THE PARASITISM OF ROSELLINIA ARCUATA.

By

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Rosellinia arcuata, though by no means the most common of tea root disease fungi, is probably the best known by name to tea planters in Ceylon. In a short account of his first experience of this fungus, Petch has described (*Tea Quarterly*, Vol. 1, p. 14) how the individual plants of a Panax hedge died in regular succession, as the fungus reached and enveloped their stems at ground level.

Its action in tea is similar, except that instead of travelling along a straight line, it usually spreads outwards along the radii of a circle, and kills the tea bushes in its path. Thus, unless the fungus is checked, an area attacked by Rosellinia becomes larger annually. The dead and dying bushes—the latter are on the perimeter of the area—bear a copious black fungus growth which is the vegetative body of the fungus *Rosellinia arcuata*.

Moreover, it is not weakly bushes only which die when attacked by this fungus; vigorous, healthy bushes also succumb. The finest tea bushes are as susceptible to attack by Rosellinia as the weaklings. Even the magnificent specimens sometimes spoken of as "show" bushes are not immune.

The rate of progress of the fungus and the speed at which the fungus will kill a tea bush are doubtlessly influenced by external environmental conditions. The constant association of a specific fungus with a definite diseased condition, its presence on dead and dying bushes, and the fact that death follows the invasion of the fungus, no matter how vigorous the bush is before the arrival of the fungus, can mean only one thing, namely, that the fungus is the primary and sole cause of the death of the bush.

The accuracy of this conclusion has recently been questioned because experimental proof has not been produced in support, and another theory has been advanced to explain the observed facts. Whatever may be said in its favour, however, the new theory suffers from a similar disadvantage, viz., it is unsupported by experimental proofs, though many attempts have been made to obtain them.

What constitutes proof of the parasitism of any specific organism ? The rules of proof were formulated by Koch, and have been appropriately termed the Canons of Koch. They may be expressed as follows (Duggar, *Fungus Diseases of Plants*, pp. 76-77) :—

(a) Under diverse conditions the fungus must be constantly and abundantly associated with the disease, or pathological state.

(b) The organism should be grown in pure cultures, when possible, and its differential characteristics well studied.

(c) The characteristic disease should be produced by infection experiments with a pure culture.

(d) The fungus associated with the disease induced should be identified as the one originally separated, and any abnormalities of host should likewise correspond.

It will be noted that rule (a) constitutes what may be termed the "field proof." Section (b) constitutes laboratory work preliminary to experiments which will furnish the requirements of sections (c) and (d). These latter constitute the "experimental proof."

As regards *Rosellinia arcuata*, the requirements of rule (a) are amply satisfied. Every dead and dying tea bush from a Rosellinia patch bears in abundance the characteristic black mycelium of the fungus *Rosellinia arcuata*. The field evidence is so strong that the mycologists of the tea stations of Java and India as well as of Ceylon, have accepted it as sufficient proof of the parasitism of the fungus. " It is true that the majority of fungi described as the causes of plant disease have not undergone experimental tests, although it will be admitted by most experienced pathologists that a large proportion of the claims are just beyond all question " (Duggar, *loc. cit.*). The field proof of the parasitism of *Rosellinia arcuata* is so clear that experimental proof in this connection has been said to be " purely academic." It is, however, the purpose of the writer to supply the " academic " interest.

Until last year *Rosellinia arcuata* was known in Ceylon as a disease of adult tea only. It had not previously been recorded as a disease of tea seedlings. In February, 1927, the disease occurred in an estate nursery. The affected plants suddenly wilted and died; the collar region and some of the roots were enveloped by a black fungus having the characters of the species *Rosellinia arcuata*.

EXPERIMENT I.

Specially selected fragments from the collar regions of seedlings naturally infected by *Rosellinia arcuata* were placed around the collar of a healthy tea seedling, growing in a pot in the laboratory verandah. The seedling was one of twelve which were well-grown basket plants four months earlier, when each was transplanted into a 12 inch pot. The plants had made good growth since transplanting, and were healthy and vigorous. As the infected material had been lying in the laboratory for a few days and had dried out somewhat, the pot in which the material was placed was covered by a bell jar for 4 days to encourage the growth of the fungus. At the end of that period it was seen that the fungus had grown out of the diseased material and had reached the collar of the seedling, so the bell jar was removed.

Two months later, this plant began to wilt; the leaves drooped and dried out. On examination, it was found that the collar region at soil level for a length of about one inch was enveloped by a black fungus which proved to be *Rosellinia arcuata*. The tissues below this fungus were dead, but all the roots were healthy.

The eleven control plants remained healthy.

EXPERIMENT II.

For this experiment a pure culture of *Rosellinia arcuata* was used. The fungus was isolated from the roots of a mature, naturally infected bush. The fungus was transferred from pure culture to sterilised blocks of tea wood about half an inch cube, and was grown on this medium for one month in sterile closed tubes. Three of these blocks were then buried just below the surface of the soil and around the collar of one the seedlings which had been used as a control in the previous experiment. Care was taken not to injure the seedling during this operation.

After two months the leaves of this seedling drooped and dried out. As the wilted plant made no sign of recovery, it was carefully removed from the pot nine days later for examination. The black mycelium of *Rosellinia arcuata* was found in abundance around the collar for a distance of about 2 ins. along the tap root. The fungus was present on some of the upper roots, along which it was growing from the tap root outwards. The majority of the roots were healthy.

Three similar but uninfected plants were kept as controls. These remained healthy, and were still healthy and vigorous 6 months after the termination of the experiment.

EXPERIMENT III.

For the third experiment, five seedlings were raised together in a 12 inch pot. Three pieces of Rosellinia-infected wood were buried, as in Experiment 2, around the collar of the central seedling. Two months later this seedling died, and the photograph shown in Plate V. was taken. The plants were left undisturbed for 5 months. During this period a fungus grew out from the drainage hole at the bottom of the pot and formed a sheet on the wooden table on which the pot was standing. This fungus proved to be *Rosellinia arcuata*. When the pot was removed to a new position, the same fungus again formed a practically pure growth on the table below the pot.

When the plants were removed from the pot, five months after the central plant died, the plants surrounding the central dead seedling were found to be quite healthy. The roots of two of these, together with those of the diseased plant are shown in Plate VI.

Mycelium of *Rosellinia arcuata* was found along the entire length of the tap root of the infected seedling, and along some of its fine feeding roots. Many of the latter, however, were apparently free of the fungus, though dead. No difficulty was experienced in recovering and identifying the original fungus (Rosellinia), used for this and the previous experiments.

It is worthy of note that although the soil was thoroughly permeated with rootlets of the surrounding plants, none of these had become infected by Rosellinia. It would appear that the feeding roots of tea are immune to this fungus. Rosellinia will attack seedlings at the collar or tap root, if sufficiently grown, and will invade the woody roots of older tea, but not the fine feeding roots. This observation is perhaps of interest in that it confirms what has been suspected to be the case from field observations.

The above experiments furnish experimental proof of the parasitism of *Rosellinia arcuata* as prescribed by the canons of Koch. A diseased condition, similar to that observed in the field, has been induced in these experiments by means of naturally infected tissue and by pure culture of *Rosellinia arcuata*, and the same fungus has been found abundantly associated with the diseased condition so caused.

It has been stated elsewhere that the generally accepted view of the parasitism of *Rosellinia arcuata* (among other fungi) is unfounded; that *Rhizoctonia bataticola* is the only parasite of present importance in the causation of root disease; and that without a preliminary attack by the latter fungus, *Rosellinia arcuata* is incapable of causing disease. These statements were kept in view during the course of the above experiments.

Rhizoctonia bataticola is said to attack the smallest feeding roots, and after establishing itself there, it grows inwards and upwards, always getting nearer the larger roots, killing as it goes. Considerable emphasis has been laid on this mode of attack.

It is also stated that attempts to reproduce Rhizoctonia infection in Uganda and Ceylon have shown that the conditions of laboratory experiments do not conduce to rapid infection.

In the above experiments the control plants were in no way different from those used for infection with Rosellinia. As, in each experiment, the seedling infected with Rosellinia died two months after inoculation while the control plants remained healthy, there appears to be no valid reason why Rhizoctonia or any fungus other than Rosellinia should be suspected to have played a part in causing the deaths.

Incidentally, no experiment has yet been published to show that *Rhizoctonia bataticola* is capable of causing the death of a tea plant, nor yet of infecting healthy tea roots. Nor is there any method of

ROSELLINIA INFECTION EXPERIMENT.

The arrow indicates the infected plant.

distinguishing a normal healthy tea plant from an apparently healthy one, which has some of its roots parasitised by *Rhizoctonia bataticola*, except by a minute examination of the whole of its root system. An examination of this nature before infection experiments is out of the question, though it is easily carried out on completion.

That was done after each experiment. The soil was carefully washed away from the roots of each dead or healthy plant to be examined, so that the roots were damaged as little as possible and no roots were lost.

In experiment 1, the infected seedling was removed from its pot immediately after the plant had definitely wilted. All the fine feeding roots were found to be alive and apparently healthy. No trace of *Rhizoctonia bataticola* could be found on them. As already stated, a black mycelium occurred around the collar region, about one inch in length, below which the tissues were dead. In order to ascertain whether *Rhizoctonia bataticola* or any fungus other than Rosellinia was present, this region, together with a little apparently healthy tissue at each end, was carefully washed in sterile water and placed on a sterile culture medium. Several fungi other than Rosellinia were isolated, but although the diseased tissue and its surrounding medium were kept under observation for several months, no sign of *Rhizoctonia bataticola* was seen. Each fungus, isolated from this tissue, was tested for parasitism on tea seedlings, but all the seedlings inoculated remained healthy, even after six months. They must, therefore, be regarded as secondary fungi which played no active part in causing the death of the seedling. The failure to find any trace of *Rhizoctonia bataticola* after very thorough investigation definitely proved that this fungus was not concerned, either primarily or secondarily, in causing the diseased condition observed.

After experiment 2, a thorough examination of all the fine rootlets, tap root, and collar of the infected plant failed to reveal the presence of *Rhizoctonia bataticola*.

In experiment 3, the infected and control plants had grown in a soil common to all, in a single pot before the experiment started. The feeding roots formed a dense mat thoroughly permeating the soil, such that individual plants could not be separated until all the soil had been washed away. Consequently, there is no reason to believe that any one of these plants would be more liable than any other to a preliminary attack by *Rhizoctonia bataticola*. Alternatively, if the roots of one seedling had been attacked by *Rhizoctonia bataticola*, the same fungus would be found on the interlacing rootlets of the other plants.

The condition of the rootlets of the plants adjacent to the Rosellinia infected seedling can be roughly judged from the photograph reproduced on Plate VI. All the roots were perfectly healthy,

and very careful examination failed to reveal the presence of *Rhizoctonia bataticola*. Neither the tap root nor the few remaining rootlets of the Rosellinia infected plant bore any symptom of Rhizoctonia attack, nor could that fungus be found.

The failure to find any symptom or trace of the fungus *Rhizoctonia bataticola* on any of the plants killed during the course of these experiments, and on the roots of the other tea seedlings grown with them (experiment 3) indicates clearly that this fungus had played no part whatever in causing the diseased condition. It also demonstrates that the claims made concerning this fungus, in its relationship to what is commonly termed the Rosellinia disease of tea, are unfounded.

The experiments prove conclusively that *Rosellinia arcuata* is a virulent parasite of the tea plant. It will attack and kill tea seedlings without the assistance of a preliminary attack by any other fungus, and in the absence of environmental conditions unfavourable to the tea plant. That Rosellinia will not attack or feed upon fine feeding roots of tea is shown in experiment 3. Its attack is always directed upon the woody parts of the plant; with tea seedlings, the collar, at or just below the soil level, is a suitable place for attack.

In short, these experiments confirm what has been known or suspected for many years from field evidence alone, concerning the parasitism of *Rosellinia arcuata*.