Fusarium diseases of conifer seedlings

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Presented at the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries), Victoria, British Columbia, Canada, August 22-30, 1990.

Abstract

Diseases caused by *Fusarium* spp. are important factors reducing conifer seedling production, particularly in western North America. These diseases are especially damaging to container-grown seedlings. Although most conifer species are affected, these diseases are particularly damaging to Douglas-fir, western larch, true fir, and spruce. Several *Fusarium* spp. are involved, the most common being *F. oxysporum* and *F. acuminatum*. Preventing high infection levels through integrated pest management approaches is the most effective way of reducing losses from *Fusarium* diseases. Consistently placing isolates in proper taxa makes it difficult to work with *Fusarium*.

Résumé

Les maladies causées par *Fusarium* spp. contribuent énormément à réduire la production de semis de conifères, notamment dans l'ouest de l'Amérique du Nord. Ces maladies affectent tout particulièrement les semis en récipients. Bien qu'elles infectent la plupart des essences de conifères, elles ravagent tout particulièrement le douglas taxifolié, le mélèze occidental, le sapin et l'épinette. Plusieurs *Fusarium* différents causent des dégâts, les plus répandus étant *F. oxysporum* et *F. acuminatum*. La façon la plus efficace de réduire les pertes causées par *Fusarium* est d'empêcher l'apparition de taux élevés d'infection grâce à l'utilisation de méthodes de lutte intégrée. Il est difficile de travailler avec *Fusarium* car il faut constamment classer les isolats dans le taxon approprié.

Introduction

Many early reports of diseases of conifer seedlings grown in nurseries described damping-off and root diseases. *Fusarium* spp. were consistently isolated from diseased seedlings and shown capable of eliciting disease in controlled pathogenicity tests (Gifford 1911; Hartley and Merrill 1914; Hartley and Pierce 1917). Most control attempts involved using agricultural fungicides and fumigants to reduce disease losses (Hartley and Merrill 1914). Many early studies were designed to determine which environmental factors in the nursery were conducive or restrictive to disease (Rathbun 1922; Tint 1945a, 1945b).

Although environmental effects were studied, epidemiology of the disease on conifer seedlings was unclear until a series of reports by Bloomberg (1971; 1973; 1976) detailed the biology of root disease caused by *F. oxysporum* Schlecht. on bareroot Douglas-fir seedlings in British Columbia. This work provided the basis for understanding the epidemiology of *F. oxysporum*; a model outlining infection processes and disease development was formulated (Bloomberg 1979). This model helped nursery growers develop more effective strategies for combating disease in bareroot nurseries. Unfortunately, not all of this information is applicable to container operations, which are increasing in importance in the western United States and Canada. *Fusarium*-associated diseases are extremely damaging in some container facilities (James 1986a). Efforts to control these diseases using common fungicides have often failed (James *et al.* 1988c; Williams 1989). To formulate more effective ways of reducing damage, investigations were initiated in the early 1980s to understand the biology of *Fusarium* spp. in container operations. Results of some of this work are briefly summarized in this paper.

Types of diseases

Fusarium spp. cause several different diseases on conifer seedlings. These fungi decay seed, thus preventing germination (Bloomberg 1981; Huang and Kuhlman 1990; Matuo and Chiba 1966). They also induce damping-off either before (pre) or after (post) seedling emergence (Bloomberg 1981; Hartley *et al.* 1914; Lock 1973). Damped-off seedlings are usually attacked at the radicle or main stem when tissues are succulent

(Spaulding 1914). Once seedling stems lignify a few weeks after emergence, they are no longer susceptible to damping-off (Rathbun-Gravatt 1925; Spaulding 1914). *Fusarium* spp. also cause root disease during the first growing season, but seldom cause disease on bareroot stock during the second growing season (Enebak *et al.* 1990; Sinclair *et al.* 1975). Several *Fusarium* spp. are capable of causing stem cankers above or just below the groundline (Brownell and Schneider 1985; Cooley 1983; Hansen and Hamm 1988); these cankers may expand and eventually girdle seedlings. Some *Fusarium* spp. also cause top blight, resulting in apical dieback of seedlings (Bloomberg 1981; Hartley *et al.* 1914; Matuo and Chiba 1966). *Fusarium* canker and top blight diseases may be associated with other pathogenic organisms (Hansen and Hamm 1988).

A major problem in dealing with *Fusarium* diseases is seedling infection without disease symptoms being produced (Bloomberg 1971, 1973; Hartley *et al.* 1914; James *et al.* 1987). Surveys indicate that many seedlings may be infected although disease symptoms are lacking (James and Gilligan 1988d), especially on container-grown seedlings (James and Gilligan 1985, 1988a; James *et al.* 1987).

Hosts affected

Most conifer species are susceptible to infection by *Fusarium* spp. (Bloomberg 1981). However, in western North American, most damage occurs on Douglas-fir, western larch, true fir, and Engelmann spruce (Bloomberg 1971; Hansen and Hamm 1988; James 1985c; James and Gilligan 1985; James *et al.* 1987). Sugar pine is also severely damaged in southern Oregon and northern California (Cooley 1983). Although commonly infected, ponderosa pine rarely displays disease symptoms (James 1985c; James and Gilligan 1988a).

Associated *Fusarium* spp.

Work with bareroot conifer seedlings identified F. *oxysporum* as the major pathogen (Bloomberg 1971, 1973, 1976, 1979; Brownell and Schneider 1985; Cooley 1983; Tint 1945a). This species is also common on container-grown seedlings, but other species frequently isolated include *F. acuminatum* Ell. & Ev., *F. avenaceum* (Fr.) Sacc., *F. solani* (Mart.) Appel & Wollenw., *F. sambucinum* Fuckel, and *F. tricinctum* (Corda) Sacc. (James *et al.* 1989b). Isolates of *F. oxysporum* and *F. acuminatum* can cause severe disease of container-grown seedlings (James and Gilligan 1984; James *et al.* 1986, 1989a). Other fusaria occasionally isolated from container-grown conifer seedlings include *F. poae* (Peck) Wollenw., *F. equiseti* (Corda) Sacc., *F. lateritium* Nees., *F. moniliforme* Sheldon, *F. proliferatum* (Matsushima) Nirenberg, *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas, and *F. sporotrichioides* Sherb. (James 1986a; James and Gilligan 1985; James *et al.* 1988c). Under certain environmental conditions, some of these species may be pathogenic to conifer seedlings (Bloomberg 1981; Rathbun-Gravatt 1925).

Epidemiology in container-grown seedlings

Conifer seed assayed prior to or just after sowing is often contaminated with *Fusarium* spp. (James 1986b, 1987b). These fungi are isolated most frequently from seedcoats, with much lower levels found internally on seed embryos (James 1987b). Extent of seed contamination seems related to seedlot, i.e., greater disease occurs within specific seedlots. Disease levels are often higher in seedlots obtained from squirrel caches than those collected directly from trees (James 1987b), although some specific lots gathered from trees may be extensively colonized with *Fusarium* (James 1985b). Investigations to locate sources of seed contamination during cone collection, transport, and processing have been lacking. However, *Fusarium* spp. may spread throughout seedlots during stratification (W.R. Littke, Weyerhaeuser Forestry Research Center, Centralia, WA., personal communication) and are often introduced into container operations on contaminated seed (James 1986a, 1987b).

Another important source of *Fusarium* inoculum in container operations is contaminated containers reused to grow several crops of seedlings. *Fusarium* spp. often colonize the inner walls of both styroblock and pine cell containers (James and Gilligan 1988b; 1988c; James *et al.* 1988a). Other possible inoculum sources include organic debris on greenhouse benches, walls, and floors and weeds growing within or adjacent to greenhouses (James *et al.* 1987). Weeds may harbor *Fusarium* spp. similar to those that attack conifer seedlings (James *et al.* 1987), although their pathogenicity to seedlings has yet to be evaluated. Investigations have shown that *Fusarium* spp. produce spores usually disseminated by water splash (Ingold 1960; Snyder 1981). However, spores of some fusaria are dispersed by air currents, particularly within greenhouses (Horst *et al.* 1970; Lukezic and Kaiser 1966; Rowe *et al.* 1977), and may be introduced through various openings. Importance of airborne inoculum in container conifer seedling production has yet to be determined.

Fusarium inoculum remains viable for long periods of time as chlamydospores (Park 1959; Price 1984). Under the right conditions of temperature, moisture, nutrients, and presence of a suitable host, i.e., exudation production by host roots, these spores will germinate and may infect seedling roots (Elad and Baker 1985;

Oritsejafor and Adeniji 1990; Price 1984). When environmental conditions are conducive, parasitic *Fusarium* species are rapid colonizers of host roots via rapid spore germination and penetration of host epidermal and cortical cells (Bloomberg 1973; Bloomberg and Trelawny 1970; Katan 1971). Pathogenic strains of *Fusarium* may be poor competitors with other soil organisms (Mitchell and Alexander 1961; Palmer and Kommedahl 1969; Sivan and Chet 1989). They are adapted to remain dormant until a suitable host is available (Gordon *et al.* 1989; Palmer and Kommedahl 1969).

Once host penetration occurs, *Fusarium* extensively colonizes cortical tissues (Bloomberg 1976), although penetration into root endodermis tissues and subsequent attack on the vascular system may be delayed (Gerik and Huisman 1985). Infections may remain quiescent in cortical tissues throughout much of the seedling growth cycle. Although present, *Fusarium* spp. may not necessarily initiate below or above-ground disease symptoms (James *et al.* 1987). Symptom production may be due to virulence characteristics of the colonizing *Fusarium* strains (Bloomberg 1971) and environmental stresses that reduce host resistance or enhance pathogen activity. Interactions of many interrelated factors probably influence disease severity (Fisher and Toussoun 1983). Unfortunately, in container-grown seedlings, root infection is an inaccurate predictor of disease levels (James *et al.* 1987). More research is needed to quantify environmental influences on disease symptom expression in conifer seedlings.

Once disease symptoms appear on host plants, *Fusarium* has extensively colonized the root system (Harling *et al.* 1988; James *et al.* 1987). For conifer seedlings, symptoms include general wilting (including twisting of needles) and needle-tip dieback followed by foliar chlorosis and necrosis (James 1985c; James and Gilligan 1985; James *et al.* 1987). Many diseased seedlings are also stunted (James *et al.* 1987). When seedlings die, orange-colored sporodochia sometimes appear on the main stem just above the groundline (James 1986a; Landis 1976). Only some *Fusarium* spp. are capable of producing sporodochia (Nelson *et al.* 1983), but seedlings infected with these species may still lack these structures (James *et al.* 1987). Disease symptoms of container-grown seedlings are most apparent for about 2-3 weeks after seedling emergence and more severe toward the end of the growth cycle when seedlings are water and nutrient stressed during bud initiation and hardening-off (James 1986a; James and Gilligan 1985; James *et al.* 1987).

Seedlings transplanted within nurseries or outplanted in forests may be stressed until they become established. Seedlings infected with *Fusarium* may become diseased as a result of this stress. This has been verified when infected seedlings are transplanted elsewhere in the nursery (James, unpublished), but may or may not be important once seedlings are outplanted in forest soils. Previous investigation (Smith 1967) showed that *Fusarium* spp. are often replaced by other microorganisms on roots once seedlings are outplanted in forest soils. *Fusarium* spp. are insignificant inhabitants of most temperate forest soils (Meyer 1967; Park 1963) and may be unable to successfully compete with other microorganisms (Mitchell and Alexander 1961; Sivan and Chet 1989). Therefore, planting infected seedlings in forest soils may not necessarily result in high disease losses.

Control options

Fusarium diseases in some bareroot nurseries have effectively been controlled with soil fumigation using standard biocides like methyl bromide and chloropicrin (Sinclair *et al.* 1975). Fungicide drenches are also periodically used to control disease in seedbeds (Bloomberg and Orchard 1969). However, fungicide treatments are usually less effective than fumigation.

Unfortunately, control of *Fusarium* diseases of container-grown seedlings has proven more difficult. An integrated program to reduce host infection levels has been the best approach (James *et al.* 1988c). Reducing levels of *Fusarium* inoculum to which seedlings are exposed is important in limiting infection. Prevention is much more effective than trying to "cure" disease once symptoms are noticed (James *et al.* 1988c; Williams 1989).

It is important to reduce inoculum carried on seed. Treating seeds with chemical pesticides has produced mixed results. Surface sterilants such as hydrogen peroxide and sodium hypochlorite (standard bleach) have been effective in reducing amounts of seedborne *Fusarium* (Barnett 1976; Dumroese *et al.* 1988; James and Genz 1981). Unfortunately, these chemicals are sometimes toxic and may adversely affect seed germination or damage young germinants (Edwards and Sutherland 1979; James 1983). Fungicides have also been tested as seed treatments, but problems with reduced germination and phytotoxicity have precluded their widespread use (Cooley 1983; Lock *et al.* 1975). Subjecting seed to running water rinses for at least 48 h. is effective in reducing amounts of *Fusarium* and other fungi colonizing seedcoats while preconditioning seed for germination (James 1987a; James and Genz 1981). Running water rinses are more effective in reducing fungal inoculum than standing water. Treating seeds with water heated with microwaves was also effective

in reducing amounts of seedborne *Fusarium* (James *et al.* 1988b).

Another potential source of *Fusarium* inoculum is the peat:vermiculite growing medium for container seedlings (James 1985a). Growing media can be fumigated or steam treated to reduce potential pathogen populations (Baker and Olson 1959). However, commercially prepared media are usually pathogen-free and may contain fairly large populations of antagonistic organisms (James 1985a). Therefore, media is not usually treated unless severe disease problems occur (James and Gilligan 1984).

One major source of *Fusarium* inoculum in container operations is reused styroblock and plastic cell containers (James et al. 1988a). *Fusarium* propagules, which increase with repeated use, are usually found at highest levels near the bottom of containers (James 1989). High-pressure steam, commonly used at many nurseries to clean containers, is usually inadequate at removing these propagules (James *et al.* 1988a). However, recent work (James and Woollen 1989) indicates that immersion of containers in hot water may effectively kill pathogens, particularly if water temperatures are above 68°C and containers are immersed for at least 3-5 min. A little detergent or surfactant added to the hot water improves contact with container surfaces. Several chemicals including sodium metabisulfite, standard bleach, and other common sterilants have been used to clean containers (Sturrock and Dennis 1988). Although some of these successfully reduce *Fusarium*, there may be problems with worker exposure to and disposal of toxic chemicals. For these reasons, many growers are implementing hot water immersion methods for cleaning containers.

When growing seedlings in greenhouses, sanitation before and during each crop is very important. Interior surfaces of greenhouses, including benches, walls, ceilings, and floors, should be washed thoroughly between crops with sterilants such as bleach. Weeds within and adjacent to greenhouses should be removed periodically, since they may harbor *Fusarium* spp. that attack nursery seedlings (James *et al.* 1987). During the crop cycle, diseased seedlings should be removed to help prevent fungal spread within greenhouses.

Unfortunately, fungicides are only partially effective in controlling *Fusarium* diseases (James *et al.* 1988c; Williams 1989). They are usually effective against damping-off, but often inadequately control root disease of older seedlings (James and Gilligan 1984; James *et al.* 1988c). This is probably because much inoculum and root infection occurs near the bottom of plugs (James 1989) and chemicals seldom reach most infection sites in sufficient concentrations to be toxic. Also, by the time above-ground symptoms become apparent, seedling root systems are thoroughly colonized and most fungicides are unable to "cure" infected plants. Another problem with repeated chemical use is potential development of fungicide resistance by *Fusarium* (Dekker 1976). When exposed to the same fungicides for a long time, selection pressure on fungi may be high enough to induce resistance. By using the lowest effective dosages and rotating several different fungicides, chances for development of resistance in resident pathogen populations may be minimized (Delp 1980).

Several environmental factors significantly affect severity of *Fusarium* diseases of container-grown conifer seedlings. Nitrogen fertilizers often stimulate succulent growth of seedlings making them more susceptible to damping-off (Sinclair *et al.* 1975; Tint 1945a). Ammonia nitrogen sources are usually more conducive to disease than nitrate nitrogen (Maurer and Baker 1965). Potassium amendments may improve host resistance to disease, at least in young seedlings (McClellan and Stuart 1947; Sinclair *et al.* 1975). Saturated conditions due to overwatering reduce oxygen interchange of roots and may help promote infection by pathogenic fungi (Hargreaves and Fox 1978). Also, too little water stresses plants and promotes infection and increased disease severity (Cook 1981). Another important factor affecting disease severity by *Fusarium* spp. is temperature (Bloomberg 1973). Several *Fusarium* spp. are considered "warm weather" fungi, growing best and inciting more severe disease at high temperatures (above 24°C) (Bloomberg 1973; Booth 1971; Nelson *et al.* 1983; Tint 1945b). By properly controlling temperature, moisture, and fertilizers, impact of *Fusarium* diseases can be reduced.

Under natural conditions, many *Fusarium* spp. are restricted by a wide range of competitive and antagonistic microorganisms. Several potential biological control organisms have been developed commercially for control of different pathogens, including *Fusarium* (Harman and Taylor 1988; Stasz *et al.* 1988). These include fungi of *Trichoderma* and *Gliocladium*, and several types of bacteria (Baker and Cook 1974; Papavizas 1985). Biological control organisms exert direct antibiosis against pathogens, compete for space, nutrients, and colonization sites, and may parasitize pathogens (Baker and Cook 1974). Biological control agents may be applied during sowing as a topical dressing or incorporated into the growing medium; they may also be applied as seed dressings. Although biological control has proven effective in many agricultural systems, adequate testing on conifer seedlings is lacking. An integrated approach using cultural, biologi-

cal, and chemical treatments is needed to effectively control *Fusarium* diseases of container-grown seedlings.

Working with *Fusarium*

One of the greatest difficulties plant pathologists have when working with *Fusarium* diseases is taxonomy (Price 1984). Isolation of organisms from diseased seedlings is fairly easy and confirming pathogenicity is usually simple. However, placing isolated fusaria into the proper taxa on a consistent basis may be quite difficult. This problem is compounded by the fact that there are several different "accepted" taxonomic treatments of *Fusarium*. Taxonomic problems make it difficult to compare findings reported in the literature because of possible isolate misidentification.

Isolation of associated *Fusarium* spp.

Standard procedures for *Fusarium* isolation include washing of plant surfaces to remove pieces of soil or organic debris. Washed specimens are surface sterilized to eliminate contaminating organisms. This is especially important for roots where rhizosphere organisms may be confused with root-infecting organisms (Parkinson 1967). After surface sterilization, tissues are rinsed with sterile water to remove residual sterilant. For quantification of root colonization, selecting root tips or randomly selecting root pieces from dissected root systems has proven useful (James 1985c; James and Gilligan 1985). Some investigators determine extent of root colonization by calculating number of *Fusarium* colonies per unit length of root (Bloomberg 1973). Several different selective media have been developed for preferentially isolating *Fusarium* spp. while keeping soil saprophytes and other microorganisms at a minimum. Most of these contain antibiotics and fungicides, such as PCNB, which restrict growth of common soil fungi and bacteria. Nash and Snyder (1962) developed their selective medium specifically for *F. solani*, but it is effective for isolating most fusaria. Komada (1975) developed another medium specifically for *F. oxysporum* and closely related species, but some *Fusarium* spp. grow poorly on this medium and may be overlooked (Nelson *et al.* 1983). However, this medium has proven very useful in isolating *Fusarium* spp. associated with conifer seedling diseases (James *et al.* 1989b).

Plates should be incubated under light to induce sporulation necessary for identification. Diurnal cycles of cool, fluorescent light are usually successful (Komada 1975). Black light also induces sporulation of fusaria (Nirenberg 1981). Plates are incubated at about 24°C (22-26°C) for 7 to 10 days to allow fungi to grow from the sample and over the agar surface. When colonies are confirmed as *Fusarium*, portions should be transferred to potato dextrose agar (PDA) or other media necessary for identification.

Identification of associated *Fusarium* spp.

Because of their importance as plant pathogens, *Fusarium* spp. have been studied extensively. Several taxonomic treatments of this genus have been formulated, each with slightly different emphasis on characteristics thought to be consistent for specific taxa. Taxonomy of the genus has been based on morphology, physiology, genetics, and molecular biology. Most practicing plant pathologists are limited by expertise or facilities to using morphological characteristics which can consistently differentiate taxa. Most taxonomic systems based on morphological characteristics use presence of microconidia and chlamydospores, morphology of macroconidia, and types of conidiogenous cells as major criteria. Less important characteristics include colony morphology and pigmentation, growth rates at specific temperatures, and production of sporodochia and sclerotia. Several of these latter characteristics vary widely among different isolates of the same taxon and mutations may occur over time. It is important that the taxonomic system used by plant pathologists emphasizes consistent characters easily determined and without excessive subjective judgment on the part of the observer.

Experience during the past several years indicates that the taxonomic system of Nelson *et al.* (1983) is relatively easy to use and provides consistent identifications of *Fusarium* spp. isolated from conifer seedlings. Another useful taxonomic system is by Gerlach and Nirenberg (1982). Both systems were developed from the original taxonomic work on the genus by Wollenweber and Reinking (1935). The Nelson system has proven more practical for identification of commonly encountered fusaria than descriptions by Snyder and Hansen (1940) or Booth (1971). Snyder and Hansen's system lumps all fusaria into only nine species. Our experience is that many isolates classified as the same species by their system are clearly different and should be separated. Booth's system seems too cumbersome and has too many individual taxa delimiting organisms with only slight variations. However, the system of Nelson *et al.* (1983) seems a reasonable compromise. Important characteristics of the Nelson system include using standard growing conditions, media, and transfer procedures. For this system, all isolates to be identified should be grown on carnation leaf agar to induce sporodochia and uniform (size and shape) production of macroconidia and microconidia

(Fisher *et al.* 1982). Plates should be incubated under diurnal cycles of cool, fluorescent light to enhance spore formation and uniform growth. Single-spore transfers should be made to reduce potential for mutation, particularly when isolates remain on agar media high in carbohydrates (such as PDA) for extended periods of time. For colony morphology, pigment production, and growth rate, potato dextrose agar is recommended. Growth on water agar amended with KCl promotes microconidial chains for isolates in the group Liseola. Several of these fungi produce microconidia in both false heads and chains, but the characteristic chains are usually absent unless grown on a medium with low water potential, such as water agar amended with KCl. Another useful technique is growth on a low nutrient medium (SNA) with exposure to continuous black light. This induces sporodochia and microconidial chain formation (Nirenberg 1981).

Several *Fusarium* spp. are notorious for mutating in culture. These mutants usually proceed from more natural sporodochial types (Waite and Stover 1960; Wellman and Blaisdell 1941). Mutants may either be mycelial, which form abundant aerial mycelium but few macroconidia, or pionnotal types, which produce little or no aerial mycelium but abundant macroconidia (Nelson *et al.* 1983). Mycelial mutant types frequently lack sclerotia, sporodochia and pigmentation; pionnotal mutant types have a slimy, wet appearance (Waite and Stover 1960). In pathogenic isolates, mutants frequently exhibit a loss in virulence and toxin production. Mutation can be minimized by single-sporing cultures, hyphal tipping (instead of mass transferring cultures), avoidance of carbon-rich media (such as PDA), and keeping subculturing to a minimum (Nelson *et al.* 1983; Wellman and Blaisdell 1941). Long-term storage of cultures is best in either liquid nitrogen or by lyophilization (Nelson *et al.* 1983).

Pathogenicity testing

Some *Fusarium* isolates commonly obtained from conifer seedlings with disease symptoms are not pathogenic (James and Gilligan 1984; James *et al.* 1989a). Non-pathogenic fungi often reside in the rhizosphere or superficially colonize roots (Parkinson 1967). In order to confirm pathogenicity, suspected isolates must be subjected to Koch's postulates (Agrios 1969). If done properly, isolates that fulfill the criteria defined by Koch's postulates can be classified as pathogens. However, care must be taken to ensure that inoculum concentrations are not excessive and that tests are not contaminated with other *Fusarium* isolates (James *et al.* 1989a).

Different inoculum types have been used for evaluating pathogenicity of *Fusarium*. Some investigators used spore suspensions, either dipping test seedlings in solutions (Walker and Foster 1946) or pouring solutions next to seedlings (Walker and Hooker 1945). Stem inoculations have also proven useful for quick screening of large numbers of isolates (Hansen and Hamm 1988). One common technique used for conifer seedlings is introduction of test isolates on young germinants in sterile test tubes (James *et al.* 1986; Vaartaja and Bumbieris 1967). Although such tests may seem quite artificial, they can provide useful information for a large number of isolates in a short period of time.

One very successful method for inoculating conifer seedlings uses inoculum composed of a cornmeal-perlite substrate colonized by *Fusarium*. This inoculum is incorporated into growing media in which seedlings are transplanted (James and Gilligan 1984; James *et al.* 1989a). This method is a variation of that described by Miles and Wilcoxson (1984) and closely mimics behavior of chlamydospores in a natural environment, i.e., the perlite substrate provides a source of fungal material which can infect roots as a response to root exudates. If refrigerated, cornmeal-perlite inoculum remains viable for a year or more.

When properly conducted, pathogenicity tests can give useful information regarding fusaria encountered when investigating conifer seedling diseases. However, such tests are time-consuming and expensive. Valuable but less costly alternatives include protein analysis (Partridge *et al.* 1984), vegetative compatibility (Puhalla 1985), and genetic tests comparing nucleic acids (Kuninaga and Yokosawa 1989). Comparisons with known pathogenic isolates could be made rather quickly, thus precluding the need for elaborate pathogenicity tests. Unfortunately, these techniques require a certain amount of expertise and equipment often unavailable to many plant pathologists. Nevertheless, these and other new techniques should be evaluated when possible to improve our understanding about *Fusarium* isolates associated with conifer seedling diseases.

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