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EFFECTS OF INCORPORATING CORN GREEN MANURE CROPS ON SOIL POPULATIONS OF *FUSARIUM*, *TRICHODERMA*, AND *PYTHIUM* - USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

An evaluation of the effects of incorporating a corn cover/green manure crop on soil populations of potentially-pathogenic *Fusarium* and *Pythium* spp. and potentially-antagonistic *Trichoderma* spp. was conducted at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. Populations of *Fusarium* and *Pythium* greatly increased as a result of incorporating the corn crop. *Trichoderma* spp. did not similarly respond; the ratio of *Trichoderma* to *Fusarium* greatly decreased as a result of addition of corn residues to soil. Populations of *Fusarium* and *Pythium* remained high for an entire year following incorporation until dazomet fumigation, which greatly reduced levels of both potential pathogens and potential antagonists. Pathogenic isolates of *F. oxysporum* comprised about 65% of the population of this important disease-causing species in corn-incorporated soil. This was a higher proportion than was found in other parts of the nursery where corn had not been grown. If a corn crop is used operationally to provide improved soil tilth and increased organic matter, fumigation will be required to reduce soil pathogen populations prior to growing conifer seedlings. Eliminating soil fumigation at the nursery is possible. However, fields will have to be fallowed prior to sowing a seedling crop and green manure crops, especially corn, cannot be used prior to sowing conifer crops if high quality seedlings are to be produced.

INTRODUCTION

Many forest nurseries that produce bareroot seedlings utilize green manure crops to maintain or increase soil organic matter and improve soil tilth (McGuire and Hannaway 1984). Several different types of green manure crops have been grown at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. In addition to supple-

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menting organic matter in soil, these crops help to reduce soil erosion and maintain proper tilth during periods when conifer seedling crops are not grown. Recently, growers at the nursery have been interested in growing corn (*Zea mays* L.: field and sweet cultivars) as a way of improving soil tilth and structure to seedling production fields. These corn crops provide deep rooting which helps make soil water permeable. Incorporating plant residues after maturity also helps maintain organic matter that may be depleted following production of conifer seedlings.

One of the main concerns with any green manure crop is increased populations of potential pathogenic fungi which utilizes incorporated plant material as a food source (Sequeira 1962; Wall 1984; Wycoff 1952). This is particularly true for species of fungi in the genera *Fusarium* and *Pythium* (Redfern 1970; Sequeira 1962), both of which are important pathogens at the nursery. For example, in recent evaluations of *Brassica* spp. as a green manure crop, levels of both *Fusarium* and *Pythium* greatly increased after crop incorporation into soil (Hamm and Hansen 1990; Hansen et al. 1990; James et al. 1996). This occurred despite the fact that certain *Brassica* spp. produce antifungal toxins (methyl isothiocyanates) that should help reduce soil populations of potential plant pathogens (Angus et al. 1994; James et al. 1996). Apparently, stimulatory effect of the added plant organic matter to soil populations of *Fusarium* and *Pythium* greatly outweighed any potential toxic effect of breakdown products.

Because of the proposed continued use of corn as a cover/green manure crop at the Coeur d'Alene Nursery, an evaluation was conducted to determine response of soil populations of three groups of fungi (*Fusarium, Pythium* and *Trichoderma*) to incorporated corn residues. Interactions of these fungi resulting from quantitative population differences may influence level of diseases on conifer crops (James et al. 1991).

MATERIALS AND METHODS

The evaluation was conducted within the southern portion of Field 9. Both sweet and field corn were sown in portions of Field 9 in the spring of 1992. Just prior to sowing the corn, ten systematic soil samples were collected, representing the area to be occupied by corn. Samples were collected with standard soil probes to a depth of 6 inches (15 cm). Within each sample location, five individual cores were collated.

Soil was analyzed for populations of *Fusarium, Trichoderma*, and *Pythium* using standard soil dilution techniques (James and Gilligan 1985, 1986, 1990). Soil was initally sieved (2 mm sieve) to remove rocks, pieces of undecomposed organic matter, and soil aggregations. From each sample, a 5 g subsample was dried at about 100°C for at least 24 h or until sample weight had stabilized (all excess moisture removed). Oven-dry weight was then calculated to provide a standard for comparison. For assay of *Fusarium* and *Trichoderma* populations, 0.5 g of field-moist soil was combined with 100 ml of 0.3% water agar and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated seven days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium and populations determined. Ratios of *Trichoderma* to *Fusarium* populations were calculated for a rough estimate of potential soil suppressiveness to pathogens (James et al. 1996). Selected *Fusarium* isolates were transferred to potato dextrose (PDA) and carnation leaf agar (Fisher et al. 1982) for identification using the taxonomic criteria of Nelson et al. (1983). For assay of *Pythium* populations, 5.0 g of soil was combined with 100 ml of 0.3% water agar. One ml of solution was placed on each of three plates of another selective medium

consisting of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene (James et al. 1990). Plates were incubated three days in the dark at about 24°C. *Pythium* colonies were identified based on their diameter after 3 days (15-20 mm), their feathery margin, and whether they grew within rather than superficially on the agar surface (James and Gilligan 1985, 1986, 1990; James et al. 1990, 1996). All fungal populations were expressed as colony-forming units per g of dry soil.

The corn crop was grown until mid September. After harvest, stalks were chopped and plowed into the soil. The field was disked periodically to help break up large pieces of plant material and enhance decomposition. Subsequent soil samples were collected at 1.5 and 6 months after incorporation. Samples were collected at the approximate locations of the first sample and analyzed as described above.

In early September of 1993 (about 1 year following incorporation of corn), the field was prepared and fumigated with dazomet using standard nursery practice; granular dazomat was applied topically at about 350 lbs./A, disked into soil, activated and sealed with irrigation). A final soil sample was collected about 1.5 months following fumigation and processed the same as other samples. For each sample period, data from the ten samples were averaged; standard deviations and total variances were calculated.

Twenty isolates of *F. oxysporum* Schlecht., randomly selected from soil samples collected after incorporation of corn residues were analyzed for potential virulence using a standard laboratory pathogenicity test (James 1996). *Fusarium oxysporum* is the most important disease-causing *Fusarium* species of bareroot conifer seedlings (Bloomberg 1971; James et al. 1991) and comprises most of the soil *Fusarium* population at the Coeur d'Alene Nursery (James 1998: James and Gilligan 1985, 1986, 1990). The basic approach of this procedure was to expose young Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) germinants to tested fungal inoculum and record production of disease symptoms. Inoculum was prepared using the techniques of Miles and Wilcoxin (1984). Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in soilless potting mixtures, was the matrix for fungal growth. 150 g of yellow cornmeal were moistened with 300 ml warm 1% PDA to which 75 g of perlite was added. The mixture was placed into glass vials (23 ml capacity) to about 2/3 capacity which were then autoclaved for 60 m at 121°C. After cooling, vials were inoculated with 10 ml spore suspension of the test *F. oxysporum* isolate (produced by adding sterile, distilled water to 14 day-old cultures grown on PDA). Vial caps were left loose to allow aeration. Vials were incubated in the dark for at least 21 days, after which test fungi had thoroughly colonized the perlite/cornmeal mixture. After incubation, inoculum was removed from vials and dried in open petri plates within a cabinet. Inoculum was dry after 5-7 days and did not become contaminated with other organisms because the food base was completely colonized by the test fungus. The medium used for pathogenicity tests was a 50:50 mixture of ground coconut husks and vermiculite. Twenty-four vials (23 ml capacity each) were used to test each fungal isolate. Each vial was filled to 2/3 capacity with dried media and autoclaved at 121°C for 60 m. The same Douglas-fir seedlot was used for all inoculations. Seeds were soaked in a 2-part bleach and 3-part water solution for 10 m (Wenny and Dumroese 1987), rinsed 48 h in running tap water and stratified for 21 days at 2-3°C. After stratification, seed was placed on filter paper moistened with sterile water. Seed was incubated under 12-h diurnal fluorescent light cycles at about 24°C and monitored daily for germination. A seed was considered germinated when its primary root was at least 3 mm long. The perlite/cornmeal inoculum for each isolate was ground to a fine powder with mortar and pestle and 0.05 g of the powder was added to each vial containing dried, autoclaved media. Inoculum was distributed throughout the media by shaking. One recently germinated seed

(germinant) was placed in each vial with its radicle placed downward into the media. Four ml sterile water was added to each vial; adding water activates inoculum (Miles and Wilcoxin 1984). For each isolate 24 vials with germinants were evaluated. Controls consisted of a set of 24 vials inoculated with sterile perlite instead of fungal inoculum.

Vials containing germinants were incubated at about 24°C on a lab bench, providing 8-12 h fluorescent light daily. Tests ran for a maximum of 14 days. After 3 days, germinants were first checked for disease; surviving germinants were checked daily after this. Initial disease was usually manifested as standard post-emergence damping-off with the germinant being attacked by the test fungus at or below the groundline (James 1996). Diseased seedlings and those surviving for 14 days were removed and placed on Komada's medium for reisolation of the inoculated isolate. A numerical rating system based on duration of germinant survival, occurrence and type of disease, reisolation of inoculated fungal isolate, and primary root growth within the vial was used for isolate comparisons (James 1996). Ratings were converted to a "virulence score" (James 1996) and assigned a category (nonvirulent, low, moderate, and high). Averages, standard deviations and variances were calculated for percent diseased germinants, average number of days survival, and average virulence score for all *F. oxysporum* isolates.

RESULTS AND DISCUSSION

Populations of *Fusarium* averaged 156 cfu/g (range = 0-468) within Field 9 prior to growing and incorporating the corn green manure crop (table 1). Levels of these potentially-pathogenic fungi increased to nearly 1700 cfu/g (range = 534-3937) by 1.5 months after incorporation of the crop. Six months after the corn had been incorporated, *Fusarium* levels were still high (average = 1350 cfu/g; range = 269-3426)(table 1). However, after the field was fumigated with standard rates of dazomat, *Fusarium* levels were lower than before the corn crop had been grown (average = 53 cfu/g; range = 0-267). The major species of *Fusarium* isolated from soil was *F. oxysporum*, which made up about 85% of all *Fusarium* isolates. Other species isolated from soil included *F. acuminatum* El & Ev., *F. equiseti* (Corda) Sacc., *F. solani* (Mart.) Appel & Wollenw., *F. sambucinum* Fuckel, *F. sporotrichioides* Sherb., and *F. avenaceum* (Fr.) Sacc.

Levels of soil *Trichoderma* did not increase in response to incorporation of the corn green manure crop as *Fusarium* did (table 1). Pre-corn levels averaged 1068 cfu/g (range = 267-1937); levels 1.5 months after crop incorporation were slightly higher at 1221 cfu/g (range = 534-2069). Six months after the crop was incorporated, *Trichoderma* levels had reduced to an average of 826 cfu/g (range = 134-1746). Dazomet also greatly reduced soil *Trichoderma;* levels were much less following fumigation than prior to growing the corn crop (average = 67 cfu/g; range = 0-334).

The ratio of *Trichoderma* to *Fusarium* in the soil (T/F ratio) gives a rough estimate of the level of potential suppressiveness of antagonistic species of *Trichoderma* toward potentially-pathogenic *Fusarium* (James et al. 1996). The higher the ratio the greater the potential suppressiveness of soil. T/F ratios started at 6.84 in soil before the corn crop was grown (table 1). After incorporation of the crop, ratios decreased significantly to 0.73 after 1.5 months and 0.61 after 6 months. The ratio increased somewhat to 1.25 after fumigation with dazomet, but did not reach pre- corn crop levels.

Pythium soil populations also greatly increased following incorporation of the corn crop (table 1). Levels started at about 31 cfu/g (range = 0-67), which is near the background level found at the nursery in untreated soil (Stone et al. 1997). Levels exploded to 455 (range = 313-567) and 585 (range = 497-605) cfu/g after 1.5 and 6

Table 1. Effects of incorporation of a corn green manure crop on soil populations of *Fusarium, Trichoderma,* and *Pythium* - USDA Forest Service Nursery, Coeur d' Alene, Idaho.[1]

Fungus	Sample 1	Sample 2	Sample 3	Sample 4
Fusarium				
Average	156	1682	1350	53
Std. Dev.	171.7	1029.4	977.3	93.4
Total Var.	29472	1059662	955126	8726
Trichoderma				
Average	1068	1221	826	67
Std. Dev.	481.5	461.4	469.2	107.7
Total Var.	231836	212934	220114	11591
T/R Ratio	6.84	0.73	0.61	1.25
Pythium				
Average	31	455	585	1
Std. Dev.	27.1	62.0	31.9	3.9
Total Var.	736	3848	1016	15

[1] Sample 1 collected prior to sowing corn crop; sample 2 taken 1.5 months after incorporation of corn crop; sample 3 taken 6 months after incorporation of corn crop; sample 4 collected following dazomet fumigation. Averages, standard deviation and total variance expressed as colony-forming units/g of dry soil.

months following crop incorporation, respectively. Dazomet fumigation eliminated most *Pythium* from the soil.

Of the 20 *F. oxysporum* isolates obtained from Field 9 after incorporating the corn green manure crop, 3 (15%) were rated as highly virulent and 10 (50%) were moderately virulent (table 2). The virulence score for all tested isolates averaged 66.2, indicating moderate virulence. Other tests evaluating virulence of *F. oxysporum* from the nursery have indicated that about 45% of the tested isolates are either highly or moderately virulent (James et al. 1998). Therefore, it appears that a larger percentage of isolates obtained from Field 9 were either highly or moderately virulent than in other parts of the nursery.

This evaluation showed that soil populations of potentially-pathogenic species of *Fusarium* and *Pythium* greatly increase in response to incorporating a corn green manure crop into soil. *Trichoderma* spp., which may potentially be antagonistic toward these pathogenic fungi (Papavizas 1985), did not respond similarly. Therefore, incorporating a corn crop may be detrimental from the standpoint of potential disease if the soil remains untreated prior to sowing the next conifer seedling crop. Soil fumigation with dazomet reduced pathogens back to low levels, allowing successful production of a conifer seedling crop.

If a corn or similar green manure crop is to be used operationally at the nursery, soil fumigation will be required prior to sowing a conifer seedling crop in affected fields. If growers want to implement a program of non-fumigation at the nursery, the use of corn as a green manure crop and to provide improved soil tilth may have to be discontinued because of increased

Table 2. Virulence of *Fusarium oxysporum* isolates from Field 9 of the USDA Forest Service Nursery, Coeur d'Alene, Idaho on Douglas-fir germinants. [1]

Isolate	Percent Dis.	Ave. Survival	Ave. Score	Rating
9245A	80.9	7.6	70.7	MODERATE
9245B	76.2	8.7	63.3	MODERATE
9245F	69.6	9.3	58.3	LOW
9245G	75	9.2	58.5	LOW
9245H	100	5.8	83.9	HIGH
9245J	66.7	10	54.4	LOW
9245K	70	8.7	65	MODERATE
9302A	100	6.3	80	HIGH
9302F	60.9	9.9	42.8	LOW
9302G	100	6.1	81.3	HIGH
9302I	75	7.1	7.08	MODERATE
9302R	62.5	11	44.8	LOW
9315C	90.9	6.9	75.7	MODERATE
9315F	85	6.6	75.5	MODERATE
9315G	90	6.6	76	MODERATE
9316F	90.5	8.4	66.2	MODERATE
9316G	85	6	74	MODERATE
9316H	91.7	9	66.2	MODERATE
9316M	76.2	9.1	59.5	LOW
9316P	80	9.5	58.5	LOW
Average	81.3	8.1	66.2	-
Std. Dev.	11.9	1.5	11.2	-
Total Var.	140.9	2.3	125.2	-
Control	20.8	14	10.4	NONVIR

[1] Based on exposing 24 germinants to test isolates for a maximum of 14 days. Percent Dis. = percent of germinants that developed disease symptoms; Ave. Survival = average number of days germinants survived before dying (maximum = 14); Ave. Score = based on disease, survival, resiolation of inoculated isolates and growth of radicle (scores range from 0 - 100).

disease pressure exerted by higher populations of *Fusarium* and *Pythium* spp. Previous work (Stone et al. 1996; Sutherland 1984) has shown that bare fallowing fields for at least 1 year maintains populations of these potentially-pathogenic fungi at relatively low levels and that high quality seedling crops can usually be produced without soil fumigation. However, adding large amounts of plant material usually causes significant increases of potential pathogens and

often results in decreased seedling crop quantity and quality (Hamm and Hansen 1990; Hansen et al 1990; James et al. 1996). If a non-fumigation regime is implemented, alternative sources of organic matter and soil tilth management will be required.

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