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EFFECTS OF A 2-YEAR FALLOW PERIOD ON SOIL POPULATIONS OF *FUSARIUM*, *TRICHODERMA* AND *PYTHIUM* SPECIES AFTER INCORPORATING CORN PLANT RESIDUES – USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Soil in Field 2 at the USDA Forest Service Nursery, Coeur d'Alene, Idaho was assayed six times over a 2-year fallow period for potentially pathogenic *Fusarium* and *Pythium* spp. following incorporation of a corn cover/green manure crop. Populations of potentially disease-antagonistic *Trichoderma* spp. were also assayed. Soil populations of *Fusarium* did not decrease over the fallow period and were at sufficient levels after two years to be of concern from a disease potential standpoint. Populations of *Pythium* were fairly low throughout the monitoring period. *Trichoderma* populations remained high, but were probably not sufficient to ameliorate *Fusarium* disease potential. *Fusarium* spp. readily colonized soil organic matter particularly roots of the previous conifer seedling crop; these fungi also frequently colonized corn organic debris. *Fusarium oxysporum* was the major potential pathogen colonizing soil and organic matter. When a corn cover/green manure crop is produced within fields destined for conifer seedling production, these fields will require soil fumigation prior to sowing seedling crops to reduce risk of *Fusarium*-associated diseases.

INTRODUCTION

For production of bareroot seedling stock at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, growers traditionally use pre-plant soil fumigation to control soil-borne pathogens and weeds (Boyd 1971; James et al. 1990). Soil fumigants are chemical biocides that non-selectively kill soil microorganisms (Boone 1988). In the past, the soil fumigant of choice was methyl bromide/chloropicrin (MBC – formulated as 66 percent methyl bromide and 33 percent chloropicrin). However, at the Coeur d'Alene Nursery, this fumigant has been replaced in recent years with dazomet (Basamid®), a powdery material applied topically, cultivated into soil, activated and sealed by overhead irrigation (Chapman 1992; James and Beall 1999). Dazomet has effectively controlled soilborne pathogens at the nursery (James et al. 1990, 1996). However, because of increasing fumigant costs and potential environmental concerns, nursery growers have a goal to produce high-quality forest tree seedlings without pre-plant soil fumigation. To this end, several field studies have been conducted at the nursery to develop alternatives to soil fumigation. One of the most promising alternatives evaluated so far is fallowing fields for at least 1 year prior to sowing a seedling crop (Stone et al. 1997). Fallowed fields are usually periodically cultivated to keep weed populations low as well as mix soil layers to facilitate death of propagules of potentially pathogenic fungi.

A major concern with prolonged fallowing is the potential for reduced soil organic matter, which directly affects soil tilth, and wind and water erosion problems. Growers have traditionally controlled erosion and maintained soil organic matter by growing a cover/green manure crop between seedling crops. Cover crops are usually incorporated into the soil a few months prior to sowing. Although different types of cover crops have been used at the Coeur d'Alene Nursery, growers have recently selected field corn because it produces relatively large amounts of organic matter and its root system helps break up water-impermeable hard pans. Normally, the corn crop is grown in the summer, chopped and incorporated in the

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fall. Fields are then fallowed for at least 1 year and fumigated with dazomet prior to sowing a seedling crop. Fumigation usually successfully reduces populations of soil fungi that normally build up on incorporated organic matter (James et al. 1990, 1996). However, without soil fumigation, high populations of potentially pathogenic fungi may persist and present a threat to the future seedling crop (James et al. 1996).

Since an important goal at the Coeur d'Alene Nursery is to eliminate pre-plant soil fumigation, a test was conducted to evaluate extended fallowing after incorporating a corn cover crop on soil populations of important disease-causing fungi. The major goal of the test was to determine how soil fungal populations responded to increased organic matter from the cover crop and how these populations changed over time in a fallowed field.

MATERIAL AND METHODS

Field corn (*Zea mays* L.) was grown in Field 2 during the summer of 1997. In September, the crop was chopped and incorporated into the soil. The goal was to mix as much of the organic material with the soil as possible. After incorporation, the field was left fallow and periodically cultivated during the next 2 years to control weed populations. The field was sampled six times over the 2-year fallow period. Sampling occurred in May '98, July '98, Sept. '98, Nov. '98, April '99, and August '99 (8, 10, 12, 14, 19, and 23 months after cover crop incorporation, respectively).

Throughout Field 2, five east-west transects were located at irrigation risers at specific intervals (each transect was four irrigation risers apart). Five approximately equidistant sample points were located along each transect (25 total sample points). Sample points were fixed by pacing along each transect. At each sample point a soil core was taken to a depth of about 8 inches (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (James 1998; James and Beall 1999; James et al. 1990,1996) were conducted to estimate populations of *Fusarium*, *Trichoderma* and *Pythium* spp. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven-dried at about 100°C for at least 24 hours until sample weight had stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3 percent 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 5-7 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; populations, expressed as number of colony-forming units (cfu) per g of oven-dried soil, were calculated. Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification using the taxonomy of Nelson and others (1983). For assay of *Pythium* populations, 0.5 g of soil was combined with 10 ml of 0.3 percent water agar. One ml of solution was placed on each of three plates containing another selective medium consisting of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene (James et al. 1990, 1996). Plates were incubated in the dark at about 24°C for 3 days. *Pythium* colonies were identified on the basis of their diameter after 3 days (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. Selected isolates were transferred to PDA for identification using the taxonomy of Waterhouse (1968). It was assumed that each fungal colony originated from one propagule.

Fusarium, Trichoderma, and *Pythium* populations were determined for each sample point and time. Averages and standard deviations were also calculated. In addition, the ratio of *Trichoderma* to *Fusarium* populations (T/F ratio) was calculated for each sample. This ratio has been useful as an approximation of potential disease suppressiveness in nursery soils (James 1998; James et al. 1996). Generally, the higher the ratio, the less potential for *Fusarium*-caused disease due to expected antagonism by *Trichoderma* spp.

During the September 1998 sample (12 months after cover crop incorporation), pieces of cornstalk debris within the soil were randomly collected and dissected into pieces approximately 5 mm x 2-3 mm in size. These pieces were washed thoroughly in running tap water for several minutes to remove adhering soil particles. They were then surface sterilized in a 10 percent bleach (0.525 percent aqueous sodium hypochlorite) solution for 10 seconds, rinsed in sterile water, blotted dry, and placed on a selective agar medium (Komada 1975) and incubated as described above. Ten debris pieces were incubated in each petri dish. Percentage colonization by *Fusarium* and *Trichoderma* spp. was calculated; selected *Fusarium* isolates were identified as described above.

During the April 1999 sample (19 months after cover crop incorporation), organic matter pieces within the soil, recovered during soil sieving, were sampled for colonization by *Fusarium* and *Trichoderma* spp. Sampled organic matter was comprised mostly of residual roots from previous conifer seedling crops; roots were randomly selected, dissected into pieces 3-5 mm in length, surface sterilized, and incubated on the selective medium (Komada 1975) as described above. Percentage colonization by *Fusarium* and *Trichoderma* spp. was calculated; selected *Fusarium* isolates were again identified as described above.

Soil populations of *Fusarium*, *Trichoderma* and *Pythium* usually vary widely within forest nursery soil (James and Beall 1999; James et al. 1990, 1996; Stone et al. 1997). Fungal propagules are often aggregated so that assays tend to give large spacial variations in population counts. Because of extreme variability, statistical analyses do not usually result in significant population differences (James and Beall 1999; Stone et al. 1997). Therefore, soil population data were not statistically analyzed. Rather, average population changes were described as trends.

RESULTS

Fusarium soil populations generally got larger with increasing time following incorporation of the corn cover crop (table 1). After the end of the first year, populations were quite high and exceeded the "disease threshold" of 1000 cfu/g (Hildebrand and Dinkel 1988; James and Beall 1999) at 20 of 25 sample points. Populations continued at high levels and remained fairly stable throughout the second fallow year. Although number of samples was limited, spring *Fusarium* populations were usually lower than fall populations. As expected, *Fusarium* populations varied greatly throughout Field 2; this variability was indicated by relatively large standard deviations (table 1).

Trichoderma populations increased faster than *Fusarium* levels after cover crop incorporation and averaged higher than *Fusarium* throughout most of the 2-year sampling period (table 2). *Trichoderma* populations varied even more than *Fusarium* populations. In some cases, e.g., during the 19-month sample, assay plates were completely covered with *Trichoderma* spp., indicating population levels beyond the detection capability of the dilution series used.

Ratios of *Trichoderma* to *Fusarium* populations throughout the 2-year sampling period are summarized in table 3. These ratios also displayed wide ranges of variability; standard deviations often exceed averages. Some ratios were at levels (i.e., below 1.00) where disease would be expected.

Pythium populations in Field 2 were usually lower than "disease threshold" levels of 100 cfu/g (Hildebrand and Dinkel 1988; James and Beall 1999)(table 4). However, during the second year, several samples had populations exceeding this threshold. As with the other assayed fungi, high population variability was found. The most commonly isolated *Pythium* species was *P. irregulare* Buisman. *Pythium ultimum* Trow. and *P. aphanidermatum* (Edson) Fitzp. were also isolated, but much less frequently.

Cornstalk debris was common within Field 2 a year following cover crop incorporation; this debris was colonized by several *Fusarium* spp., but at much lower levels than *Trichoderma* spp. (table 5). However, *Fusarium* spp. were much more commonly isolated from other soil organic matter, comprised mostly of roots of previous conifer seedling crops (table 6).

The soil *Fusarium* population isolated from Field 2 was quite diverse (table 7). By far the most common species within soil (table 7) and colonizing organic matter (tables 5 and 6) was *F. oxysporum* Schlecht. The second most common species in soil was *F. equiseti* (Corda) Sacc. All other *Fusarium* spp. were usually isolated at very low levels. Exceptions were *F. solani* (Mart.) Appel & Wollenw., isolated during the second sampling period (10 months after cover crop incorporation), and *F. avenaceum* (Fr.) Sacc., isolated during the first sampling period (8 months after incorporation).

Table 1. Changes in soil *Fusarium* populations following incorporation of the corn cover crop in Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample	Months After Incorporation[3]							
Location[2]	8	10	12	14	19	23	Average	STDDEV[4]
1	1219	336	1080	736	267	604	707.0	385.7
2	68	880	747	1138	334	2483	941.7	847.2
3	68	67	1150	471	869	1946	761.8	722.9
4	549	67	6059	469	602	1412	1526.3	2263.4
5	204	136	1487	536	67	604	505.7	528.0
6	406	203	1759	2011	1003	3226	1434.7	1132.0
7	270	135	674	4159	1273	1480	1331.8	1485.0
8	340	134	1214	2286	535	2686	1199.2	1068.3
9	407	135	2708	2084	1003	1273	1268.3	983.3
10	202	337	475	1612	334	1473	738.8	630.0
11	686	338	1750	2015	1804	1814	1401.2	703.3
12	880	405	2181	4923	1471	2419	2046.5	1601.1
13	480	677	2304	3619	1004	3225	1884.8	1355.5
14	480	472	2563	1274	1604	739	1188.7	810.7
15	341	67	2284	1543	2209	336	1130.0	1004.9
16	553	137	1221	404	534	604	575.5	358.2
17	614	1083	2366	1475	1607	269	1235.7	750.6
18	1658	1222	1012	1954	606	871	1220.5	504.5
19	206	272	1341	404	2149	738	851.7	760.1
20	684	204	1421	2229	1474	1609	1270.2	718.2
21	1243	405	2374	8680	1675	1809	2697.7	3003.4
22	1232	136	1289	604	1205	536	833.7	475.8
23	343	0	810	1947	535	536	695.2	669.1
24	343	337	1348	674	2485	202	898.2	880.7
25	137	203	403	604	1609	335	548.5	544.6
Average	544.5	335.5	1680.8	1914.0	1130.3	1329.2	1155.7	967.5
STDDEV[4]	403.3	310.6	1108.1	1805.4	664.8	927.4	870.0	918.7

[1] Numbers in table are colony-forming units per g oven-dried soil (cfu/g).
[2] Samples collected in approximately the same location along 5 transects (5 plots per transect) throughout Field 2.
[3] Months after incorporating the corn cover crop into soil.
[4] Standard deviation.

Table 2. Changes in soil *Trichoderma* populations following incorporation of the corn cover crop in Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample	Months After Incorporation[3]							
Location[2]	8	10	12	14	19	23	Average	STDDEV[4]
1	0	336	135	1071	67	67	279.3	404.7
2	274	68	204	402	468	201	269.5	146.0
3	274	202	271	605	1404	604	560.0	449.4
4	69	0	0	2146	1404	874	748.8	892.3
5	68	341	68	872	469	134	325.3	312.7
6	203	1622	203	8046	8023	4638	3789.2	3665.6
7	270	6414	809	4298	8040	2354	3696.7	3114.1
8	204	7325	2697	4236	8025	3828	4385.8	2917.4
9	1085	67	203	2286	8026	737	2067.3	3025.4
10	540	269	2037	2620	3879	469	1635.7	1455.5
11	480	338	606	201	2072	1478	862.5	743.8
12	948	5880	68	742	8022	2285	2990.8	3222.8
13	411	135	135	1407	8032	3896	2336.0	3134.8
14	686	2765	540	2280	8021	5242	3255.7	2892.2
15	1024	1013	739	1275	3748	4841	2106.7	1737.9
16	207	0	1017	404	467	1140	539.2	450.3
17	205	1151	4124	4024	8037	3022	3427.2	2750.3
18	138	1018	4117	1011	8074	1005	2560.5	3029.5
19	549	408	5030	2829	4366	1408	2431.7	1966.6
20	2873	0	3585	0	670	1006	1355.7	1519.2
21	414	6813	1628	67	1273	3416	2268.5	2516.2
22	685	136	475	336	603	469	450.7	195.5
23	343	8149	1350	604	2609	1608	2443.8	2907.6
24	343	6481	1955	3910	604	2082	2562.5	2303.6
25	961	68	3896	67	603	2147	1290.3	1489.6
Average	**530.2**	**2040.0**	**1435.7**	**1829.4**	**3880.2**	**1958.0**	**1945.6**	**1743.1**
STDDEV[4]	**566.9**	**2789.2**	**1534.1**	**1866.9**	**3363.0**	**1570.1**	**1948.4**	**1845.751**

[1] Numbers in table are colony-forming units per g oven-dried soil (cfu/g).
[2] Samples collected in approximately the same location along 5 transects (5 plots per transect) throughout Field 2.
[3] Months after incorporating corn cover crop into soil.
[4] Standard deviation.

Table 3. Changes in *Trichoderma/Fusarium* soil population ratios following incorporation of the corn cover crop in Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample	Months After Incorporation[3]							
Location[2]	8	10	12	14	19	23	Average	STDDEV[4]
1	0	1.00	0.12	1.45	0.25	0.11	**0.49**	**0.59**
2	4.03	0.08	0.27	0.35	1.40	0.08	**1.04**	**1.55**
3	4.03	3.01	0.24	1.28	1.62	0.31	**1.75**	**1.51**
4	0.13	0	0	4.57	2.33	0.62	**1.28**	**1.84**
5	0.33	2.51	0.05	1.63	7.00	0.22	**1.96**	**2.65**
6	0.50	7.99	0.11	4.00	8.00	1.44	**3.67**	**3.61**
7	1.00	47.51	1.20	1.03	6.32	1.59	**9.78**	**18.60**
8	0.60	54.66	2.22	1.85	15.00	1.42	**12.63**	**21.29**
9	2.66	0.50	0.07	1.10	8.00	0.58	**2.15**	**3.00**
10	2.67	0.80	4.29	1.62	11.61	0.32	**3.55**	**4.19**
11	0.70	1.00	0.35	0.10	1.15	0.81	**0.69**	**0.40**
12	1.08	14.52	0.03	0.15	5.45	0.94	**3.70**	**5.67**
13	0.86	0.20	0.06	0.39	8.00	1.21	**1.79**	**3.07**
14	1.43	5.86	0.21	1.79	5.00	7.09	**3.56**	**2.78**
15	3.00	15.12	0.32	0.83	1.70	14.41	**5.90**	**6.93**
16	0.37	0	0.83	1.00	0.87	1.89	**0.83**	**0.64**
17	0.33	1.06	1.74	2.73	5.00	11.24	**3.68**	**4.04**
18	0.08	0.83	4.07	0.52	13.32	1.15	**3.33**	**5.09**
19	2.66	1.50	3.75	7.00	2.03	1.91	**3.14**	**2.05**
20	4.20	0	2.52	0	0.45	0.62	**1.30**	**1.70**
21	0.33	16.82	0.69	0.01	0.76	1.89	**3.42**	**6.60**
22	0.56	1.00	0.37	0.56	0.50	0.87	**0.64**	**0.24**
23	1.00	0	1.67	0.31	4.88	3.00	**1.81**	**1.85**
24	1.00	19.23	1.45	5.80	0.24	10.31	**6.34**	**7.37**
25	7.01	0.33	9.67	0.11	0.37	6.41	**3.98**	**4.21**
Average	**1.62**	**7.82**	**1.45**	**1.61**	**4.45**	**2.82**	**3.29**	**4.46**
STDDEV[4]	**1.70**	**14.04**	**2.12**	**1.82**	**4.32**	**3.90**	**4.65**	**4.55**

[1] Numbers in table are ratios of *Trichoderma* to *Fusarium* populations.
[2] Samples collected in approximately the same location along 5 transects (5 plots per transect) throughout Field 2.
[3] Months after incorporating corn cover crop into soil.
[4] Standard deviation.

DISCUSSION

In the production of bareroot seedlings in forest nurseries, diseases caused by soil-borne pathogens can quickly cause substantial damage. *Fusarium*-associated diseases are probably the major diseases of conifer seedlings at the Coeur d'Alene Nursery (James et al. 1987, 1990, 1996). In bareroot production areas, these diseases have traditionally been controlled by pre-plant soil fumigation with general biocides such as methyl bromide/chloropicrin and, more recently, dazomet (James et al. 1990, 1996). Fumigation is usually effective in greatly reducing or eliminating soil populations of potential pathogens (Boone 1988; Boyd 1971; James et al. 1990, 1996). However, such treatments are expensive and may cause environmental problems. Bare fallowing for at least 1 year prior to sowing a seedling crop is fairly effective in reducing soil pathogen populations, resulting in production of satisfactory seedling crops at the Coeur d'Alene Nursery (James et al. 1996; Stone et al. 1997).

To provide necessary soil tilth and help break up water impermeable layers, managers at the Coeur d'Alene Nursery started to grow field corn cover/green manure crops a few years ago. These crops improved soil characteristics necessary to grow high-quality forest seedlings. However, incorporating such large amounts of plant organic material into soil resulted in tremendous increases in microbial populations, including organisms capable of causing conifer seedling diseases (James 1998). Without taking steps to reduce potential pathogen populations, such as soil fumigation, subsequent seedling crops could be expected to suffer from increased diseases (James 1998; James et al. 1996).

Table 4. Changes in soil *Pythium* populations following incorporation of the corn cover crop in Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Sample	Months After Incorporation[3]							
Location[2]	8	10	12	14	19	23	Average	STDDEV[4]
1	34	0	67	60	53	60	**45.7**	**25.1**
2	27	0	54	54	13	47	**32.5**	**22.8**
3	41	0	81	34	60	67	**47.2**	**28.8**
4	55	0	108	27	20	27	**39.5**	**37.9**
5	41	7	41	33	20	67	**34.8**	**20.5**
6	0	0	0	20	13	34	**11.2**	**14.0**
7	0	0	27	20	7	13	**11.2**	**10.9**
8	7	0	13	60	27	20	**21.2**	**21.2**
9	14	7	20	60	33	13	**24.5**	**19.5**
10	7	0	14	74	47	20	**27.0**	**28.1**
11	14	0	34	40	53	7	**24.7**	**20.8**
12	14	0	68	142	40	108	**62.0**	**55.1**
13	7	13	41	87	100	7	**42.5**	**41.7**
14	0	0	54	94	47	40	**39.2**	**35.7**
15	27	0	27	121	74	47	**49.3**	**42.8**
16	0	0	54	142	67	107	**61.7**	**56.9**
17	34	0	34	87	60	33	**41.3**	**29.4**
18	76	0	20	81	27	54	**43.0**	**32.5**
19	7	7	33	81	74	54	**42.7**	**32.3**
20	7	14	81	209	107	54	**78.7**	**74.5**
21	41	88	95	87	188	47	**91.0**	**52.7**
22	14	20	95	87	74	87	**62.8**	**36.2**
23	7	47	47	107	33	13	**42.3**	**35.8**
24	21	40	27	74	248	81	**81.3**	**85.0**
25	14	41	27	60	94	121	**59.5**	**41.1**
Average	**20.4**	**11.4**	**46.5**	**77.6**	**63.2**	**49.1**	**44.7**	**36.0**
STDDEV[4]	**18.9**	**20.9**	**28.1**	**42.5**	**54.8**	**32.6**	**33.0**	**34.5**

[1] Numbers in table are colony-forming units per g oven-dried soil (cfu/g).
[2] Samples collected in approximately the same locations along 5 transects (5 plots per transect) throughout Field 2.
[3] Months after incorporating corn cover crop into soil.
[4] Standard deviation.

Table 5. Colonization of cornstalk debris by selected fungi 12 months after incorporation into soil – Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fungal Species	Percent Colonization
Fusarium oxysporum	11.0
Fusarium sporotrichioides	4.0
Fusarium culmorum	1.0
All *Fusarium* spp.	16.0
Trichoderma spp.	54.0
Others (Unidentified)	30.0

Table 6. Colonization of pieces of organic matter within soil by selected fungi 19 months after incorporation of the corn cover crop – Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fungal Species	Percent Colonization
Fusarium oxysporum	51.0
Fusarium equiseti	3.0
Fusarium solani	2.0
Fusarium acuminatum	2.0
All *Fusarium* spp.	58.0
Trichoderma spp.	40.0

Table 7. *Fusarium* species isolated from soil following incorporation of the corn cover crop – Field 2, USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Fusarium	Sampling Period – Months After Incorporation[3]						
Species[2]	8	10	12	14	19	23	Average
FOXY	78.4	61.9	80.5	82.9	93.3	89.3	83.9
FEQU	2.0	4.0	10.3	13.3	5.4	5.9	8.3
FACU	3.0	0.8	5.5	0.8	0.2	1.4	2.2
FSOL	0	23.0	0.6	1.3	0.9	0	1.8
FAVE	12.1	4.8	0.3	0.8	0	0.4	1.6
FSPO	0	0.8	2.2	0.6	0	1.0	1.0
FSAM	3.5	0	0	0	0	2.0	0.7
FPRO	1.0	0	0.5	0	0	0	0.2
FCUL	0	3.2	0	0	0	0	0.2
FCHL	0	1.6	0	0.3	0	0	0.2
Isolates[4]	199	126	622	626	422	495	2490

[1] Numbers in table are percent of *Fusarium* isolates identified from soil samples.
[2] *Fusarium* species: FOXY = *F. oxysporum*; FEQU = *F. equiseti*; FACU = *F. acuminatum*; FSOL = *F. solani*; FAVE = *F. avenaceum*; FSPO = *F. sporotrichioides*; FSAM = *F. sambucinum*; FPRO = *F. proliferatum*; FCUL = *F. culmorum*; FCHL = *F. chlamydosporum*.
[3] Months after incorporating the corn cover crop.
[4] Number of isolates examined for each sampling period.

One possibility to reduce soil pathogen levels without fumigation after cover crop incorporation is prolonged fallowing (James et al. 1996; Stone et al. 1997). However, results of this evaluation indicated that after 2 years of fallowing, soil populations of *Fusarium* were still above disease threshold levels and had remained fairly stable during the second fallow year. There was still evidence of high amounts of corn and seedling root residues within the soil after 2 years; this material was commonly colonized by *Fusarium* spp. Therefore, since the 2-year fallowing did not result in extensive reductions of *Fusarium* populations, the soil would require fumigation prior to sowing a conifer seedling crop to ensure low disease levels.

Soil populations of *Trichoderma* spp. also increased and remained high during the 2-year fallow period. However, their levels may not have been sufficient to ameliorate disease potential due to high *Fusarium* populations (Papavizas 1985; Papavizas and Lumsden 1980). Therefore, some level of *Fusarium*-incited disease would be expected even though *Trichoderma* populations were higher than *Fusarium*.

Pythium populations in Field 2 were generally low and did not greatly increase over the 2-year fallow period. Normally at the Coeur d'Alene Nursery, *Pythium* root disease is fairly uncommon and localized in inadequately drained portions of seedbeds (James 1982).

This evaluation confirmed previous investigations at the Coeur d'Alene Nursery (James 1998; James et al. 1989, 1990, 1996) indicating that many different *Fusarium* spp. reside in the soil. Ten species were isolated from both mineral soil and organic matter. Most species were present at very low levels. *Fusarium oxysporum* comprised the largest proportion of the population, but relatively high, isolated populations of *F. equiseti*, *F. solani*, and *F. avenaceum* were also found. The most probable pathogenic species on conifer seedlings were *F. oxysporum* and *F. solani* (Gordon and Martyn 1997; Hartley et al. 1918). *Fusarium equiseti* and *F. avenaceum* are common soil inhabitants of many agriculture-type soils, particularly those of warm-temperate areas (Burgess 1981; Burgess et al. 1988). *Fusarium equistei* is usually considered saprophytic or secondary colonizer of diseased plants (Francis and Burgess 1975). However, it has rarely been associated with pine seedling diseases (Delucca et al. 1982).

The proportion of the soil *Fusarium* population made up of fungal strains capable of eliciting diseases on conifer seedlings was unknown. Many isolates were probably saprophytic. However, without further testing, the pathogenic/saprophytic ratio cannot be known. *Fusarium oxysporum* occurs in soil as both pathogenic and saprophytic populations (Gordon and Martyn 1997; Gordon and Okamoto 1992a; Kistler 1997). If most of the population increase resulting from incorporation of corn organic matter were saprophytes, there would be little concern. However, if pathogenic strains responded to higher levels of organic matter like the saprophytic population, there would be greater disease potential. There is indirect evidence from previous tests to indicate that overall *Fusarium* population increases adversely affect subsequent seedling crops (James et al. 1996; Stone et al. 1997). Therefore, it is expected that at least some of the increased population was comprised of pathogenic strains. Unfortunately, current sampling protocols do not distinguish pathogenic from saprophytic *Fusarium* isolates. Molecular analyses can be used to help characterize pathogenicity on the basis of genetic polymorphisms (Gordon and Martyn 1997; Gordon and Okamoto 1992b, 1992c; Kistler et al. 1987, 1991). If such techniques are applied to *Fusarium* populations in forest nursery soils, we may be in a position to better predict disease potential from soil population levels.

In conclusion, this evaluation indicated that fallowing a field for 2 years following incorporation of a corn cover/green manure crop did not reduce soil population levels of potentially-pathogenic *Fusarium* spp. As a result, soil fumigation will probably be necessary to reduce *Fusarium* populations to levels of low potential disease impact prior to sowing subsequent seedling crops.

LITERATURE CITED

Boone, A.J. 1988. Soil fumigation in forest tree nurseries. *In*: Proceedings: 1988 Southern Forest Nursery Association. pp. 33-38.

Boyd, R.J. 1971. Effects of soil fumigation on production of conifer nursery stock at two northern Rocky Mountain nurseries. USDA Forest Service, Intermountain Forest & Range Experiment Station. Research Paper INT-91. 19p.

Burgess, L.W. 1981. General ecology of the Fusaria. *In*: Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). *Fusarium*: Diseases, Biology & Taxonomy. The Pennsylvania State University Press, University Park. pp. 225-235.

Burgess, L.W., P.E. Nelson, T.A. Toussoun and G.A. Forbes. 1988. Distribution of *Fusarium* species in sections *roseum*, *arthrosporiella*, *gibbosum*, and *discolor* recovered from grassland, pasture and pine nursery soils of eastern Australia. Mycologia 80:815-824.

Chapman, W. 1992. Alternative treatments to methyl bromide. *In*: Proceedings: 1992 Southern Forest Nursery Association. pp. 33-38.

Delucca, A.J., J.J. Dunn, A. Ciegler and E.G. Kuhlman. 1982. The inability of *Fusarium* species extracts to induce dieback in pines. Mycopathologia 79:105-108.

Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151-153.

Francis, R.G. and L.W. Burgess. 1975. Surveys of Fusaria and other fungi associated with stalk rot of

maize in eastern Australia. Australian Journal of Agricultural Research 26:801-807.

Gordon, T.R. and R.D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35:111-128.

Gordon, T.R. and D. Okamoto. 1992a. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82:73-77.

Gordon, T.R. and D. Okamoto. 1992b. Variation in mitochondrial DNA among vegetatively compatible isolates of *Fusarium oxysporum*. Experimental Mycology 16:245-250.

Gordon, T.R. and D. Okamoto. 1992c. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. Canadian Journal of Botany 70:1211-1217.

Hartley, C., T.C. Merrill and A.S. Rhoades. 1918. Seedling diseases of conifers. Journal of Agricultural Research 15:521-558.

Hildebrand, D.M. and G.B. Dinkel. 1988. Evaluation of methyl bromide, Basamid granular, and solar heating for pre-plant pest control for fall-sown eastern redcedar at Bessey Nursery. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest, and Cooperative Forestry Management. Technical Report R2-41. 13p.

James, R.L. 1982. Pythium root disease of Douglas-fir and grand fir seedlings at the Coeur d'Alene Nursery, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 82-10. 10p.

James, R.L. 1998. Effects of incorporating corn green manure crops on soil populations of *Fusarium, Trichoderma*, and *Pythium* - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 137. 8p.

James, R.L. and K. Beall. 1999. An evaluation of the effects of dazomet on soil-borne diseases and conifer seedling production - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-9. 15p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1989. Occurrence, characteristics, and descriptions of *Fusarium* isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-4. 23p.

James, R.L., R.K. Dumroese, D.L. Wenny, J.F. Myers and C.J. Gilligan. 1987. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region, Forest Pest Management. Report 87-13. 22p.

James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-5. 16p.

Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87:474-479.

Kistler, H.C., P.W. Bosland, U. Benny, S. Leong and P.H. Williams. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77:1289-1293.

Kistler, H.C., E.A. Momol and U. Benny. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. Phytopathology 81:331-336.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-34.

Papavizas, G.C. and R.D. Lumsden. 1980. Biological control of soilborne fungal propagules. Annual Review of Phytopathology 18:389-413.

Stone, J.K., D. Hildebrand, R.L. James and S.J. Frankel. 1997. Alternatives to chemical fumigation in bareroot forest nurseries: effects on pathogen levels and seedling density, mortality and quality. *In*: James, R.L. (ed.). Proceedings of the Third Meeting of IUFRO Working Party S7.03-04 (Diseases and Insects in Forest Nurseries). USDA Forest Service, Northern Region, Forest Health Protection. Report 97-4. pp. 59-69.

Waterhouse, G.M. 1968. The genus *Pythium* Pringsheim. Commonwealth Mycological Institute, Kew, Surrey, England. Mycological Papers No. 110. 70p.