SECTION - IV

BIOLOGICAL INTERACTION BETWEEN
FOOT-ROT PATHOGENS AND SOIL ANTAGONISTS
INTRODUCTION

In recent decades the phenomenon of antagonism between microorganisms has received much attention. Many studies have been made in Petri-dishes and cultures in tumblers and pots. With the discovery of gliotoxin (Weindling and Emerson, 1936) from Trichoderma viridae, the interest in antibiotics produced by soil fungi has received added impetus and after that several other antibiotics like viridin from T. viridae (Brian and McCown, 1945) and griseofulvin from Penicillium nigricans (Brian, 1949) have been discovered. By now there is ample evidence to the fact that a number of interactions take place among the microbial populations in the soil, which are of extremely complex nature because of the large number of organisms which inhabit the soil and which differ much in physiological characteristics. Obviously these interactions have to do much in connection with the root diseases which are located in soil. A root disease fungus has to face this microbial environment which may act for or against it. Among the antagonistic organisms T. viridae has been found to be specially active by a number of workers (Johnston and Greaney, 1942; Anwar, 1949; Bliss, 1951 and Rishbeth, 1951/52) and so it has received a lot of attention. It should be mentioned here that observations in Petri-dishes or in sterilized soils are not comparable to what may be
happening in the unsterilized soil environment because of the high complexities prevailing there. Secondly, the observations recorded in connection with one case may not be applicable to others as has been seen by the conflicting observations by different workers; e.g., *T. viridae* was seen to have further deteriorating effect on the host in some cases (Stakman, 1923; Simmonds and Ledingham, 1937; Bruehl, 1952). Finally the same antagonist may have several strains with different properties and behaving differently with the pathogen so that the results are not comparable unless the same strains of host and parasites are used.

As was noted in the last section, *T. viridae* was found to occur regularly in the rhizoplane of wheat variety Ry 65. It had some deleterious effect on the pathogenicity but on the whole failed to control the disease.

In the present section interactions between the footrot pathogens and strains of *T. viridae* were studied in agar cultures as well as in sterilized and unsterilized soil. Some experiments were also performed with other antagonists namely *P. nigricans* and a bacterial species. The last two organisms were known to exercise antibiosis against soil saprophytes (Lily, 1961). The wheat variety used in all the experiments was Ry 65, which has been referred in earlier tables.
EXPERIMENTAL

Methods: In these experiments six strains of T. viridae, one bacterial species and one strain of P. nigricans were used. These organisms had the following sources.

Trichoderma 1. from rhizosphere of wheat.
Trichoderma 2. from rhizoplane of wheat.
Trichoderma 3. from betel vine orchard (Dr. Mehrotra's isolate).
Trichoderma 4. Cambridge isolate (from Katering loam, culture brought by Dr. Saksena).
Trichoderma 5. from stored wheat grains (Mr. Thammayya's isolate).
Trichoderma 6. from grassland soil (Dr. Lily's isolate).

In general characteristics, the strains 1, 2 and 3 resembled each other. These were lighter in shade producing bigger spore balls and did not produce any coloration in the medium. The strains 4, 5 and 6 formed another group which were darker in shade with smaller spore balls and produced yellowish brown coloration in the medium. This coloration was most prominent in isolate no. 6. As will be seen later these two groups of strains behave differently with respect to their interactions against test organisms.

An isolate of P. nigricans from wheat fields was used in these experiments. This fungus was found to be antagonistic
against soil saprophytes in alkaline conditions (Lily, 1961). A bacterial species which was found to be active against soil saprophytes was used to study the reaction against pathogens and *T. viride*. Experimental details of the methods used are given below at appropriate places.

Isolates of *Trichoderma* and their characteristics:

**Experiment No. 1** - This experiment was devised to note the interaction between different strains of *T. viride* and bacterium. The object was to note if different strains of *T. viride* have different reactions.

**Method:** PDA dishes were used in this experiment. 4 mm. discs of young *Trichoderma* colonies and a needle touch from the bacterial colony served as inocula which were put 5 cm. apart in the dishes. The dishes were incubated at 25°C and were observed daily.

**Observations:** Final observations recorded after about a week were as follows. Strains 1, 2 and 3 behaved more or less similarly. The fungus grew rapidly and surrounded the bacterial colony from both the sides, meeting again on the distal side. A little later it grew over it and covered it up completely. No inhibition zone was observed at all. Strains 4, 5 and 6 behaved as another type in which clear zones of inhibition were produced around the bacterial colony. Later, a slight invasion
of the inhibition zone by the fungus could be observed. Strain number 5 produced a rather smaller inhibition zone so that it could be referred to have an intermediate behaviour between the two types.

**Interaction between Trichoderma and the foot-rot pathogens on the agar plate:**

**Experiment No. 2** - In this experiment the 6 strains of *T. viride* were tested in dishes against the five foot-rot pathogens viz. *G. rothsii*, *B. bicolor*, *Rhizoctonia* sp., *C. graminis* and *C. geiculata*. The method adopted was the same as in the first experiment.

**Observations:** These observations were similar in the case of *B. bicolor*, *C. graminis* and *C. geiculata*. In case of these pathogens, the strains 1, 2 and 3 of *Trichoderma* surrounded the test fungus from the two sides and later grew upon it. The colony of the test fungus did not grow much in size and was suppressed. Direct parasitism by *Trichoderma* could be noted in case of *B. bicolor*. Strains 4, 5 and 6 produced definite zone of inhibition. Later some hyphae of *T. viride* were seen to grow towards the test fungus through the inhibition zone. The colonies of the test fungi did not grow much.

Colonies of *Rhizoctonia* sp. in case of strains 1, 2 and 3 were overrun by the growing colonies of the antagonists, as a result of which the test fungus got suppressed. In case of
strains 4, 5 and 6 both the colonies grew towards each other almost at the same rate so that in the middle of Petri dish a yellowish line was produced at the junction of the two species (Pl. XI-A). Some hyphae of the antagonist spread over the test fungus but the latter was not suppressed. Some sclerotia could be seen after about 10 days. These were tested after 31 days and were found to be viable. It is clear that in this particular case strains 1, 2 and 3 proved better antagonists than the strains 4, 5 and 6.

In *S. rolfsii*, in case of strains 1, 2, 3 and 5 the test fungus and the antagonist grew towards each other and formed a yellowish line of junction in the middle of the Petri-dish. After this *I. virida* was seen to overgrow the test fungus and was also seen to cause direct parasitism. The test fungus was suppressed in course of time. Interaction with strains 4 and 6 was rather peculiar. After meeting at the centre, the colony of *S. rolfsii* was seen to overrun that of the antagonist and grew over it forming strands. After a week's time it produced typical sclerotia. In about 10 days a rather rapid lysis of the hyphae and sclerotia of the test fungus started and by the 14th day no living hyphae or sclerotia were left. It is obvious that the metabolites secreted by these strains of *I. virida* acted after accumulation and were responsible for the process. It may be mentioned that such observations could never be made by the author when *S. rolfsii* was grown alone.
Since these observations were found to be rather peculiar and abnormal a further test was made by devising another experiment with the help of cellophane paper. Cellophane paper, after due treatments (after boiling for 10 minutes in water and then sterilizing in autoclave) was placed in a dish of plated agar. A small disc of strain 6 of *T. viridae* was placed at the centre of the paper and allowed to grow for 4, 8 and 15 days in three dishes. The experiment was run in duplicate. The paper along with the *Trichoderma* was then lifted away from the dish and a small disc of freshly growing *S. rolfsii* was placed at the centre of the dish. *S. rolfsii* grew to a small extent for 3 or 4 days after which the lysis started and the colony dissolved away. All the three time treatments (4, 8 and 15 days) were almost equally effective and produced the same results.

**Interactions between the various antagonistic organisms and the foot-rot pathogens with respect to host:**

**Experiment No. 3** - This experiment was devised to know the effect of the pathogens on wheat plants when the roots of the latter were heavily impregnated with *T. viridae*. Earlier work in the laboratory had shown (Lily, 1961) that the population of *Trichoderma* boosted up in the soil which is acidulated with sulphuric acid.

**Method:** Pots of sterilized soil were acidulated with 15 ml. of normal sulphuric acid per 200 gm. of soil and inoculum of *Trichoderma* was given to it. Soaked and surface sterilized
seeds were sown in these pots and when the seedlings were five days old, they were transplanted to other pots with unsterilized soil to which 10% sand-oat meal culture of the three pathogens, *E. bicolor*, *Rhizoctonia* sp. and *S. rolfsii* had been added. Records for disease were taken after three weeks. The causal fungi were searched, in washed roots after and before surface sterilization and by root dissections.
Twenty such root pieces were examined in each case.

**Observations:** Plants from *S. rolfsii* infested pots were severely damaged, 30% of them being dead. Plants from *Rhizoctonia* sp. pots showed lesions at the coleoptile and base but did not die. In case of *E. bicolor*, severe root lesions and stunting of plants were noted. The results of root platings are given in the following table.

Table No. XIII.

Fungi isolated from 20 pieces of surface washed roots, expressed as percentage occurrence.

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<th>Fungi appeared</th>
<th>% occurrence in root pieces</th>
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<tr>
<td></td>
<td><em>E. bicolor</em></td>
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<td></td>
<td>Seminal roots</td>
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<tr>
<td>Trichoderma</td>
<td>100</td>
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<tr>
<td>Fusarium sp.</td>
<td>60</td>
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<tr>
<td>Curvularia sp.</td>
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<tr>
<td>Sterile black</td>
<td>5</td>
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<tr>
<td>Rhizopus sp.</td>
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</table>
It is remarkable to find that no pathogen could come out of any root piece while *Trichoderma* was collected from all pieces. Even when affected root pieces were surface sterilized with mercuric chloride, washed and plated, only *Trichoderma* and *Fusarium* were found to come out. However, on dissecting the root pieces some typical *R. bicolor* and *Rhizoctonia* sp. hyphae could be seen, but even these could not develop in agar due to the preponderance of *T. viride*. It is clear that *Trichoderma* is unable to suppress the disease and the pathogens are able to enter the roots in spite of its preponderance on root surface. Another indication is that the necrotic portions provide a ready substrate for *Trichoderma* which grows and obstructs the appearance of the pathogens in the dish.

**Experiment No. 4** - This experiment was devised to confirm further the results obtained in the last experiment with slight modifications and with *Q. graminis*, as an added pathogen. In this the inoculation of *T. viride* was provided by soaking the surface sterilized seeds in a heavy spore suspension. These seeds were then sown in duplicate series/pots of unsterilized soil of which one set was acidified with sulphuric acid as described earlier. Unsterilized soil was used because the sterilized soil was thought to result in very abnormal conditions. Two sets of pots in each series were incorporated with pathogen in the form of sand-oat meal cultures. In one set which acted as control, normal surface sterilized seeds were sown and in the other set the *Trichoderma* incorporated seeds
were sown for comparison. There were two more pots in each series. One was without any treatment and the other had only Trichoderma soaked seeds without any pathogen. Ten seeds per pot were sown. The disease ratings were scored after 15 days according to the method described on page 32. The results are tabulated below.

Table No. XIV.

Showing the infection rating of different sets in interaction between pathogens and Trichoderma.

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<th>Setting type</th>
<th>Infection rating (%)</th>
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<td>Control</td>
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<td>Unacidified series</td>
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<td>Controls</td>
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<td>Trichoderma and pathogen</td>
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<td>Acidified series</td>
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<td>Controls</td>
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<td>Trichoderma and pathogen</td>
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A perusal of the table shows that in the unacidified series there is no substantial difference in any case by the incorporation of Trichoderma, but on the other hand in one case (S. rolfsii) there is a marked increase in disease incidence. In case of G. graminis and R. solani there is a slight increase while in B. bicolor there is a slight depression. When the
soil is acidified (when it is known that the population of *Trichoderma* in soil gets boosted up) there is a marked depression of disease in case of *S. rolfsii* and *R. solani*. In the other two cases there is no change. It may be noted that *R. solani* and *S. rolfsii* are those pathogens which kill the host at a very early age. On the whole the results are in corroboration with that of the last experiment proving that *Trichoderma* is not able to suppress the pathogen especially in normal soils.

Surface flora from the roots of all the types of plants was determined by plating method. From the controls (without *Trichoderma*) all the pathogens were readily isolated (except for *S. rolfsii*). In case where *Trichoderma* was incorporated, in acidulated soils, pathogens could be isolated from the pieces during early stages of infection, with increase in duration, the quantity of pathogens coming out rapidly diminished so that only *Trichoderma* was collected during later stages. In case of unacidulated soils, though the sequence remained the same it took a longer time for the *Trichoderma* to dominate and pathogens could be collected till a later stage. In case of *O. graminis* runner hyphae could be seen on the surface of such partially damaged roots. This further confirms the view that *T. viridae* multiplies profusely on the necrotic portions of the roots caused by the pathogen and finally overwhets the latter. This is particularly marked in acidulated soil. This phenomenon of *Trichoderma*
was particularly noted with *S. rolfsii* and to a certain extent with *B. solani* even in acidulated soils. In earlier experiment (p. 68) it was demonstrated in connection with rhizosphere studies that these two fungi tend to promote the activity of *T. viride* around the roots. This is thought to be due to the fact that these fungi are able to decrease the pH of the surrounding soil, thus promoting the activity of *Trichoderma*. These have been the observations of Abeygunawardena and Wood (1967) also in Petri-dish cultures of *S. rolfsii*. By the following test the present writer further confirmed it. *S. rolfsii* was inoculated on wheat seedlings and allowed to develop in test tube in Knop's solution. The solution became more and more acidic and the pH fell from 7.7 to 5.4. In case of *B. solani* it fell to 6.9.

**Experiment No. 5** - In this experiment an effort was made to study the interactions of such antagonists as *T. viride* and *P. nigricans* with foot-rot pathogens on root surface by direct observations.

**Method** Surface sterilized seeds were grown in Petri-dishes with blotting papers to act as moist chambers and duly sterilized. On the germinating seeds, 2-3 drops of heavy spore suspensions of the two antagonists, *T. viride* and *P. nigricans*, were added, and in one case a pinch of normal soil was placed to provide normal flora. Small agar discs of two pathogens *P. bicololor* and *S. rolfsii* were then placed
in contact with the young roots. Observations were recorded from time to time.

Observations: In case of *R. bicolor* brown necrosis was produced on the roots while in case of *S. rolfsii* rapid rotting was produced on the roots and basal portion and the seedlings succumbed to the attack. The observations were concordant in all the three cases of *T. virida*, *P. nigricans* and soil.

It is evident from the observations that *T. virida* and *P. nigricans*, under the conditions of this experiment failed to check the growth of the pathogens under investigation.

Experiment No. 6 - In the past experiments a deleterious effect was sometimes noted on the health of the plants when *T. virida* was added as an inoculum against pathogens. As has been seen, it was often difficult to judge the exact role of *T. virida* except that it failed to produce the desired antagonism. In the present experiment *T. virida* was used alone to act as a 'pathogen' in a culture tube experiment. All the six isolates of *T. virida* were used.

Method: Seedlings were grown by the usual procedure in culture tubes and small agar discs of *T. virida* were placed in contact with young germ tubes. The experiment was run with control which received no fungal inoculum. Observations were recorded from time to time.
Observations: In all the cases a deleterious effect on the health of the plants was noted. A fair amount of browning and rotting of the roots was induced. This was particularly noticeable in strain no. 6.

It is evident from the above that Trichoderma itself can exert a deleterious influence on wheat plants and this point must be kept in mind while judging its role against the root pathogens. Similar observations about the effect of Trichoderma on wheat were earlier recorded by Simmonds and Ledingham (1937).

Experiment No. 7 - Besides T. viride, P. nigricans and the bacterium referred earlier were also found to be active against soil saprophytes in alkaline soils (Lily, 1961). It was, therefore, thought desirable to know their interaction on the lines adopted for T. viride with the root disease fungi of wheat.

Method: In this experiment normal field soil was used. The antagonists were incorporated by dipping the surface sterilized and germinated seeds in concentrated spore suspension. Inoculum of pathogens was given in the form of sand-oat meal culture as done in earlier experiments. Plants were scored for infection rating after 15 days. The root surface flora of twenty root pieces was recorded as per usual method. The results are presented in the following table.
Table No. XV.

Showing the percentage appearance of rhizoplane microflora and the infection ratings.

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</table>

Infection rating 0 25 62.5 70 72.5 62.5 67.5 62.5 100 100 100 5 7.5 10
It will be seen from the table that there was no checking of disease in any case as is clear from the infection ratings. *P. nigricans* was found to enter into the rhizoplane of wheat and was isolated as such, but exerted no untoward reaction against any of the disease fungi. No rhizoplane flora was recorded in case of *R. solani* which totally killed the young germlings and *Fusarium* produced no disease. In the control of *P. nigricans* alone, no disease effect was noted. In case of bacterium the infection rating was twenty five because *R. solani* (from the soil) attacked some of the plants.
SUMMARY AND DISCUSSION

In this section the biological interactions between the foot-rot pathogens and some important soil antagonists have been studied. As is well known, T. viride acts as an antagonist against some of the root disease fungi. Earlier studies in this laboratory showed P. nigricans and the bacterium sp., to be antagonistic to other soil saprophytes (Lily, 1961). It was, therefore, thought worthwhile to investigate the reaction between these microorganisms and the foot-rot pathogens. Six different strains of T. viride available in the department were derived from different sources. At first, as a preliminary measure, it was thought desirable to know their differential reaction against a common test organism. The strains were tested against the bacterium and the reactions were noted. The first three strains produced similar reaction in which no inhibition zone was formed and the fungal colonies could grow upon the bacterial colonies overrunning them. The other three strains formed another group in which a definite inhibition zone was produced with a few hyphae traversing further. Strain no. 6 was specially pronounced in this respect, while no. 5 was the weakest of the three.

In the next experiment the six strains of T. viride were tried in dishes against the five important foot-rot pathogens viz. G. rolfsii, B. bicolor, Rhizoctonia sp., O. graminis
and C. geniculata. The reactions were similar in the case of
R. bicolor, O. graminis and C. geniculata. The colonies of
the test fungi in case of strains 1, 2 and 3 of Trichoderma
were completely overrun. Direct parasitism by Trichoderma
could be noted in case of R. bicolor. The strains 4, 5 and
6 induced a definite zone of inhibition. In Rhizoctonia sp.
the colonies were overrun by strains 1, 2 and 3, but in case
of strains 4, 5 and 6 they grew towards each other meeting at
a demarcated line. The behaviour of S. rolfssii was rather
peculiar. The strains 1, 2 and 3 behaved in the usual way
but in case of strains 4 and 6, S. rolfssii was seen to overrun
the colony of the antagonist forming stands and sclerotia.
Later there was a rapid lysis of the entire colony of S. rolfssii
including the sclerotia. The behaviour of S. rolfssii is
peculiar. It appears that because of the rapid growth rate
this fungus at first grows rapidly against Trichoderma. The
antibiotics produced by the latter seem to have little influence
on S. rolfssii in the initial stages. These antibiotics,
however, get accumulated in course of time and then produce
lysis of the test fungus. This was further corroborated by
another experiment in which Trichoderma was grown on a cello-
phane paper in a dish and the effect of diffused antibiotics
was noted by growing S. rolfssii in the dish.

In the next experiment the effect of pathogens on wheat
plants was noted when the roots of the latter were heavily
impregnated with T. viride by growing seedlings in acidulated
and infested soil. The seedlings were then transferred to pots which were inoculated with pathogens. Three pathogens, *R. bicolor*, *Rhizoctonia* sp. and *S. rolfsii*, were tried in this way. The results showed that *T. viride* was not able to suppress the disease, though it was difficult to isolate the pathogens from the roots due to preponderance of *T. viride*. In another experiment the same results were confirmed. In this the inoculation of *T. viride* was provided by putting the soaked and surface sterilized seeds in concentrated spore suspension of *Trichoderma* for a couple of hours. These seeds were sown in the pathogen infested pots. Two series of pots were used, one acidulated with sulphuric acid and the other without it. It was known by earlier experimentation that such acidulation is conducive to the development of *T. viride*. Four pathogens viz. *C. framinis*, *R. solani*, *B. bicolor* and *S. rolfsii* were tried in this way. Some depression of the disease was noted in the acidulated pots in case of *S. rolfsii* and *R. solani*. In other cases no substantial change could be recorded. Reisolation of the pathogens from roots was possible in all the cases during earlier stages excepting in those of *S. rolfsii* and *R. solani* where the seedlings were totally killed at an early stage but were overwhelmed by a very profuse growth of *T. viride*. These two fungi specially *S. rolfsii* were found to lower the pH of the medium during its growth period which is favourable for the growth and development of *Trichoderma*. 
In still another experiment an effort was made to study the interaction between the antagonists (\textit{T. viride} and \textit{P. nigricans}) and the foot-rot pathogens. In this case surface sterilized seeds were grown in Petri-dishes on blotting papers forming moist chambers. On the germinating seeds drops of spore suspensions of the antagonists were put and then agar discs of pathogens were placed in contact with roots. Here again, the results were negative and disease symptoms were produced.

During all these experiments though variations in observations were obtained, it was more or less established that \textit{T. viride} is unable to check the foot-rot pathogens on the root surface. Since it was also suggested that in some cases (\textit{S. rolfsii}), \textit{T. viride} increases the disease, it was thought desirable to know the effect of \textit{T. viride} alone on wheat seedlings. In view of this, all the strains of \textit{T. viride} were tried as 'pathogens' in culture tube experiments. It was found that in almost all cases a deleterious effect on the health of the seedling by way of rotting of roots was induced. It was specially pronounced in case of strain no. 6. These observations are in accord with those of Simmonds and Ledingham (1937).

The other two antagonists (\textit{P. nigricans} and species of \textit{bacterium}) were also tested against the foot-rot pathogens. Trials were made by growing germinated seeds of wheat which were soaked in suspensions of antagonists, in pots to which
pathogens were added. The results were negative and no check in the development of the disease could be noted.

Concluding from all these series of experiments it can be said that *T. viride* which is a noted antagonistic against some root disease fungi is not active against the foot-rot pathogens of wheat.