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Sprays for Control of Sycamore Anthracnose

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Fungi of Two Forest Soils of Johnson County

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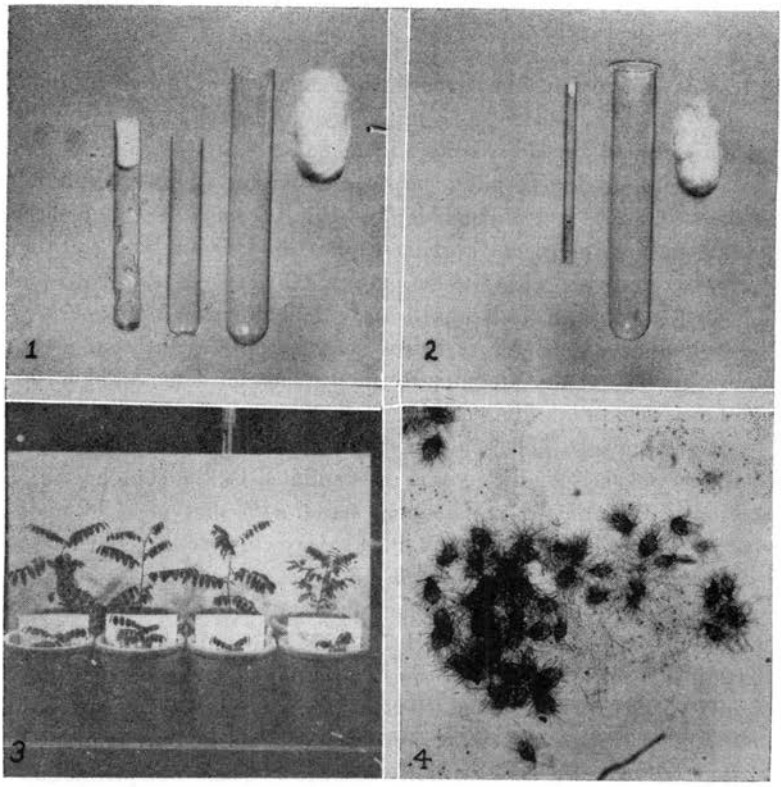
It was noted that the undergrowth under a stand of *Robinia Pseudo-Acacia* was sparse as compared with that under an adjoining area dominated by elms, oaks and hickories. It was thought that perhaps this difference might be reflected in the fungal population of the two soils. The soil under *R. Pseudo-Acacia* was found to be loosely packed, moist and to have a pH 6.89-7.00 for both soil and overlying humus. The mosaic pattern of the trees did not seem sufficiently dense to limit significantly the amount of light reaching the ground line. Therefore it was conjectured that the sparse vegetation under the tree was due to the presence of some antagonistic substance that was either exuded from the roots or leached out of the fallen leaves. This contention that limited growth was not due to environmental conditions was substantiated by the comparison of soil conditions of the neighboring stand with that of *R. Pseudo-Acacia*, and from the results obtained by growing seedlings of *Ulmus americana* and *R. Pseudo-Acacia* in crocks containing soil from either their own stand or from the other stand. The reaction of the mixed, or *Ulmus*, stand was pH 7.0-7.1. The soil texture was that of tightly packed clay. The mosaic pattern did not seem to vary significantly from that of *R. Pseudo-Acacia*. In short, the two soil environments appeared to be similar. Also, the fungus population of the two proved to be essentially identical. There was distinct difference, however, in the growth of seedlings in the two soils. *R. Pseudo-Acacia* grew poorly in its own soil, but luxuriantly in soil from the mixed stand (fig. 3). The average height of the six seedlings grown in *R. Pseudo-Acacia* soil was 9.2 cm., and the average number of branches was 4.6. The average height of the same species grown in *U. americana* soil was 29.2 cm., and the average number of branches was 8.2.

Ulmus americana appeared to grow better in its own soil than in *R. Pseudo-Acacia* soil. However, hail damage to the seedlings of *U. americana* made it impossible to interpret relative growth. It is perhaps noteworthy that seedlings of *R. Pseudo-Acacia* seldom grow in the stand of the parent, but instead succeed in growing only on the margin of the stand. It may be that an antagonistic substance is present but that it affects the flowering plants and not the fungi. further investigation into the true nature of the inhibiting agent was not made since emphasis was placed on the fungus populations and

PLATE I



1. Soil immersion tube, left, and two outer jackets. Hyphae pass through the invaginated apertures of the tube into the medium. Inner jacket prevents loss of agar when the tube is being sterilized.
2. Glass corer tube containing soil and outer jacket.
3. Front row: *Robinia pseudo-acacia* in R. Soil.
Back row: *Robinia pseudo-acacia* in U. soil.
4. *Chaetomium subterraneum.*

the methods for their determination. This report lists the fungi isolated from the two soils during this investigation, which started in May, 1950, and was terminated in December, 1950.

MATERIALS AND METHODS

Much attention was given to the selection of media, methods of inoculation and method of collecting the soil samples. The instrument adopted for the collection of soil was a modification of the core tube principle. A 6. mm. glass tube five inches long was plugged with cotton at both ends, placed in a plugged test tube and sterilized in an autoclave. Soil was collected by inserting one end of the glass tube into the desired depth of soil and withdrawing a

cylinder of soil about two inches long. The tube was returned to the test tube and brought to the laboratory. At this time the tube was filed and broken at a point one-fourth of the distance from the end of the soil sample. The exposed end was flamed and placed under the lid of a sterile petri dish. One-fourth of the remaining soil core was forced into the petri dish by ejection with a glass plunger inserted at the upper end of the tube. Inoculations were made within 48 hours of collection.

The selection of media was made with three factors in mind: (a) bacterial growth must be discouraged, (b) spreading of fungal colonies must be limited, (c) nutrient environmental requirements of fungi must be met.

It was recognized that all of these factors could not be incorporated into one medium for all of the fungi. Consequently several media were sought, each of which would possess one or more of the desired qualities. The following media proved to be the most efficient of those tested:

1. Hemp seed in 0.8% agar.
2. *Ambrosia* stem in mineral agar.
3. Bleached duck fiber in mineral agar.
4. Soil and humus extract in mineral agar.
5. Rose bengal-streptomycin solution in glucose-peptone-mineral agar.

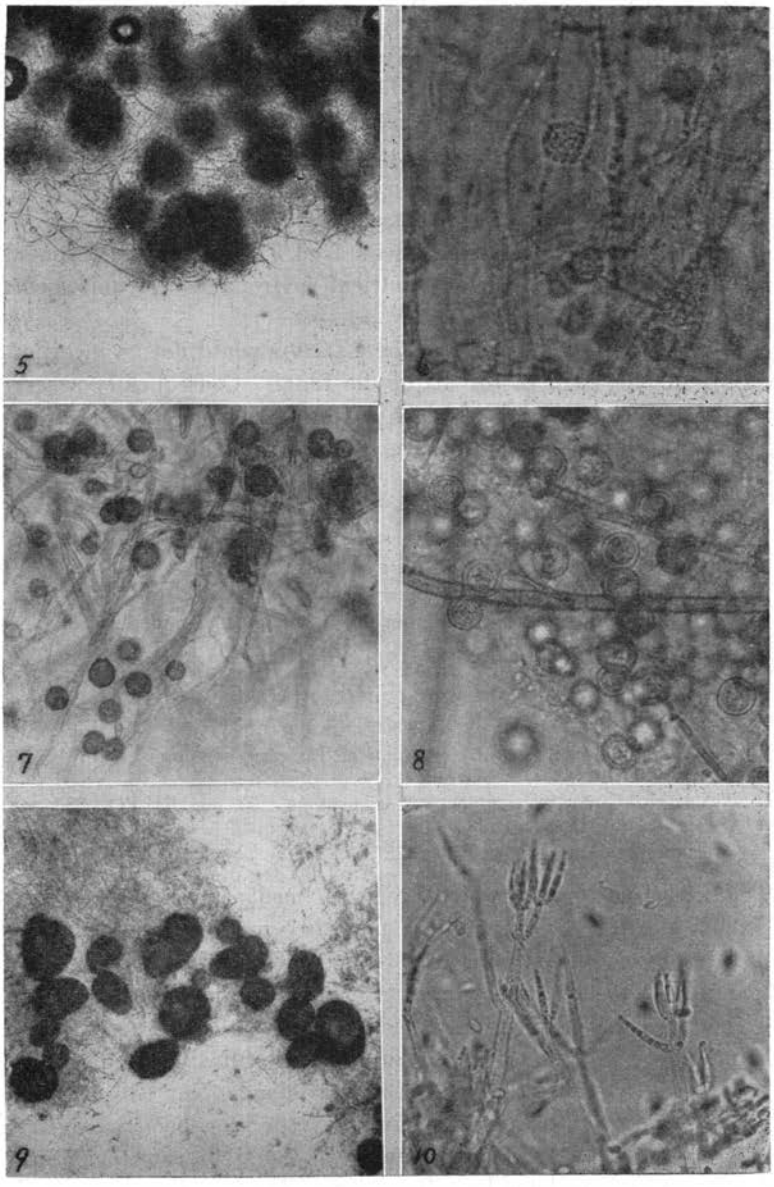
The last medium was adopted from an article by J. P. Martin (8). The following proportions were found to be satisfactory:

a. 0.1 gram Rose bengal dye in 900.0 cc water.
b. add to above 1.0 cc of a solution of 1.0 gram streptomycin in 100 cc water.
c. add to above: 10. g. glucose, 2. g. peptone, 250. mg. KH_2PO_4, 250. mg. $MgSO_4$ and 15. g. agar.
d. make up to a volume of 1.0 liter.

The first four media, which are weak media, were suggested by G. W. Martin. Their merit is that they are characterized by a proper physical substratum or by being sufficiently low in nutrient materials to discourage bacterial growth and to limit fungal growth, or both. The fifth medium is rich. The rose bengal-streptomycin solution serves both to inhibit bacterial growth almost completely and to restrict fungal growth to vertical elongation. This medium is far superior to acidified medium for checking bacterial growth and permitting a maximum number of fungi to grow. Medium No. 5 can be inoculated by soil solution. The first four media must be inoculated with dry soil particles or by streaking dry soil over the plate.

An attempt was made to determine which fungi were present in the soil in the mycelial state by burying a soil immersion tube

Plate II



5. *Pseudogymnoascus roseus.*
6. *Cephalosporium acremonium.*
7. *Humicola* sp.
8. *Coccospora sp.*
9. *Penicillium sclerotiorum.*
10. *Gliocladium roseum.*

(fig. 1) containing nutrient agar in the soil. This technique, adopted from Chesters (3), failed because the mucors quickly overran the agar.

List of Fungi Isolated from Both Soils

1. *Zygorrhynchus vuilleminii*
2. *Zygorrhynchus moelleri*
3. *Chaetomium subterraneum*
4. *Cunninghamella elegans* R
5. *Humicola* sp.
6. *Cephalosporium acremonium* R
7. *Hormodendron olivaceum*
8. *Mucor hiemalis*
9. *Mortierella* sp.
10. *Coccospora* sp.
11. *Spicaria violacea*
12. *Spicaria elegans*
13. *Gliocladium vermoensi*
14. *Gliocladium roseum*
15. *Gliocladium fimbriatum* R
16. *Trichoderma koningi*
17. *Trichoderma lignorum*
18. *Streptomyces* sp.
19. *Diplodinia* sp.
20. *Torula* sp. U
21. *Verticillium* sp.
22. *Botrytis cinerea* R.
23. *Fusarium orthoceros*
24. *Fusarium* sp.
25. *Penicillium clavigerum* U
26. *Penicillium sclerotiorum*
27. *Penicillium spiculisporum*
29-35. *Penicillium* sp.
36. *Aspergillus terreus* R
37. *Aspergillus fumigatus* R
38. *Pseudogymnoascus roseus* U
39. *Alternaria humicola*
40. *Acrostalagmus* sp.
41. *Humicola* (brown sp.)

The letter following a name indicates that the fungus was found only in that soil. R represents *R. Pseudo-Acacia* soil. U represents *U. americana* soil. It can readily be seen that the fungus population of the two soils was essentially identical. Actually, more genera were isolated from the *Robinia* soil than from the *Ulmus* soil.

According to Gilman (4) the following isolates have not previously been reported from Iowa: *Chaetomium subterraneum, Cunninghamella elegans, Humicola sp., Mortierella sp., Cephalosporium acremonium, Coccospora sp., Diplodinia sp., Torula sp., Botrytis cinerea, Penicillium clavigerum, Penicillium sclerotiorum, Pseudogymnoascus roseus.*

The fungus determined as *Pseudogymnoascus roseus* has been repeatedly isolated from Amana Colony soil in a current investigation. According to Gilman this fungus has been reported from soil only in the U.S.S.R.

The data collected so far from the Amana Colony investigation suggests that the method of isolation described here is capable of distinguishing between the mycobiota of a pine stand and an adjacent uncultivated strip.

Grateful acknowledgment is made to Professor G. W. Martin for his suggestions in the selection of media and for supervision in the identification of the fungi, and to S. C. Damon for assistance in the determination of many of the fungi.

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