SOIL FUNGI FROM CHRYSANTHEMUM PLANTINGS [1]

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As a part of a study of soil-inhabiting plant pathogenic fungi, qualitative fungal analyses were made of soil samples collected at regular intervals during one year from commercial and experimental plantings of *Chrysanthemum morifolium* Ram. in Florida. The data presented primarily concern the identity rather than numbers of isolated fungi.

Previous surveys of the kinds of fungi in soils of the southeastern United States are limited to Louisiana and Georgia and were reviewed by Miller, *et al.* (1957). In Florida, studies have been directed toward estimating changes in numbers of soil microorganisms resulting from agricultural practices.

Three soil types were present in the 6 areas sampled. Two plantings were on St. Lucie fine sand, 3 were on Leon-Immokalee fine sand, and 1 was on Bradenton fine sand. These soils were, in every case, amended by the addition of peat and had been used for the culture of chrysanthemums during the previous year. The soil in each sampled area had been treated with steam, Vapam, or Mylone prior to planting to reduce the numbers of nematodes, fungi, and weeds. The pH values of the soils were between 5.6 and 6.8. The soil pH did not fluctuate more than 0.4 units in any area during the sampling period.

MATERIALS AND METHODS

Samples from chrysanthemum beds were taken monthly, beginning prior to planting or within the first week after planting. Only 3 or 4 samples could be obtained during the crop period. Samples were collected in 2 series, the first during September-December from 2 commercial Yellow Iceberg plantings and 1 experimental mixed variety planting. The second series samples were collected during January-March from 3 commercial Iceberg plantings and 1 experimental mixed variety planting. All samples were collected monthly within a 10-day period.

Each soil sample, consisting of a pooled lot of 7 sub-sample

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cores, was taken from 1 bed in each area sampled. A soil sampling tube 1 inch in diameter was used to extract a cylindrical sample 6 inches long. The sub-sample cores were obtained from the same locations within the bed on successive sampling dates. Plastic bags, used to contain the samples, were disinfested before use and the sampling tube was immersed in 10% Clorox before each sample extraction.

In the laboratory each sample was mixed thoroughly and 12.5 g portions were placed in 3 separate flasks containing sterile 1% methyl cellulose solution. After roiling 15 seconds with an electrical mixer, one further dilution was made bringing each replicate dilution to approximately 1 in 5000. The moisture content of each sample was determined later and final soil dilutions were calculated. Sample data were adjusted to a dry-soil weight basis. Three Petri dishes, each containing 1 ml of the final soil dilution and approximately 10 ml of Rose Bengal-Streptomycin (RB-S) medium, were prepared from each sample replication. Aseptic techniques were used throughout laboratory processing. The cultures were placed at 24° C for 5 days after which they were marked to define the position of original colonies. Fungi were identified within 14 days.

The RB-S medium was similar to that used by Martin (1950) except that the Rose Bengal concentration was decreased to 1 in 67,000 and 60 μg of streptomycin sulfate per milliliter of medium were used. Czapek's medium was used for the identification of species of *Aspergillus* and *Penicillium*.

Results

A soil dilution of 1 in 5,000-6,000 was satisfactory in most instances and permitted discrete growth of fungal colonies, especially those developing from samples collected soon after soil treatment. Mean estimated numbers of fungal bodies per gram of dry soil ranged from 12,000 (all initial samples) to 56,000 (all terminal samples). Fungal populations increased in all areas during the sampling periods but the rates of increase varied among sampled areas.

Identification of all fungi from the 49 samples was not possible since the routine nature of the study did not permit extensive subculturing. RB-S medium, while permitting the growth of a wide variety of fungi, was unfavorable for the sporulation of many fungi.

Eighty-five per cent of all fungi isolated were identified as to genus. Many isolations in various genera did not conform to the extant species descriptions available and were enumerated in a genus category. A tabulation of all identified fungi is given in table 1 with the percentage species frequency and the percentage genus frequency and abundance. Species frequency is the ratio of the number of samples in which the species occurred to the total number of samples. Genus frequency was calculated similarly. Genus abundance is the ratio of the total number of colonies of a genus in all samples to the total number of identified fungus colonies in all samples. Genus frequency and abundance are listed opposite the first entry of the genus.

A comparison of the genera of fungi from the 3 soil types revealed that only the genera *Penicillium*, *Fusarium*, *Trichoderma*, *Cladosporium*, and *Curvularia* were common to all soils during the spring sampling period. The numbers of genera in all soils were approximately equal but estimated fungal populations were consistently higher in samples from the planting on Bradenton fine sand. This soil has a greater moisture retention and is more compact than the other soils sampled. *Aspergillus*, *Mucor*, and *Rhizopus* were found in samples of Bradenton fine sand but were not found in other soils during the spring. The lighter sandy soils of the St. Lucie and Leon-Immokalee types had 8 genera in common. Three of these, *Phoma*, *Cephalosporium*, and *Phymatotrichum* were not found in samples of Bradenton fine sand.

Discussion

The most abundant genera in the samples were *Penicillium*, *Fusarium*, and *Trichoderma* which comprised more than half of the fungal colonies identified. These genera were also the most frequent. *Syncephalastrum*, *Aposphaeria*, and *Pullularia* ranked 4-6 in abundance but none of these genera had a frequency of more than 10 per cent since they were restricted in distribution to several related samples. *Cladosporium*, *Curvularia*, *Aspergillus*, *Mucor*, and *Cephalosporium*, ranking 4-8 in frequency, were not represented by many colonies in the samples in which they occurred. Of the 49 genera listed in table 1, 84 per cent belong to the Fungi Imperfecti while the remaining few are distributed among the 3 other classes. Only 66 per cent of the 61 genera listed

by Miller *et al.* (1957) are classified in the Fungi Imperfecti and relatively larger numbers of Phycomycetes and Ascomycetes were found in their samples than in chrysanthemum soil samples.

The abundance ranking of genera from treated chrysanthemum soils is different from rankings given by workers in Georgia, Louisiana, and Texas. Surveys of soil fungi in these states revealed that *Aspergillus* ranked first or second in abundance. Miller *et al.* (1957) suggested that *Aspergillus* species are dominant in warm climates. A comparison between the order of abundance of fungi from treated Florida soils and from untreated cultivated or forest soils of other states is not entirely valid, since the presence or abundance of some fungi may be due to cultural practices. A soil treatment, by reducing the fungal population, favors the rapid growth of some surviving fungi. In addition fungal populations are influenced by the introduction of many genera of fungi on the roots and foliage of cuttings.

TABLE 1

SUMMARY OF FUNGI ISOLATED FROM SOILS OF CHRYSANTHEMUM PLANTINGS

	% Genus frequency	abundance*	% Species frequency
Phycomycetes			
Blakeslea trispora Thax.	2	R	
Mucor spp.	16	1.1	
M. fragilis Bain.			2
M. racemosus Fres.			2
Rhizopus nigricans Ehrenb.	2	R	
Syncephalastrum racemosum (Cohn) Schroet.	6	10.0	
Ascomycetes			
Arachniotus citrinus Massee & Salm.	6	0.3	
Chaetomium spp.	6	R	
Neocosmospora vasinfecta E. F. Smith	2	R	
Basidiomycetes			
Rhizoctonia solani Kuhn	4	R	

TABLE 1—(Continued)

SUMMARY OF FUNGI ISOLATED FROM SOILS OF CHRYSANTHEMUM PLANTINGS

	% Genus frequency - abundance*		% Species frequency
Fungi Imperfecti			
Acremonium sp.	6	R	
Acrotheca sp.	2	R	
Alternaria tenuis Nees ex Fr.	14	0.4	
Aposphaeria sp.	8	5.3	
Aspergillus spp.	33	2.6	
A. flavipes (Bain. & Sart.) Thom & Church			4
A. flavus Link			8
A. fumigatus Fres.			2
A. melleus Yuk.			10
A. niger van Tiegh.			10
A. ustus (Bain.) Thom & Church			10
A. versicolor (Vuill) Tirab.			4
A. wentii Wehmer			2
Bispora sp.	12	1.0	
Botryosporium pulchrum Corda	2	R	
Botrytis cinerea Pers.	2	R	
Calcarisporium sp.	2	R	
Cephalosporium spp.	16	1.2	
C. acremonium Corda			4
Chalaropsis sp.	2	R	
Cladosporium spp.	43	3.4	
C. herbarum Link ex Fr.			33
C. epiphyllum Pers.			8
Coniothyrium sp.	2	R	
Curvularia spp.	35	1.0	
C. geniculata (Tracy & Earle) Boedj.			6
C. lunata (Wakk.) Boedj.			8
C. pallescens Boedj.			8
C. tetramera (McKinney) Boedj.			2
Diplodia sp.	2	R	
Fusarium spp.	45	13.1	
F. lateritium Nees ex Fr. emend. Sny. & Hans.			2
F. moniliforme Scheldon emend. Sny. & Hans.			4
F. nivale (Fr.) Ces. emend. Sny. & Hans.			2

TABLE 1—(Continued)

SUMMARY OF FUNGI ISOLATED FROM SOILS OF CHRYSANTHEMUM PLANTINGS

	% Genus frequency	abundance*	% Species frequency
F. solani (Mart.) Appel & Wr. emend. Sny. & Hans.			20
F. roseum Link emend. Sny. & Hans.			22
Gliocladium sp.	2	R	
Gliomastix sp.	4	R	
Gonatobotryum sp.	2	R	
Harpographium sp.	2	R	
Heterosporium sp.	2	R	
Humicola sp.	10	0.4	
H. brevis (Gilm. & Abb.) Gilm.			4
Masoniella grisea (Smith) Smith	4	R	
Melanconium sp.	10	0.3	
Nigrospora sp.	8	R	
N. sphaerica (Sacc.) Mason			6
Oospora sp.	2	R	
Paecilomyces varioti Bain.	4	0.4	
Penicillium spp.	84	35.3	
P. brefeldianum Dodge			4
P. charlesii Smith			2
P. citrinum Thom			10
P. decumbens Thom			14
P. herquei Bain. & Sart.			14
P. janthinellum Biourge			2
P. lanosum Westling			8
P. lilacinum Thom			2
P. oxalicum Currie & Thom			12
P. paxilli Bain.			8
P. simplicissimum (Oud.) Thom			4
P. velutinum van Beyma			2
P. wortmanni Klock.			14
Phoma sp.	22	2.0	
P. hibernica Grimes, O'Conner & Cummins			10
Phymatotrichum spp.	14	1.1	
Pullularia sp.	4	5.0	
P. pullulans (de Bary) Berkh.			2
Pyrenochaeta sp.	4	R	
Scopulariopsis spp.	4	R	
S. brevicaulis Bain.			2

TABLE 1—(Continued)

SUMMARY OF FUNGI ISOLATED FROM SOILS OF CHRYSANTHEMUM PLANTINGS

	% Genus frequency - abundance*		% Species frequency
Sporotrichum sp.	4	0.3	
S. pruinosum Gilm. & Abb.			2
Spicaria sp.	4	0.4	
S. simplicissima Oud.			2
Stysanus medius Sacc.	2	R	
Stemphylium botryosum Wallr.	8	0.2	
Trichoderma spp.	53	12.7	
T. glaucum Abb.			4
T. viride Pers. ex Fr.			53
Trichothecium roseum Link	2	R	
Trichocladium sp.	2	R	
Zygosporium sp.	2	R	

* Genus abundance of less than 0.2% is listed as rare (R).

The relative paucity of *Aspergillus,* Phycomycetes, and Ascomycetes in treated soils may reflect differences in susceptibility of fungi to soil treatments as well as response to edaphic conditions.

Ascomycetes were absent from most samples. *Arachniotus citrinus* was isolated in several related samples in the early fall. The genera *Talaromyces* C. R. Benj. and *Carpenteles* Langer., listed by Gilman (1957) as the ascigerous stages of *Penicillium wortmanni* and *P. brefeldianum,* were isolated occasionally.

Fungi Imperfecti were the most numerous fungi in all samples. Several genera, apparently infrequently reported from soil, were isolated during the study. The genera *Melanconium, Aposphaeria, Diplodia, Gonatobotryum, Harpographium, Zygosporium, Calcarisporium, Bispora,* and *Acrotheca* are not included among the genera listed by Gilman (1957).

Isolations of *Trichoderma,* with the exception of *T. glaucum,* could not be separated on any basis other than occasional color and growth rate differences. This separation was not reliable since these differences occurred in a series of gradations. Therefore all isolates, except *T. glaucum,* were referred to *T. viride,* considered

by Bisby (1939) to include *T. lignorum* Tode ex Harz and *T. koningi* Oud.

Rhizoctonia solani was isolated on only a few occasions; no other proven chrysanthemum pathogens were found. The relationships that may have existed among numbers and kinds of fungi and such variables as soil treatment methods, other cultural practices, varieties, and climatic conditions were not explored.

Summary

Soil fungi were isolated from a total of 49 samples collected in 6 chrysanthemum plantings in Florida. The soils of all areas were treated before planting and sampling to reduce numbers of nematodes, fungi, and weeds. Forty-nine genera of fungi were identified, of which 41 were Fungi Imperfecti. The 3 most abundant and frequent genera were *Penicillium, Fusarium,* and *Trichoderma.* Approximately equal numbers of genera were isolated from 3 soil types sampled, but estimated fungal populations were greater in samples of Bradenton fine sand.

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