; there is often little or no relationship between soil population levels of *Fusarium* and *Pythium* and levels of disease on seedlings. This is primarily due to our inability to distinguish between pathogenic and non-pathogenic strains of these fungi. Pathogenic and non-pathogenic strains can be morphologically similar (Bloomberg 1966; Booth 1971) so total populations are not accurate disease predictors. Recently refined molecular techniques may be valuable for differentiating among fungal genera, species, and strains (Kerr 1987; Young 1990). Pulsed-field gel electro-

phoresis, polymerase chain reaction, random primed amplified DNA, and vegetative compatibility groupings will be used to measure genetic differences of fungi (Gordon and Okamota 1992; Puhalla 1985). These techniques will be adapted for use in assays to quickly differentiate pathogenic and non-pathogenic fungal strains within soil and infected plant material without having to resort to burdensome pathogenicity tests.

This project will answer four major questions:

1 Can alternative cropping techniques including bare fallow, cover crops, and soil amendments, be used to manage soil-borne diseases in high-volume production nurseries?

2 Can biocontrol techniques, including beneficial microorganisms and suppressive soils adequately control soil-borne diseases in bareroot forest nurseries?

3 Can recent developments in biotechnology be applied to genetically differentiate pathogenic and non-pathogenic populations of *F. oxysporum* Schlecht. in forest nursery soils and on seedling roots?

4 Can immunoassay techniques be developed for detecting *Cylindrocladium* and *Macrophomina spp.* which are as effective as existing cultural or other techniques of detection.

METHODS

In 1993, eight nurseries set up alternatives to fumigation trials in the western United States: three in California, one in Washington, and two each in Oregon and Idaho. One Florida nursery was included; nurseries in Minnesota, North Carolina and South Carolina will be added in 1994. The project will span four years to allow for bareroot seedling production in the western and northern regions: one year for pre-sowing treatments, two years for crop production, and one year for post-lifting analyses. In the southern region, crops are grown annually; therefore, several crop cycles are possible during this timeframe.

Field treatments tailored to each nursery are being tested. Locally available soil amendments, cover crops adapted to local growing conditions and available equipment will be used to create soil conditions that minimize damage from soil-borne organisms. Treatments include the following organic amendments: sawdust, sewage sludge, composed mushroom media, pine bark, yard waste, and pine needle mulches. Most nurseries will test bare fallowing with periodic cultivation because of its reported success in British Columbia and Oregon and Washington (Stone 1993). Standard chemical soil fumigation will be included for comparison. Treatments will be compared for seedling establishment, disease incidence and severity, seedling growth, and production of shippable seedlings per unit area.

Plots were set up using a complete randomized block design with buffer areas between treatments. Operational nursery procedures for fertilization, irrigation, and weed control are being used. One conifer species of one seedlot will be sown into all treatments to reduce inter-seedlot variability.

Fusarium populations are being monitored and estimates of pathogenic species will be made for each treatment (proportion of the *Fusarium* population that consists of *F. oxysporum).* Soil samples will be processed by one laboratory to reduce variability in assay procedures. Several samples of forest soil will be evaluated for inherent suppressiveness to soil-borne pathogens through greenhouse screening followed by field testing.

Because of its wide geographic distribution and importance in forest nurseries (James and others 1990), *F. oxysporum* will be used for most of the detection protocols. Representative *Fusarium* isolates will be maintained and techniques developed to rapidly elucidate pathogenicity. Pathogenic strains and host range variabilities will be identified and evaluated using molecular techniques to determine the genetic component of pathogenicity. Vegetative compatibility grouping, pulsed-field gel electrophoresis, and polymerase chain reaction to amplify probes will be tested to determine their ability to distinguish pathogenic and non-pathogenic strains. Population genetics of F. oxysporum within selected nurseries will be elucidated and quick assay techniques developed for determining the

proportion of pathogenic strains within a population.

For detection of C*ylindrocladium* and *Macrophomina.* enzyme-linked immunosorbent assays (ELISA) will be developed. The development and testing of ELISA kits will occur in three phases. In the first phase, antibody titers for the immunoassay will be produced from rabbits inoculated with antigens and Freund's complete adjuvant. Three types of testing will be conducted in the second phase: cross-reactivity with other common root pathogens and soil saprophytes, comparison studies with existing detection techniques for *Cylindrodadium* and *Macrophomina,* and determination of sensitivity of the immunoassays. In the final phase, ELISA detection kits will be field tested.

COOPERATORS

This project will include several cooperators from government, universities, and private research laboratories. A listing of cooperators is in Table 2.

EXPECTED PRODUCTS

At the conclusion of this project, the following will be available:

1. Customized recommendations of cultural and biological treatments for management of soil-borne diseases without chemical fumigation.

2. Protocols for differentiating pathogenic and non-pathogenic populations of

Table 2. Cooperators investigating alternative technologies for management of soil-borne diseases in bareroot forest nurseries in the United States

USDA Forest Service
Forest Pest Management
Region 1 (Northern Region)
Region 6 (Pacific Northwest Region)
Region 5 (Pacific Southwest Region)
Region 8 (Southern Region)
Northeast Area - State and Private Forestry
Research
North Central Forest Experiment Station
Southeastern Forest Experiment Station
State Organizations
Florida Division of Forestry
California Department of Forestry
Oregon State **Department of Forestry**
Universities
Oregon State University
University of British Columbia
Clemson University
Private Laboratories
B. C. Research (Vancouver, British Columbia, Canada)
PENINSU-LAB (Poulsbo, Washington)

F. oxysporum and ELISA detection kits for *Cylindrocladium* and *Macrophomina.*

3 Information on the genetic makeup of *Fusarium,* nursery soil suppressiveness, and soil ecosystem interactions.

CONCLUSIONS

The transition from a reliance on chemical soil fumigation to growth regimes without fumigation will be difficult. Most cultural and biological techniques may have limited effectiveness and growers may have to accept higher than usual losses or lower seedling quality. Eventually, pest-caused losses should decline as soils become more suppressive (Toussoun 1975). Improved detection tools will aid in understanding soil ecosystems and allow pathologists to predict effects of cultural treatments on disease development, thereby enabling growers to minimize losses.

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