Effects of Fumigation on Soil Pathogens and Beneficial Microorganisms[1]

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Abstract.--Soil fumigation with broad-spectrum biocides is a non-selective means of killing soil-borne pathogens in forest seedling nurseries. Beneficial microorganisms (antagonists, competitors, pathogen parasites and mycorrhizal fungi) are also killed by most fumigants. Organisms are killed by direct contact with fumigants. Dormant structures of microorganisms are usually more resistant to fumigant action. Specific fumigants are more effective against certain microorganisms. Fumigant effects on populations of selected pathogens *(Fusarium, Pythium, Phytophthora, Rhizoctonia, Macrophomina,* and *Phoma)* and certain antagonistic fungi, bacteria, and mycorrhizal symbionts are discussed.

INTRODUCTION

Using soil fumigation to control soil-borne diseases has recently increased in importance at many forest tree seedling nurseries in the United States. Techniques using broad-spectrum soil fumigants to enhance plant production by reducing impacts of pathogenic fungi were first developed for agricultural crops (Miller and Norris 1970). Operational soil fumigation over a relatively large area was probably first successfully used for strawberry production in California (Wilhelm and others 1974). Techniques and products developed for agriculture have been implemented at many forest tree nurseries. Soil fumigation has usually improved the number and quality of seedlings produced (Klock and Benson 1975; Norris 1983; Norris and Hessburg 1985; Smith and Bega 1966) while reducing weeds and soil insect problems.

Over the years, several chemicals have been tested for use as soil fumigants. However, consistent beneficial effects have only been obtained with a few products and formulations (Munnecke and Van Gundy 1979; Wensley 1953). Combinations of several different fumigants are often more effective than single chemicals (Smith and Bega 1966), as is the case with methyl bromide and cholorpicrin (MBC). MBC is the most commonly used fumigant combination for forest nurseries, and although different formulations have been tested, the most popular and effective solution for controlling soil-borne pathogens is 67 percent methyl bromide and 33 percent chloropicrin. Most fungi are more susceptible to cholorpicrin mixtures than the methyl bromide alone (Ebben and others 1983; Munnecke and Van Gundy 1979). Other fumigants used less frequently include metam-sodium (Vapam®) and dazomet (Basamid®).

All fumigants kill soil microorganisms non-selectively through direct contact (Boone 1988). Susceptibility of microorganisms to fumigants is variable, especially at reduced or 'sub-lethal' dosages. However, all microorganisms are susceptible if fumigant concentrations are high enough. Fumigant action is largely affected by soil temperature, i.e., most chemicals are more effective at higher temperatures (Gandy and Chanter 1976).

ECOLOGY OF SOIL MICROORGANISMS

Nursery soil is an extremely conducive habitat for a variety of microorganisms. Their relative numbers may fluctuate widely and are greatly affected by season, cropping history, and types of amendments. Normally, microorganisms interact with each other and compete for substrates. Many pathogenic fungi are rapid initial colonizers of suitable substrates, such as host roots. This provides them a better competitive position since many do not compete well with more free-living, saprophytic soil organisms (Papavizas 1985).

Most soil organisms are either actively colonizing substrates or dormant. Substrates are colonized by a succession of organisms. As indicated above, pathogens often are initial colonizers and are followed by other organisms that are better competitors. Competition by soil microorganisms is often intense; many are capable of producing powerful 'antibiotics which give them competitive advantages (Papavizas 1985), while some parasitize other organisms. Another competitive advantage is rapid spore germination and growth when new substrates become available.

Many pathogens produce dormant 'resting' structures which are stimulated into activity by presence of a suitable host. Roots of most plants exude amino acids which may stimulate germination of spores as well as provide directional gradients for motile spores and growing hyphae of certain pathogenic fungi (Rovira 1970). Most dormant structures are fairly long-lived and resistant, although they respond readily to host root exudates and may be

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susceptible to damage by biocides like soil fumigants.

FUMIGATION EFFECTS

General

Most soil fumigants are non-selective in their action against microorganisms, but rarely are all organisms killed during standard treatments. Organisms tightly bound in soil aggregates and those deeper in the soil below the zone of effective fumigation (greater than 25-30 cm) may escape undamaged (Fuller and others 1980; Kolbezen and others 1974; Marshall 1985). Some investigations (Baines and others 1966; Ebben and others 1983) found that different organisms have different sensitivities to commonly used fumigants. These tests were usually done at lower fumigant concentrations. Most of these investigations showed that microorganisms which produce more resistant dormant structures (such as fungal sclerotia) are more resistant to fumigants.

Once fumigated, soil is conducive to rapid reinvasion by microorganisms (Danielson and Davey 1969; Munnecke and Van Gundy 1979). A 'biological vacuum' may occur and any organism initially introduced into fumigated soil often expands rapidly to produce abundant populations because of the lack of competition from other organisms. Fumigation also releases an abundance of nutrients (from death of previous organisms) which may provide an important source of growth substances for invading organisms (Munnecke and Van Gundy 1979). Organisms commonly reinvading fumigated soil produce air-borne propagules, are located deeper in the soil, or reside within adjacent non-treated fields (Danielson and Davey 1969). Important re-invaders may also be introduced on seed, nursery implements, or in water.

Effectiveness of soil fumigation is often monitored by assaying for selected microorganisms before and afterfumigation (Johnson and Zak 1977; Marshall 1983). The two most common genera of fungi assayed are Fusarium and Pythium. Soil dilutions on selective agar media are made and the number of propagules (colony forming units) per unit weight of soil (usually grams) are calculated. These assays are for total populations of these fungi and do not necessarily determine levels of pathogens. There are pathogenic and saprophytic strains of both Fusarium and Pythium. Therefore, it may be difficult to correlate soil populations of either of these organisms with disease (Bloomberg 1965), although predictions of losses based on soil assays are sometimes made (Hildebrand and Dinkel 1988). Further, most soil assays do not include levels of potential antagonists (such as bacteria, Actinomycetes, *Trichoderma, etc*.). Levels of these competitors may be more important in predicting disease than the levels of Fusarium and Pythium in the soil (Marshall 1983; Papavizas 1985).

Effects on Fusarium

Fusarium spp. are widely diverse fungi which are very important plant pathogens (Nelson and others 1983). Although important pathogens of agricultural crops and forest seedlings in nurseries (Sutherland and others), Fusarium spp. are not often pathogens in natural forest stands. In soil, most pathogenic species of Fusarium produce dormant structures called chlamydospores. However, species which do not normally produce chlamydospores produce other resistant-type structures in the soil (Hargreaves and Fox 1977). Most chlamydospores, other types of spores (macroconidia) and other resistant structures are fairly long-lived in the soil; their longevity depends on level of microbial antagonism and presence of suitable host substrates. Chlamydospores germinate when stimulated by host root exudates (Rovira 1970). Rapid colonization of host material after spore germination is important for this group of fungi.

Fusarium spp. are generally reduced by soil fumigation (Gillman 1977; Roberts and others 1988). However, they are less sensitive to some fumigants, such as methyl bromide, as some other fungi (Ebben and others 1983; McCarter and others 1978; Munnecke and others 1978; Norris 1986). Dry macrocondia (Weststeijn 1973) and mycelium (Munnecke and others 1978) may be quite resistant to fumigants. Metam-sodium may (Corden and Young 1965) or may not (Ben-Yephet and Frank 1985; Campbell and Kelpsas 1988) be as effective as MBC in reducing Fusarium levels. Dazomet reduces Fusarium levels in soil, but usually not as effectively as MBC (Campbell and Kelpsas 1988). Surviving propagules may quickly reinvade dazomet treated soil (Hoffman and Williams 1988). Vorlex® (methyl isothiocyanate and chlorinated hydrocarbons) did not significantly reduce numbers of Fusarium propagules in two studies (Manning and Vardaro 1977; Sinclair and others 1975). However, in another study (Marois and others 1983), this fumigant eliminated Fusarium from the top 20 cm of soil, although the pathogen quickly reinvaded fumigated soil from below.

Except in dazomet treated soil, Fusarium spp. may be rather slow recolonizers of fumigated soil (Danielson and Davey 1969; Johnson and Zak 1977). However, if introduced on seed or from adjacent non-fumigated fields, these fungi may increase to levels higher than those found before fumigation (Young 1940).

Effects on Pythium and *Phytophthora*

Pythium and *Phytophthora are* two very important plant pathogens. These 'water molds' produce motile zoospores which move through soil in water and seek out host roots to infect. As such, they are usually more damaging in poorly drained soils. They can inhabit water supplies and be introduced through irrigation water. Many agricultural crops and forest seedlings in nurseries are attacked by these fungi (Sutherland and others 1989).

Dormant Structures of Pythium and *Phytophthora are* either asexual (sporangia and chlamydospores) or sexual (oospores). These thick-walled spores can remain viable in soil for extended periods of time and withstand periods of desiccation. When soil moisture is adequate, sporangia will germinate to produce zoospores capable of attacking plant roots. Oospores and chlamydospores germinate to produce a mycelium that may grow toward host roots in response to root exudates (Rovira 1970).

Pythium and *Phytophthora are* more sensitive to most fumigants than several other plant pathogenic fungi (Gillman 1977; Munnecke and others 1978; Norris 1986). Their mycelium is readily killed by fumigants, even at relatively low concentrations (Roberts and others 1988; Smith and Bega 1966). Oospores and chlamydospores are probably the most resistant to fumigant action, but at concentrations usually employed at forest nurseries, they are readily killed as well (Munnecke and others 1978). One problem in controlling these fungi with fumigation is the rapidity with which they reinvade treated soil (Campbell and Kelpsas 1988; Johnson and Zak 1977; Tkacz 1983; Vaartaja 1967). Reinvasion may occur from large populations existing

below the zone of fumigation and/or through irrigation water. Experience indicates that they are usually detected in higher numbers than Fusarium in recently fumigated soil (Tkacz 1983). Dazomet is more effective in reducing populations of Pythium than Fusarium (Tanaka and others 1986).

Effects on *Rhizoctonia*

Rhizoctonia solani is an important pathogen of many agricultural crops and causes damping-off of conifer seedlings in nurseries (Sutherland and others 1989). This organism is well adapted to the soil environment. For example, it rapidily colonizes organic material introduced into soil before many other organisms, and it is rather resistant to microbial competitors. *Rhizoctonia is* also capable of producing sexual spores (basidiospores) which may be disseminated long distances in air or water.

Rhizoctonia is usually more sensitive to fumigants than Fusarium, but less sensitive than either Pythium or *Phytophthora* (Munnecke and others 1978), although responses of *Rhizoctonia* spp. are not often assayed in nursery soils (McCarter and others 1978; Smith and Bega 1966).

Effects on *Macrophomina*

Macrophomina phaseolina causes charcoal root disease of several conifer species in forest seedling nurseries (Smith 1975). The fungus produces abundant sclerotia which may remain viable in the soil for long time periods. The only effective way of reducing these soil propagules is by fumigation, particularly with MBC (Cordell 1982; Rowan 1981; Smith and Bega 1966).

Effects on *Phoma*

Phoma comprises a diverse group of soil-borne fungi that attack a wide range of host plants. Most species are considered relatively weak pathogens, but some Phoma spp. can cause serious root and stem rots and tip dieback of bareroot seedlings (James and Hamm 1985). Most species produce several spore stages; dormant structures in soil are either chlamydospores or dictyochlamydospores. On suitable substrates under moist conditions, Phoma spp. produce sporophores called pycnidia which ooze spores capable of moving in the soil.

Assays for Phoma in fumigated soil are not usually conducted. However, experience with styroblock containers indicates that these fungi may be difficult to kill with standard sterilants (James and Woollen 1989). It is likely that most fumigants, especially MBC at dosages normally employed, effectively kill most propagules of Phoma in nursery soil.

Effects on Beneficial Microorganisms

Bacteria

Many diverse groups of bacteria commonly inhabit nursery soil. Several species are antagonistic toward common soil-borne pathogens (Cornwall 1985). Some soil bacteria form dormant spores relatively resistant to environmental degredation. Some species, such as Bacillus, may produce spores resistant to fumigants, at least at low chemical concentrations (Altman 1970). Bacteria are also very rapid recolonizers of fumigated soil (Ingestad and Nilsson 1964; Martin 1963; Wensley 1953).

Actinomycetes

This group of primitive fungi are common soil inhabitants and many species are antagonistic toward other soil fungi (Cornwall 1985). Members of this group may remain dormant in the soil for long periods of time; however, most members are readily killed by commonly used fumigants. They will reinvade fumigated soil, but slower than some other types of fungi (Cornwall 1985).

Trichoderma

Trichoderma spp. are common soil-borne fungi that reside in many soil types, including those from forest nurseries (Papavizas 1985). They exist saprophytically on a wide variety of organic substrates, readily competing with or being antagonistic toward many plant pathogenic fungi, including Fusarium, Pythium, Phytophthora, and Rhizoctonia. Some species of *Trichoderma produce* powerful chemicals toxic to other fungi; other species are parasitic on certain groups of soil fungi (Papavizas 1985). *Trichoderma s*pp. are usually less sensitive to common soil fumigants than many soil-borne pathogens (Gandy and Chanter 1976). These fungi are often the first to be detected at high levels after soil fumigation (Danielson and Davey 1969; Ingestad and Nilsson 1964; Vaartaja 1967; Wensley 1953), often reaching higher population levels than in nonfumigated soil (Marshall 1986; Martin and Pratt 1958; Sinha and others 1979; Vaartaja 1967). *Trichoderma often* is the dominant microorganism in fumigated soil (Bollen 1961; Martin and others 1957; Warcup 1957).

Endomycorrhizal Symbionts

Endomycorrhizal fungi are important in production of many hardwood tree seedlings. They are not disseminated readily because their spores are soilborne. Most endomycorrhizal symbionts are quite sensitive to fumigants and are readily killed at concentrations normally used. These fungi are more sensitive to low doses of methyl bromide than many soil-borne pathogens (Menge 1982), although not all propagules are usually killed, especially those below the zone of effective fumigation (McGraw and Hendrix 1984). Endomycorrhizal fungi are usually very slow to infest fumigated soil because of their subterranean sporulation (Menge 1982). Growth depression of crop plants following fumigation has been at least partially due to reduction of endomycorrhizal inoculum in the soil (Munnecke and others 1978; Wilhelm and others 1974). In cases where endomycorrhizal fungi are necessary for satisfactory production of seedling stock, they must be reintroduced manually following fumigation.

Ectomycorrhizal Symbionts

Ectomycorrhizal fungi, common inhabitants of conifer seedling nurseries, are usually reduced the first year following fumigation, but often return to pre-fumigation levels the second year (Johnson and Zak 1977; Peterson 1970). Although most ectomycorrhizal fungi are susceptible to most fumigants, Rowan (1981) reported that *Thelephora terrestris* is somewhat resistant to MBC because fumigation did not affect soil-borne inoculum of this species. Ectomycorrhial fungi were not significantly reduced in Vorlex® treated soil (Sinclair and others 1975). Although retardation of mycorrhizal formation may occur in fumigated soil, seedling response may still be greater than in non-fumigated soil because of reduced pathogen levels (Hacskaylo and Palmer 1957; Laiho and Mikola 1964). Ectomycorrhizal fungi are readily disseminated by air-borne spores and usually reinfest fumigated soil if there are large conifer trees near nurseries or if adjacent fields harbor inoculum (Cordell 1982).

In cases where inoculum is not readily available, these fungi may have to be reintroduced manually following soil fumigation.

CONCLUSIONS

Communities of soil microorganisms tend to stabilize quantitatively and qualitatively in the absence of biocides that may preferentially inhibit certain species. When susceptible hosts are introduced into soil, certain pathogens may proliferate unless restricted by the action of competitors or antagonists. As indicated earlier, spores of pathogens are stimulated into activity by the presence of host roots. However, ff resident populations of antagonists are sufficient, pathogens may not be able to cause disease. Over time, pathogens and competitors/ antagonists tend to come into 'balance' and will remain so until that balance is upset. Several factors can upset this balance, including introduction of extensive amounts of susceptible host material, introducing pathogen populations (such as on seed), and treatment of soil with biocides.

Because most fumigants are non-selective in their action, their use results in a soil habitat colonized most by the organisms first reintroduced following treatment. If these initial colonizers are 'good' fungi, i.e., those competitive with or antagonistic toward pathogens, any pathogenic fungi inadvertently introduced will not proliferate and little disease will likely result. Conversely, rf pathogens are the first to be reintroduced into fumigated soil, such as on seed, they will proliferate and reach higher levels than before fumigation and disease losses could be extensive. One problem with soil fumigation is that once this practice is implemented, it usually has to be repeated before each successive crop because the biological balance of microorganisms in the soil has been disrupted.

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