CHAPTER XIV

ANTIBIOSIS IN RELATION TO PYTHIUM ROOT-ROT OF WHEAT

INTRODUCTION AND HISTORICAL REPERT

In this section the effect of antagonists on the development of Pythium root-rot has been studied. The organisms used were selected on the basis of earlier experiments (Chapter V) in which they were tried and found active in agar plate tests. In this series 6 organisms were tried as inoculum in soil along with the pathogen and results assayed by means of wheat seed germination tests.

Attempts have been made by several workers to make use of the phenomenon of antibiosis in plant disease control since the early days of this century. Antagonistic microorganisms have been used to control disease in various cases and the decrease recorded in the severity of the disease has been attributed to the sensitivity of plant pathogen to the toxic products of the antagonists or to such other factors as competition for food, unfavourable substrate reaction and growth rate etc. Antagonists from various groups of bacteria, actinomycetes and fungi have been known to act in the above manner.

Hardly, as early as in 1921, found that the inoculation of steamed soil with various saprophytic forms resulted in a decrease of the parasitic activity of Pythium debaryanum on
forest coniferous seedlings. Weindling, in 1932, 1934, demonstrated that Trichoderma lignorum markedly reduced the parasitic activity of R. solani and Phytophthora parasitica on citrus seedlings under sterilized soil conditions.

In 1932, Tims discovered actinomycetes antagonistic to a Pythium which was a root parasite of sugarcane. He found that the saprophytic actinomycetes reduced the amount of root rotting in young cane and corn plants. LeBeau, in 1939, found isolates of Trichoderma which were antagonistic to the same root parasite.

Johnson, in 1952, studying the control of damping-off of alfalfa, concluded that in sterile loam both T. lignorum and Streptomyces sp. gave control of disease when the soil was amended with 1 per cent glucose or ground oat straw. No control was obtained, however, when this antagonist was added to natural soil artificially or naturally infested with the pathogen. He (Johnson, 1954) further studied the effect of soil organisms on Pythium root-rot of sugarcane and corn, and better control was obtained by the use of actinomycetes than with bacteria or fungi. The control was attributed to the result of secretion of antibiotic substances.

Pestinskaya, 1956, reported control of P. debaryanum by use of antagonistic saprophytic microorganisms like Penicillium, Aspergillus and Trichoderma.

In our laboratory attempts were made to control root-rot and foot-rot fungi by using antagonistic actinomycetes,
bacteria and fungi (Thampi, 1964; Churde, 1962; Mehrotra, 1961) with positive results.

**METHODS USED**

In the previous experiments (Chapter V) *P. ultimum* PF-12 was found to be inhibited by a number of antagonistic microorganisms. From among these, those organisms which showed strong activity were selected and used in the present tests. Further, an effort was made to select representatives from the various groups, bacteria, actinomycetes and fungi. These were *B. cereus* var. *mycoides* B-44, *S. erythraea* A-15, *S. anulatus* A-71, *A. candidum* F-81, *C. trilaterale* F-75 and *T. viride* T-10.

**Preparation of inocula:**

**Pathogen:** The pathogen was tested for its virulence before actually using in the experiments. Sterile soils, in 125 ml. bottles, were inoculated with *P. ultimum* PF-12, 1-3 discs to each bottle. The moisture content of soil was adjusted to 45 per cent. After incubation, wheat seeds (HY65), surface sterilized for 4 minutes in 1 in 1,000 alcoholic mercuric chloride (Thammayya, 1961) and washed well in sterile water, were sown, 8 seeds in each bottle. After one week of growth, the symptoms and severity of the disease were recorded. It showed to cause about 90 per cent mortality; and then it was cultured on oatmeal yeast extract agar plates for 7-14 days, to serve as inoculum by cutting discs of 8 mm. diameter. These discs, which were cut to measure, were used as quantitative units given as inoculum. In one set of treatments only one disc was added and
in other set, which was more heavily inoculated, 3 disc were
given to each bottle.

**Antagonist:** Prior to preparation of inoculum, the
antagonists were also tested for their activity in pilot exper-
iments by cross-streak assay method. It will be recalled that
these antagonists were found to be active against *P. ultimum* PF-12
in the experiments on antimicrobial spectra. These results are
extracted from these observations and given in Table XXXVII, page
154 for ready reference. The antagonists were grown on agar media
as follows: *B. cereus* var. *mycoides* B-44 on nutrient agar,
*P. erythraeus* A-15 and *A. tri.terale* F-75 on oatmeal yeast extract
agar, *P. anulatus* A-71 on Emerson's medium, and *I. viride* T-10 and
*A. candidus* F-81 on pot to dextrose agar. The cultures were incu-
bated at 26°C. for 14 days. And from these cultures heavy spore
suspensions were prepared in sterile distilled water for inoculation.
In preparing a suspension of the antagonist for inoculation,
one-fourth of the agar plate was cut away and placed in a separate
sterile dish and flooded with 20 ml. of water. With the help of
a needle the scraping was taken and agar removed. The whole thing
was then stirred and used for one experimental bottle of soil.

**Preparation of soil containers for experiments:**

Wide mouth glass specimen bottles of 125 ml. capacity
were used as soil containers in experiments. 100 g. of soil
was weighed in each bottle and moisture content adjusted finally
to 45 per cent. This moisture content includes water which was
added along with the inoculum of the antagonist. The bottles
having soil were sterilized (15 lb. pressure for 45 minutes) at
Table - XXXVII.

Table showing inhibition of *Pythium ultimum* PF-12 on agar medium by antagonists.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antagonist</th>
<th>Average clear inhibition zone (in mm.)</th>
<th>Average radius of the antagonist (in mm.)</th>
<th>Total inhibition zone (in mm.)</th>
<th>Persistence of inhibition zone (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>B. cereus</em> var. <em>mycoides</em> B-44</td>
<td>10.0</td>
<td>20.0</td>
<td>30.0</td>
<td>40.0</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. anulatus</em> A-71</td>
<td>19.0</td>
<td>8.0</td>
<td>27.0</td>
<td>40.0</td>
</tr>
<tr>
<td>3.</td>
<td><em>S. erythraeus</em> A-15</td>
<td>12.0</td>
<td>4.5</td>
<td>16.5</td>
<td>30.0</td>
</tr>
<tr>
<td>4.</td>
<td><em>C. trilaterale</em> F-75</td>
<td>16.0</td>
<td>12.5</td>
<td>28.5</td>
<td>10.0</td>
</tr>
<tr>
<td>5.</td>
<td><em>A. candidus</em> F-81</td>
<td>17.0</td>
<td>16.0</td>
<td>33.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>
25 per cent moisture content so that the total moisture content could be adjusted after adding 20 ml. of inoculum in water.

For each antagonist under test the following treatments were undertaken:

1. In the case of each of the 3 replicate bottles, one agar disc of the pathogen and then 20 ml. of the suspension of the antagonist were added. The contents were stirred thoroughly at every stage. After one week, 8 wheat seeds, which were surface sterilized and washed, were sown equidistantly in the bottle.

2. All the treatment was as above, excepting that in these 3 bottles 3 discs of the pathogen were added instead of one (stronger inoculum potential).

3. The treatment for the 3 bottles was the same as in No. 1 excepting that no antagonist was added (Control 1).

4. The treatment for the 3 bottles was the same as in No. 2 excepting that no antagonist was added (Control 2).

5. In these 3 replicate bottles seeds were sown without mixing any pathogen or antagonist (Control 3).

In this way 15 bottles (3 of each type) were run for each treatment. Since the last 3 treatments were common for all the antagonists, they were run only in a single set for all. In this way $6(=3+3) \times 6($antagonists$) + 9($common controls$) = 45$ bottles were run in all.

The bottles were kept in a moist chamber and protected from external contamination.
Observations were recorded 14 days after sowing of the seeds in each case. The number of seedlings which emerged out and the state of their health were recorded and photographed. The seedlings and the unemerged seeds were then taken out, underground parts washed, and each was examined with the help of lens or, if necessary, with a stereoscopic microscope. Records were then taken for the number of root-rot cases or pre-emergence blight. These observations have been recorded in Table XXXVIII, page 157.

Summary, Discussion and Conclusions

In this section, the antagonistic activity on the development of Pythium root-rot disease of wheat has been described. The experiments were done in glass bottles in which 100 g. of soil was taken and sterilized. The antagonist was added in the form of suspension in water and the pathogen in the form of agar discs. 8 wheat seeds were sown in each bottle and observations were recorded after 14 days (Table XXXVIII, page 157). Proper controls were run side by side. The following conclusions could be drawn with respect to each antagonist.

1. A. candidus F-81 (Plate XVIII A & B):

This antagonist produced marked control of the disease. When the inoculum was of 1 disc the survival of the plants was 98.5 per cent. Out of these only 12 per cent suffered from severe root-rot and rest of the plants showed normal growth with slightly
Table - XXXVIII.

Effect of the six antagonists on the occurrence of root-rot of wheat caused by *Pythium ultimum* PF-12.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment of the soil in bottles</th>
<th>Amount of inoculum of pathogen</th>
<th>Percent survival of plants</th>
<th>Degree of the disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>None (Control)</td>
<td>None</td>
<td>98.0</td>
<td>No disease. Healthy plants.</td>
</tr>
<tr>
<td>2.</td>
<td>None (Control)</td>
<td>1 disc</td>
<td>12.0</td>
<td>Preemergence blight. Survived plants suffered from root-rot and stunted growth.</td>
</tr>
<tr>
<td>3.</td>
<td>None (Control)</td>
<td>3 discs</td>
<td>10.0</td>
<td>Preemergence blight. Survived plants suffered from root-rot and stunted growth.</td>
</tr>
<tr>
<td>4.</td>
<td><em>A. candidus</em> F-81</td>
<td>1 disc</td>
<td>95.8</td>
<td>Preemergence blight. 12% of the survived plants suffered from severe root-rot and stunted growth. Rest of the plants healthy with slightly reduced root system.</td>
</tr>
<tr>
<td>5.</td>
<td><em>A. candidus</em> F-81</td>
<td>3 discs</td>
<td>93.7</td>
<td>Preemergence blight. 40% of the survived plants suffered from root-rot. Rest of the plants normal with slight reduction in root system.</td>
</tr>
<tr>
<td>6.</td>
<td><em>C. trilatereale</em> F-75</td>
<td>1 disc</td>
<td>12.5</td>
<td>Preemergence blight severe root-rot.</td>
</tr>
<tr>
<td>7.</td>
<td><em>C. trilatereale</em> F-75</td>
<td>3 discs</td>
<td>10.0</td>
<td>Preemergence blight, severe root-rot.</td>
</tr>
<tr>
<td>8.</td>
<td><em>T. viride</em> T-10</td>
<td>1 disc</td>
<td>75.0</td>
<td>Preemergence blight, 22.2% of the survived plants suffered from severe root-rot and stunted growth.</td>
</tr>
<tr>
<td>9.</td>
<td><em>T. viride</em> T-10</td>
<td>3 discs</td>
<td>56.2</td>
<td>Preemergence blight. 25% of the seedlings suffered from severe root-rot and stunted growth.</td>
</tr>
<tr>
<td>10.</td>
<td><em>B. cereus</em> var. <em>mycoides</em> B-44</td>
<td>1 disc</td>
<td>58.3</td>
<td>Preemergence blight. 50% of the survived plants suffered from severe root-rot and stunted growth.</td>
</tr>
<tr>
<td>11.</td>
<td><em>B. cereus</em> var. <em>mycoides</em> B-44</td>
<td>3 discs</td>
<td>50.0</td>
<td>Preemergence blight. Almost all suffered from disease.</td>
</tr>
<tr>
<td>12.</td>
<td><em>S. anulatus</em> A-71</td>
<td>1 disc</td>
<td>71.0</td>
<td>Preemergence blight. 65% of the survived seedlings suffered from root-rot and stunted growth.</td>
</tr>
<tr>
<td>13.</td>
<td><em>S. anulatus</em> A-71</td>
<td>3 discs</td>
<td>65.5</td>
<td>Preemergence blight. 80% of the survived plants suffered from root-rot and stunted growth.</td>
</tr>
<tr>
<td>14.</td>
<td><em>S. erythraeus</em> A-15</td>
<td>1 disc</td>
<td>44.0</td>
<td>Preemergence blight. 57% of the survived plants suffered from severe root-rot and stunted growth.</td>
</tr>
<tr>
<td>15.</td>
<td><em>S. erythraeus</em> A-15</td>
<td>3 discs</td>
<td>18.5</td>
<td>Preemergence blight. All the plants suffered from severe root-rot.</td>
</tr>
</tbody>
</table>
reduced root system. When the inoculum was increased to 3 discs the survival of the plants was 93.7 per cent, out of which 40 per cent suffered from root-rot and the rest of the plants were normal with little reduced root system.

It may be noted that these observations are in accord with observations recorded in experiments on agar media in Petri-dishes.

These observations suggest that some form of antagonism developed between A. candidus F-81 and the pathogen in the soil, to the detriment of the latter. In the absence of A. candidus F-81 (controls 1 and 2) the pathogen caused severe mortality (90 per cent) and root-rot of wheat. But in the presence of this antagonist very good control was obtained. It may be mentioned that A. candidus F-81 produced an antibiotic substance both in agar and liquid media (Chapters V & VI), and in all probability the control of the pathogen was due to secretion of an antibiotic substance in soil.

2. C. trilaterale F-75:

This organism has not produced control of the disease. The plants suffered from both pre-emergence blight as well as root-rot, almost to the same extent in the control bottles.

These results are rather surprising because the fungus was found to be very active against P. ultimum FF-12 in Petri-dish tests, though the exudate was found to be unstable and did not remain active for long. Further, in the Petri-dish tests it was
found antagonistic against a whole array of organisms, both bacteria and fungi. Though found inactive in these experiments, the organism deserves further special studies.

3. *T.viridae* T-10 (Plate XVIII C & D):

This fungus produced significant control of the root-rot as well as pre-emergence blight. When inoculum of the pathogen was 1 disc, the survival of the plants was 75 per cent, out of which only 22.2 per cent suffered from severe root-rot and the rest of the plants showed normal growth with slightly reduced root system. But, when the inoculum of the pathogen was 3 discs the survival of the plants was only 56.2 per cent, out of which 25 per cent suffered from root-rot.

The observations indicate that *T.viridae* T-10 developed definite antagonism against the pathogen. It may be mentioned that *T.viridae* T-10 produced antibiotic substances in liquid media (Chapter VI) and in soil (Chapter XII) and the present observations are in conformity with it. In the literature there are many more reports about the suppressive activity of *T.viridae* against many other pathogens (Ludwig and Henry, 1947; Wood, 1951; Wright, 1956). It may be noted that Wright (1954) demonstrated production of gliotoxin in sterilized soils, whose pH was 7.1-8.3, though Jeffreys (1952) has reported that gliotoxin and viridin are inactivated at pH above 5.0. The pH of the present soil under test was 7.2.

This organism produced a limited control of the disease. When the inoculum of the pathogen was 1 disc the survival of the plants was 58.3 per cent, out of which 50 per cent suffered from severe root-rot. When the inoculum of the pathogen was 3 discs, the survival of the plants was 50 per cent, but all of them suffered from severe root-rot.

It may be mentioned here that *B. cereus* var. *mycoides* B-44 inhibited the same pathogen on agar media, it produced an antibiotic substance in liquid media (Chapter VI) but no substance could be extracted from soil (Chapter XII). It may be that the active principle is not stable in soil and is immediately adsorbed on soil colloids.

5. *E. anulatus* A-71 (Plate XIX A & B):

This antagonist produced only some reduction in the severity of the disease. When the inoculum of the pathogen was 1 disc the survival of the plants was 71 per cent, out of which 65 per cent suffered from severe root-rot and the rest of the plants showed normal growth, though the root system was slightly reduced. When the inoculum of the pathogen was 3 discs, 65.5 per cent plants survived, out of which 80 per cent suffered from severe root-rot and the rest were normal with slightly reduced root system. On the whole, we find that there was a substantial check of the pathogen though a total control did not occur.

It may be recalled that this antagonist produced an antibiotic substance in liquid media (Chapter VI), but no
antibiotic substance could be extracted from the soil (Chapter XII). It exerted antagonistic activity against *T. virida* T-10 in soil (Chapter XI) where it was attributed to other antagonistic effects such as competition for food and space. From all these observations it appears that the antagonism developed here was due to such factors as competition for food or for space.


The decrease in the severity of the disease was not significant in this case. When the inoculum of the pathogen was 1 disc, the survival of the plants was only 44 per cent out of which 57 per cent suffered from severe root-rot and rest of the plants showed normal growth with slight reduction in the root system. When the inoculum of the pathogen was 3 discs the survival of the plants was only 18.5 per cent and all the plants suffered from severe root-rot.

Even the limited control of the severity of the disease indicates that there was suppressive activity against the pathogen. It may be mentioned that *S. erythraeus* A-15 produced an antibiotic substance in liquid media (Chapter VI) and in sterile soil (Chapter XII). It exerted activity against soil microorganisms and *T. virida* T-10 in sterilized soils (Chapters X & XI). From all these findings it may be concluded that the antagonism developed here was due to secretion of an antibiotic substance, though the amount of control produced was not of the same intensity as was observed in the earlier experiments on agar media.

In conclusion, we can say that all the organisms tried
were active against the pathogen in some measure or the other, excepting C. trilaterale F-75 which was almost totally ineffective. This was rather an unexpected observation because this fungus had proved to be both antifungal and antibacterial in its activity, though the active principle involved was not very stable under the agar plate conditions. May be, the active principle secreted was readily degraded in soil in this case.

Out of the others, A. candidus F-81 and T. viride T-10 proved very effective against the pathogen. It is very interesting to note that here, there are no such reports about A. candidus against the plant pathogenic fungi. This fungus could not be tried in earlier experiments because it was discovered during the preliminary screening very late, when other experiments had progressed a long way. Its performance in this series of experiments has been extremely remarkable and further work on it is under contemplation. Regarding T. viride T-10 much need not be said, since strains of this fungus are well known to produce antibiotic substances and their activity has been reported against several other pathogenic fungi.

Out of the remaining 3 antagonists, S. annulatus A-71 gave best performance and needs further investigation. The other two, S. erythraeus A-15 and B. cereus var. mycoides B-44, were only moderately effective. It may be mentioned here that though all these three organisms were found to be effective in agar plates and also in liquid media experiments, only S. erythraeus A-15 was found to produce an active substance in soil which could be extracted out. It is now well-known that these substances
which are often produced in fair quantities on agar plates or liquid media are not often extracted from soil and, as has been stated earlier, this may be due to a variety of reasons such as non-production, degradation of the substance due to biochemical activities in the soil or their adsorption on the soil colloids in a irreversible way.
SECTION - E

INDUCTION OF MUTATIONS IN SELECTED SPECIES