

the student needs a well-equipped laboratory for his investigations. In 1909 Professor A. H. R. Buller published his "Researches on Fungi," which is an invaluable work embodying the results of original research on the physiology of our fungi and it is to be hoped that he will favour us with another volume on the same subject.

I have now passed in brief review the most important features of our mycological progress that has been made since the foundation of our Society. I think that we can congratulate ourselves on the fact that, although many of our junior members were called away during the war, we managed to carry on, to hold our autumn forays and to publish our Transactions as usual. We have emerged from that trying time stronger in number than we ever were before and I feel confident all our members will continue to advance our knowledge and uphold our motto "Recognosce notum, ignotum inspicie."

THE PARASITISM OF NECTRIA CINNABARINA (CORAL SPOT), WITH SPECIAL REFERENCE TO ITS ACTION ON RED CURRANT.

With Plate I.

By J. Line, M.A.

GENERAL.

This subject has been investigated by a number of workers, but although the very familiar fungus is regarded in this country as a frequent parasite on many broad-leaved trees, no critical investigation appears to have been made, nor has it been considered as the cause of any serious damage.

Mayr⁽¹⁾ demonstrated by actual infection experiments as long ago as 1883 that the fungus could become parasitic on maple: although he did not use pure cultures of the fungus there seems no reason to doubt the general accuracy of his results.

He pointed out that the normal method of infection was through a wound, and that the fungus could not penetrate the living phloem and cortex. He also showed that the fungus when established in a side branch could readily pass over into the healthy wood of the main stem, and that the pathological effect was primarily due to the blocking of the vessels by the hyphae, causing death of all parts above the point affected. The stem so killed was then easily and rapidly invaded by the fungus, although the first blocking process might take two years or considerably more.

It is almost impossible to reconcile these results with those of Wehmer⁽²⁾ who from observations on the lime and hornbeam in 1894 concluded that the fungus became parasitic, but that it was strictly confined to the cortical regions, never entering the woody tissues. He considered that Mayr was completely mistaken in his observations or that he was working with another fungus.

Durand⁽³⁾ in 1897 described the appearance of the fungus on red currant in the U.S.A., his descriptions of the disease tallying exactly with the symptoms observed during 1919 and 1920 in this country. He considered that it was acting as a parasite, but although he performed infection experiments with pure cultures of the fungus, he does not record any of his results. It is well known that coral spot often occurs on dead branches of otherwise healthy red and black currant bushes in this country. It was pointed out to the writer in 1919, by Mr F. T. Brooks, that it had never been shown how much of the die-back observed was due to the primary action of the *Nectria*, and how much to the action of one or other of the fungi often associated with it.

An investigation was therefore started in order to settle if possible

- (1) to what extent the fungus may be regarded as a parasite, particularly on the red currant;
- (2) its normal method of infection and method of growth in the host tissues;
- (3) whether any differences in power of infection could be detected between different strains of the fungus.

FIELD OBSERVATIONS.

A number of orchards were kept under fairly close observation during the years 1919 to 1921, and a very large number of the bushes were found bearing the stromata of *Nectria* on one or more of their branches. Other commonly occurring fungi were *Collybia velutipes* and *Fomes Ribis*. A few bushes were found bearing *Stereum purpureum* and *Botrytis*. No experimental work was done with these fungi, but a short summary of observations made on them will be found elsewhere.

During this preliminary examination of the bushes, single branches on apparently healthy bushes were often found showing signs of wilting of the leaves. In some cases this was observed just after the leaves had expanded, but others did not become wilted until about flowering time. Later on branches bearing nearly ripe and quite normal fruit were found suddenly to become wilted (Figs. 1 and 2). Such branches when left on the bush were observed to lose all their leaves during the summer,

and to become completely dried up: in all about fifty branches were kept under close observation from the time wilting was first observed, and in every case the branch which had lost its leaves became covered with the pink stromata of *Nectria* during the following winter.

A number of similar branches were removed from the bushes as soon as the wilting was observed. When cut open it was found that a brownish green region was present in the wood at the base, extending almost across the branch, leaving a small area of white and healthy wood, with a corresponding area of healthy cortex external to it.

This discoloured portion could always be traced either to a wound in the branch itself or more commonly to a dead side branch; in many cases these dead portions already bore stromata of *Nectria*.

The discoloured portions of wood and cortex were found to be full of fungus mycelium extending up to the edge of the white and healthy wood, the vessels and tracheids being choked up in the darker parts with hyphae and gummy material. This characteristic discoloration of the wood was observed by Mayr in the wood of Norway maple, and he considered the gummy liquid represented the product of solution of part of the cell wall by the fungus. Fig. 3 shows the hyphae in the wood cells taken from the edge of the discoloured zone; it is seen that in the early stages of invasion the fungus appears incapable of invading the living ray cells; later on these also become filled with hyphae. It was never found that the fungus advanced in the cortex before the wood was blocked up, in fact there seemed no evidence that it could effect an entrance into any living cell in a normal healthy condition.

EXPERIMENTAL WORK.

A. *In the laboratory.*

Twelve typical branches were selected with discoloured portions attached to them, but no visible stromata or signs of the presence of any fungus upon them; after thorough scrubbing they were placed under sterile conditions in jars of water under bell jars. In six weeks every branch began to show the pink stromata of *Nectria* breaking through the bark, commencing round the discoloured regions. (Control branches developed a little *Cladosporium* under these conditions.)

Portions of the wood from different parts of the discoloured regions were removed from another batch of branches under sterile conditions and were transferred to Petri dishes of sterile agar jelly.



FIG. 1.



FIG. 2.

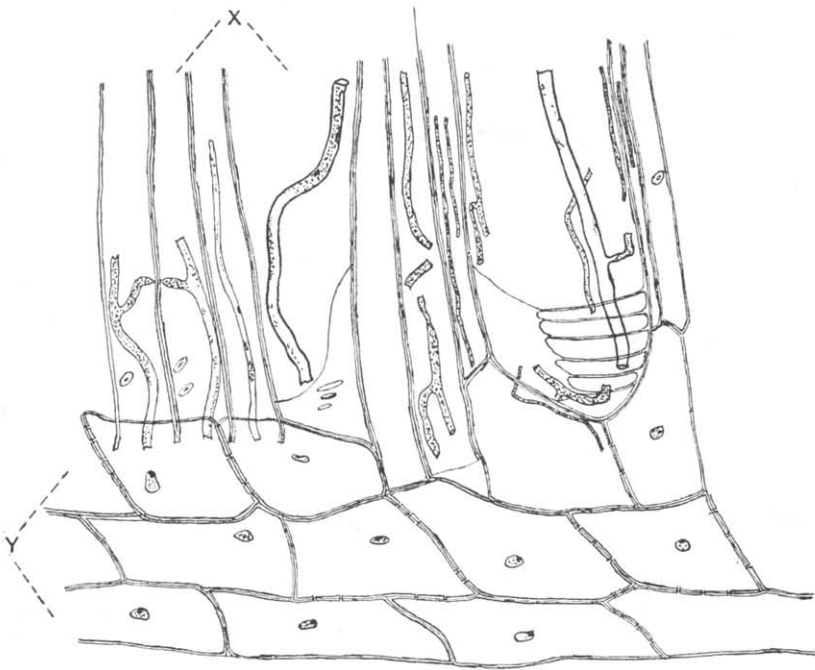


FIG. 3.

FIG. 1. First signs of wilting of twig of red currant.

FIG. 2. Later stage of wilting of twig of red currant. Note the nearly ripe fruit on this branch.

FIG. 3. Long, radial section of wood of red currant. Mycelium of *Nectria cinnabarina* invading the healthy wood. Note absence of hyphae from the ray cells. $\times 350$ approx.

X = xylem elements.

Y = ray cells.

From a number of these dishes nothing developed, but in all cases where the piece of wood had been taken from the edge of the dark zone a vigorous fungal mycelium grew out from the chip into the jelly. Portions of this mycelium could be readily removed to other media, and in all such cases the mycelium proved to be that of *Nectria*.

From these observations and experiments it seemed probable that the *Nectria* was able to cause the wilting and death of branches on the currant when once established in the bush. It is worth recording that in no single case did *Botrytis* develop from the cultures made from the branches, and it was observed comparatively rarely in the orchards. *Collybia velutipes* was extremely common in one orchard on older bushes, and was then frequently associated with *Nectria*; it was rarely found on younger bushes and did not develop on any of the bushes from which wilted branches were removed. About a dozen bushes in all were found bearing fructifications of *Fomes Ribis*; these were in every case sickly looking plants stated to be at least forty years old.

B. *Infection Experiments in the field.*

The common occurrence of the fungus on so heavily pruned a plant as the red currant, as well as its behaviour on other plants, would lead one to suppose that its normal method of entering the host was through a wound; in view of some recent work on the method of infection of the apple canker fungus it was decided to test the power of the coral spot fungus to penetrate uninjured branches, through either the leaf-scars, lenticels or bud scales.

It was thought possible that different strains of the fungus might be found which would show different powers of infection. A series of cultures of the fungus was started with the usual precautions as to freedom from other fungi, bacteria, etc. No difficulty was experienced after a time in making single spore cultures from both conidia and ascospores, and these were obtained from a number of different sources. Cultures were set up on many different media under a variety of conditions in the hope that the formation of perithecia might be induced. So far none have been obtained, although nodules exactly resembling them in appearance and to some extent in structure are obtained in old cultures. These cultures are being carried on with other media at the present time, in the hope that the success of Miss Cayley with *Nectria galligena* may be repeated.

This fungus shares with *Nectria cinnabarina* the character that in many gelatinous media the mycelium buds off spores laterally in great profusion.

Infections were made on red currant (200), fig (20), lime and horse-chestnut (20) in a number of different ways and at different times of the year. Wounds made were of course protected from chance contamination from outside sources.

The cortex and wood were inoculated at varying depths; mycelium and spores were placed on leaf-scars of different ages and between the scales of resting buds. In most cases the mycelium from wood block culture was used, but conidia and mycelium from agar cultures, ascospores and conidia from natural sources were also used.

It was found in the case of the red currant that the fungus made very little progress indeed in the cortex and phloem, or in the wood of a healthy branch. When severely wounded by a deep incision some headway was made, but in two cases only did the fungus establish itself and produce fructifications on the branch.

As a rule a small discoloured area was formed round the point of inoculation and this did not increase. Callus formation rapidly covered the wound with healthy tissue, and by the following year the only trace of the inoculation was a small dark area in the wood.

In the case of the horse-chestnut and lime several branches were found in which the fungus made rapid headway, fructifications of *Nectria* being formed in about three months. In other cases on the same trees the fungus was apparently isolated and unable to make headway.

The observations on naturally occurring infections suggest that the fungus can attack healthy wood when it is established in a dead portion adjoining the healthy part. A series of infections were therefore made on artificially killed branches projecting from healthy branches. Some were killed by severe longitudinal slitting, others by means of a steam jet or the flame of a spirit lamp. All were covered up for some days before inoculation.

The fungus was found to establish itself fairly readily in these killed side shoots, stromata being developed sometimes in six weeks. After a period which was variable, but never less than six months, the fungus began to work its way into the main stem, stromata appearing on the side from which the shoot projected. In time the whole stem was blocked, exactly the same symptoms being observed as have been described previously from field observations.

CONCLUSIONS.

It would seem probable that this fungus resembles others which have been described recently in that it cannot establish

itself directly in healthy tissues, but that it can do so after a period spent on a dead portion of the host.

It is thought that the harmful action of the fungus is entirely due to its growth in the xylem elements, causing death of living cells above the infected area owing to water shortage.

No evidence was obtained that it could ever enter a living cell until the cell was at any rate partially cut off from its water supply. No ill effects appear to be felt by the leaves and flowers on a stem, which may be almost completely blocked with *Nectria* at a lower level, until they suddenly show signs of wilting. It therefore would seem improbable that any toxic substance is secreted by the fungus which can affect living cells in advance of the fungal hyphae. Experiments are being conducted with a view to further elucidation of this point.

It is commonly observed that the attacks of the fungus are much more frequent on red currant than on either black currant or gooseberry. The former, which is normally somewhat heavily pruned, furnishes a number of dead spurs each year, and these are observed to be the starting-points of the fungus in the great majority of cases. The time of the actual invasion of the main stem is not apparently related to the time at which the first infection took place. Observations show that the older bushes suffer more serious damage from the fungus than younger and more vigorous bushes of the same variety, but it is plain that the fungus has in most cases been growing for several years on the older bushes before actual death of branches on a large scale occurs.

Very little information could be obtained as to the names of varieties in the orchards, although it was certain that differences in the extent of attack by the fungus under the same conditions did exist. Further work is being done in collecting evidence as to the different varieties, and the effect of soil conditions.

SUMMARY.

(1) The fungus was not found capable of effecting an entry into uninjured plant tissues.

(2) It can occasionally establish itself when introduced into a wound in certain woody plants, more readily in the case of the lime and horse-chestnut than in the case of the red currant, but it usually failed to do so under the conditions of the experiments.

(3) Its normal method of attacking the red currant is by spreading through the wood cells from a dead portion into the healthy wood.

(4) Its harmful action in the first place is due to the stoppage

of the wood cells by the fungal hyphae, thus causing wilting and death of all parts above the point affected.

(5) No differences in power of infection or in behaviour in culture were observed between strains of the fungus isolated from different sources.

I should like to take this opportunity of thanking Mr F. T. Brooks for the time he has spent in directing the work and for many suggestions and criticisms, and Professor Seward for laboratory accommodation.

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Other references to literature will be found in the 3rd edition of P. Sorauer's *Handbuch der Pflanzenkrankheiten*, II, p. 205 (1906).

ORCHID MYCORRHIZA*.

With Plates II—VII.

By J. Ramsbottom.

INTRODUCTION.

One of the most interesting phenomena in biology is that generally known as *symbiosis*—the living together of two organisms in close association. It is usually considered that this intimate relationship is of benefit to both components. Many examples occur in the plant kingdom. The lichen is probably the best known of these, being a composite plant formed of a fungus and an alga in definite union. Other well-known examples are the bacteria (*Pseudomonas radicolica*) living in the root nodules of Leguminosae, and the Ginger-beer plant†, of which the lumps are composed of a yeast (*Saccharomyces pyriformis*) and a bacterium (*Bacterium vermiforme*). An intimate union can also occur between plant and animal, as in the case of the marine worm *Convoluta*, in the body of which an alga is always present,

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† The Ginger-beer plant is, at the present time, being widely distributed over the country as "Californian Bees," "Macedonian (Salonika) Bees," "Mesopotamian Bees," "Palestine (Jerusalem) Bees," "Wine Bees," "Water Bees," "Balm of Gilead," etc.