Sanitation Methods and Monitoring Progress Reduce Disease in British Columbia Container Nurseries[1]

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Abstract.--Conifer seedling nurseries in B. C. have reduced incidence of disease, and applications of pesticides. Sanitation methods that have been implemented and monitoring programs that have been developed for nursery diseases are discussed.

In B. C. there has been a significant reduction in pesticide use at forest seedling nurseries. Over the last 3-5 years, use of chemical pesticides has decreased by 80%. Some nurseries in the province are producing stock entirely pesticide free, and at most facilities there is at least one stock type that is produced free of pesticides each year. There are no herbicides applied to container seedlings, which currently represents over 90% of the stock produced. A rough estimate has shown that approximately 30% of all stock currently produced in the province has not been treated with any chemical pesticide. This of course will vary among nurseries, years and crops.

There are several reasons for the reduction in the use of pesticides. Over the last 10 years Integrated Pest Management programs that utilize biological, cultural, physical and regulatory control, in addition to chemical control have been developed. Many insects are hand removed and destroyed. Biological control agents are continuing to be tested. Improvements are being realized in nursery sanitation, crop nutrition, irrigation regimes, crop spacing, and growing media, and pest monitoring programs are being, and have been developed. Stock destined for the 1+0 summer ship program is grown early in the year when many pest populations are inactive and, as a result, is usually pesticide free. Requests for this stock type are increasing each year.

Specifically there have been significant advances in nursery sanitation methods and disease monitoring programs. This paper will discuss the sanitation methods that have been implemented, as well as the monitoring programs that have been developed for pathogens. Information is presented in a chronological order as it would be used throughout the growing season.

MEDIA TESTING

Even before the seedlings are sown, samples of the growing media are analyzed for presence of pathogens. Nurseries in B. C. are fortunate in having a plant disease clinic operated by John Dennis at Forestry Canada's Pacific Forestry Centre. Over the years, nurseries have sent in numerous samples of growing media for pathogen analysis. Recently, samples of grit used to cover container sown seed were found to contain Fusarium and low levels of Pythium. To assay the grit, approximately 50 granules were sprinkled onto a petri dish with Komada's medium. The plates were incubated at room temperature under natural light and checked at 5 and 10 days. Plating dilution samples was ineffective, possibly because the Fusarium was bound tightly to the grit[3].

Assays showed that 80% of the grit pieces tested contained Fusarium. All Fusarium contaminated grit came from the same supplier. Washing the grit with a solution of bleach helped to drastically reduce but not eliminate this pathogen. The best solution appears to be choosing a supplier who has a Fusarium-free supply of grit.

Samples of peat have also been tested for presence of Fusarium, Cylindrocarpon and Pythium. To assay for Pythium, 1.25 gms of peat are added to 50 mLs of 0.1% water agar and then plated at 1 mL per plate on a V8 juice medium. They are incubated at room temperature in the dark. To assay for Cylindrocarpon and Fusarium 1.25, gms of peat are added to 200 mLs of 0.1% water agar, then plated at 1 ml per plate on Komada's medium. Plates are incubated in the light at 20 - 25° C for Fusarium and 15° C for Cylindrocarpon[4].

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[3,4]Dennis, John 1991. Personal conversation. Nursery and Reforestation Pest Technician, Pacific Forestry Centre, Forestry Canada, Victoria, B. C.

A recent survey showed that 10% of peat samples tested contained Fusarium. Presence of the fungus varied drastically among bales of peat. There seems to be a trend toward higher levels of Fusarium in peat. This may be due to the use of coarser peat with a larger particle size. Larger pieces of peat could provide a larger growing medium thereby sustaining Fusarium for a longer period of time. However, when peat was plated on a particle size basis, the larger pieces did not contain more Fusarium than the smaller pieces. Once the grower is aware of this pathogen being present, they may choose to alter their peat supply.

SEED TESTING

Before sowing begins information about pathogens on the seed is also available. The Ministry of Forests has established a monitoring program for seed held at the Tree Seed Centre to determine if the pathogens Sirococcus and Fusarium are present. Presently, a list of seedlots contaminated with Sirococcus is available to all growers in the Province. For the Sirococcus survey, 500 seeds are collected from each seedlot. These are surface sterilized for 30 minutes in 30% H_2O_2, rinsed twice in distilled water and air dried on sterile towels in a laminar flow hood. They are then plated on 1.5% water agar using 25 seeds per plate. Plates are incubated at 20°C day and 16°C night with an 8 hour 900 lux photoperiod. They are checked after 8 days, then twice weekly for 2 weeks, and then weekly for another 3 weeks[5].

Sirococcus is usually present inside the seeds so a program of surface sterilization for seed would be ineffective. However, this information is helpful to nursery staff. When infested seedlots are sown, they are closely monitored and a fungicide applied as soon as symptoms appear. Diseased seedlings are rogued and burned when practical. The practice of sowing multiple seeds per cavity may allow the disease to spread from seed to seed in containers. This may explain the higher incidence of the disease in containers than in field culture.

Surveys of seed for presence of Fusarium are being initiated at the Tree Seed Centre in 1991. Fusarium inoculum is usually present both inside and on the surface of the seed. Surface cleaning methods have been effective at reducing levels of this seed-borne pathogen. Surveys of Douglas-fir seed in storage have shown that in some seedlots up to 23% of the seed can be infested with Fusarium. However, this is not common. For the Fusarium survey, 500 seeds are collected from each seedlot. They are plated onto medium using 25 seeds per plate, and incubated at room temperature (25 - 28°C) with either normal or constant light. Plates are checked for Fusarium after 7 and 14 days[6].

In 1990 a trial was conducted using five Douglas-fir seedlots to determine if cleaning or disinfecting treatments would reduce the levels of Fusarium. A 24 hour running water soak instead of standing water prior to stratification reduced the incidence of Fusarium on the seed. A four hour soak in 3% hydrogen peroxide or a 10 minute soak in 2.1% sodium hypochlorite followed by a 48 hour running water rinse after stratification reduced the Fusarium levels further, but the results were not consistent. A three minute soak in 70% ethanol reduced germination by 50% in lab and field assessments. Based on these results we feel that running water treatments are an effective way to reduce seed-borne pathogens. Plans have been made to change the operational system for seed imbibition prior to stratification at the Tree Seed Centre.

There is good evidence to suggest that seed-borne Sirococcus and Fusarium cause pre and post-emergence damping off. We do not know however, if they cause foliar blights and root rots later in the growing season. Melody Neumann[7] at B. C. Research is conducting vegetative compatibility trials in 1991 - 92 to determine if the Fusarium that causes root rots was the same as that on the seed. Seed and root isolates will be grown on a chlorate media to create mutations. Mutant isolates from the seeds and roots will then be grown on minimal media containing only one of three sources of nitrogen to characterize them. To determine if the seed and root isolates are of the same vegetative compatibility group (VCG) they will be paired on minimal media. Those nitrogen mutants that are of the same VCG will form a heterokayon and grow profusely.

STYROBLOCK SANITATION

Attempts at ensuring that peat, grit and seeds are pathogen free would be fruitless if they were loaded into contaminated containers. Algae and pathogenic fungi that can inhibit the growth of nursery seedlings are often transmitted from year to year through poor block sanitation. Blocks are used for up to 5 years, and significant levels of inoculum can be found especially in the lower third of the seedling cavity. Pathogens such as Pythium, Fusarium, Phoma, Cylindrocarpon, many species of algae and liverwort spores can all be isolated from used containers.

In 1990, over 100 different sanitation treatments were investigated for control of pathogen inoculum on styroblocks. The most effective methods for reducing fungal and algal propagules on used containers were: steam (95°C for 1 minute); heated soaps (Safer's De-moss or Ivory soap, 10-second dip in 5% solution at 80°C);

[5,6] Dennis, John 1991. Personal conversation. Nursery and Reforestation Pest Technician, Pacific Forestry Centre, Forestry, Canada, Victoria, B. C.

[7] Melody Neumann, Masters Candidate, Faculty of Graduate Studies, Department of Forest Science, University of British Columbia.

bleach (10-second dip in a 0.5% solution buffered to pH 7.0); hydrogen peroxide (10-second dip in 10% solution); sodium metabisulphite (10-second dip in 5% solution); and sulphur dioxide fumigation (Peterson, 1990). Operationally, most nurseries in B. C. are using heated water, bleach or sodium metabisulphite. Sodium metabisulphite provides the best control of fungi, algae and weeds, but it is difficult to use and nursery workers must be careful to wear full protective clothing.

PALLET SANITATION

After sowing, blocks are moved to either greenhouses or open compounds where they are placed on wooden pallets. Root diseases in containers can be favoured by poor drainage, especially where blocks are placed on flat wooden pallets. This results in the cavities becoming blocked at the bottom and this frequently encourages disease. To eliminate this problem, angled runners were designed for the blocks to rest on, allowing free drainage.

Recently a Rhizoctonia problem on lodgepole pine was traced to pallets contaminated with this fungus by a previous poinsettia crop. The greenhouse had been bleached but the wooden pallets had not. In cases where seedling roots are known to be infected with Pythium or Fusarium, these pathogens can also be isolated from samples of wood taken directly beneath the styroblocks. We do not know if the disease came from the styroblocks first or whether the pallets could be transmitting the fungi to the stock. Some nurseries have a program of washing the pallets with bleach between crops.

GREENHOUSE SANITATION

Greenhouse floors, walls, roofs and benches can also be a source of disease fungi and should be cleaned. The facility may first be washed with a high pressure hose followed by an application of household bleach (6%) at 1 part bleach/10 parts water. Some nurseries leave the bleach solution in the house and turn up the heat to obtain a fumigant effect. Algae and some weeds can be controlled on greenhouse floors using a slurry of copper sulphate at 20 pounds copper sulphate to 100 gallons of water. Care should be taken to keep the solution away from metal because it can be corrosive. The advent of large outdoor compounds for production of container stock has caused an increase in weed problems, particularly liverworts. It is important to keep the ground free of weeds in these areas. Asphalt or cement are obvious choices, but often the expense is prohibitive. Ground covers such as black propex have been effective. Herbicides such as glyphosate, simazine and gramoxone are applied on compound floors, and in and around greenhouses to control weeds.

[8,9] Dennis, John 1991. Personal conversation. Nursery and Reforestation Pest Technician, Pacific Forestry Centre, Forestry Canad, Victoria, B. C.

DISEASE MONITORING

To reduce disease spread throughout the growing season, all crops are monitored closely. Diseased seedlings are rogued and the affected area noted. Roguing is particularly effective at reducing Sirococcus and Fusarium caused foliar blights. A fungicide tolerance test is used to determine resistance by Botrytis, Rosellinia, Cylindrocarpon, and Fusarium. Aerial condidia or sporodochia are innoculated onto 5 marked areas on PDA medium ammended with 50 ppm of the fungicide to be tested. When observed under a compound microscope, fungicide sensitive strains will either not germinate or produce abnormal germ tubes and hyphae. Resistant strains will grow normally although somewhat slower[8].

WATER TESTING

Finally, at most nurseries supplies of water used to grow the stock are monitored. Nurseries obtain their water for irrigation from a variety of sources such as rivers, lakes, wells or municipal water systems. Water can be a source of disease carrying moulds and it is important to check any water source before it is used. This can be accomplished through baiting with an unripe pear. The pear is placed in a mesh bag and submerged in an area where water is flowing such as near the surface of an inlet or outlet. This allows a large volume of water to be sampled. The length of time the pear is left in the water depends on the temperature of the water:

40 - 50 F	7 Days
50 - 60 F	5 Days
60 - 70 F	4 Days
80 - 90 F	2 Days

Swimming zoospores of Pythium and Phytophthora infect the pear tissue and produce a brown dry, corky-type rot. The pear is then sent to the lab where the brown spots are cultured from the decay margin onto PDA or CMPV (Cornmeal agar containing Vancomycin 20 ppm and Pimaricin 20 ppm) agar[9].

If pathogens are discovered the nursery can investigate alternate water supplies or install a chlorination system on site. The use of injected chlorine gas in irrigation water can reduce the disease spread. A level of free chlorine of 2 ppm for a minimum of one minute will kill spores of Phytophthora cinnamomi. The recommended residual level of chlorine in irrigation water is 0.5 - 1.0 ppm (C. Barnett, 1990).

LITERATURE CITED

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