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Observations on the Parasitism of Isaria farinosa (*Dicks.*) Fr. with special reference to the Larch Sawfly (Nematus erichsonii *Hartig.*)

BY

H. T. GÜSSOW, Dominion Botanist.

OTTAWA
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1911

VII.—*Observations on the Parasitism of Isaria farinosa* (Dicks.) *Fr. with special reference to the Larch Sawfly* (*Nematus erichsonii* Hartig).

By H. T. Gussow, Dominion Botanist.

(Communicated by Dr. Wm. Saunders, F.R.S.C., and read 27th September, 1910.)

The fungus on which I wish to record a few observations is, as no doubt you are aware, one of the commonest of those found growing on insects, at some time or other during different stages of their development. Far greater importance has been attached to these entomogenous fungi in recent years owing to their being regarded as natural factors in the control of insect pests. This question has interested both Entomologist and Botanist alike and, owing to the alarming increase at the present day of the larch sawfly on both sides of the Atlantic, a special study of this particular fungus has been made more opportune. There were several points of interest on which further research was desired. Was the fungus known to occur on the larch sawfly cocoons in Europe identical with that observed in Canada? This is, however, one of the minor points at issue. Still more prominent was the question —Does this fungus attack the living cocoons, or the pupating larvæ or is it only secondary, growing saprophytically on the dead cocoons? The third point of interest was to ascertain how infection took place.

I.

The first point was easily settled. Material i.e. larch sawfly cocoons collected by Dr. C. Gordon Hewitt in Canada, showed small patches of a whitish fungus growing on the surface. The cocoons were kept under suitable conditions for further study of the fungus. Some three weeks later the growth had become elongated and had taken the shape of the characteristic forked or tongued sporophores with which the investigator is quite familiar. These measured from 2-3 centimeters in length; their stalks were orange coloured at the base and about two-thirds of the upper portion was covered with a white farinaceous mass. On microscopic examination of these filaments the fungus was identified as belonging to the genus *Isaria*, being the conidial form of the ascigerous (pyrenomycetous) fungus *Cordiceps militaris*. To establish, however, the identity of the Canadian species with the European one it was thought advisable to make a series of pure cultures. I succeeded, with the necessary care, in obtaining immediately a pure culture by removing a small portion of the farinaceous mass composed of spores by means of a sterilized platinum needle. The spores at that

time densely covered the erect stroma. They were transferred to a sterile petri dish containing Standard Nutrient Gelatine. After 36 hours, small radiating colonies became visible to the unaided eye. Previously, the germination of the colourless, oval-shaped conidia was observed with the aid of the microscope. After 6 hours the first signs of germination occurred, the spore sending forth one or two fine mycelial tubes. Sometimes the germination and development reminded, on account of the much enlarged spherical cells produced, of the development of common yeast spores; at other times the germination resembled more an ordinary hyphomycetous fungus. The growing colonies formed beautiful objects. Radiating from the central spore the hyphae produced circular rays, which appeared slightly iridiscent when holding up the dish and looking at the colony through the medium. After a few days the surface of the petri dish and of "slants" in test-tubes became covered with a dense mass of tufty hyphae of a creamish or pale orange tinge. Small portions of these were carefully removed and examined. Abundant spores had been produced in the meantime. They are born in long chains from 2-11 on finely drawn out flask-shaped sterigmata which are produced from the main or lateral branches of the mycelium in "whorls" from 2 to 7. They were, however, also observed singly. The spores produced in the cultures sowed themselves all around the growing fungus masses and new colonies were constantly observed and watched. The spores and sterigmata were measured and were found identical in every respect with those grown in pure cultures from European material. The fungus on the Canadian cocoons hence was identified as *Isaria farinosa*.

II.

There has long been the conception that *Isaria farinosa* grows parasitically on insect larvae of various kinds. All textbooks of mycology agree on that point. In the absence, however (at least I was not able to discover any records), of demonstrating experiments, these statements did not exclude the possibility that the *Isaria* may occur secondarily. It was just as likely that it grew saprophytically on cocoons or larvae that had died previously. In view of the fact that the larch sawfly was increasing here and elsewhere, it was thought advisable that the parasitism of the fungus, if such existed, would play an important role in the control of this enemy of tamaracks.

I must thank here my friend and colleague, Dr. Hewitt, for placing material in form of *Isaria* covered larch sawfly cocoons at my disposal. These cocoons were placed together with the moss in which they were imported from England into a flat glass dish. The moss was moistened and a well-fitting lid preserved the moisture satisfactorily. The cage

was kept in the dark under ordinary laboratory temperature. In about 22 days a considerable quantity of sporophores of the *Isaria* were produced. Originally 23 cocoons showing the white patches of the fungus were placed in the cage. No adult insect emerged from these cocoons. Some of the cocoons were dissected at intervals and were found to contain a blackened or dirty yellowish adult. The dissected cocoons were replaced and the *Isaria* developed further. I then obtained a handful of cocoons which were carefully examined and which showed no signs of an infection whatever. They were divided in equal numbers, 30 cocoons serving in each of the following experiments:—

Experiment A.

These sound living cocoons were introduced into a breeding chamber and were carefully kept free from external infection from *Isaria* spores. It was sought to ascertain how many of the cocoons would produce living adults and those emerging were carefully recorded. Ten adult larch sawflies emerged in the course of ten days. Eleven parasitic insects were also found to emerge from the cocoons; the remainder of the cocoons did not "hatch" at all. Some of these were found, on dissection, empty. Some showed remains of a dead adult. Only one cocoon showed signs of *Isaria*.

Experiment B.

The same number of cocoons were used. These were placed, together with infested moss and *Isaria* spores bearing cocoons, into a small breeder. After 10 days 6 adults and 6 parasites were observed and were left confined in the cage. One of the first peculiar symptoms observed in these cocoons was a darkening in colour of 16 of them. The colour of the normal cocoons being light chocolate, while in these cases the colour was of a pronounced dull chocolate tinge. Four more adults emerged on subsequent occasions. Although no signs of *Isaria* were then noticeable on the darkening cocoons some of them were dissected and microscopically examined. Two of the examined cocoons showed the interior walls lined with white fungus hyphae; others showed fungal hyphae in the dead adults' body. Later on white fluffy patches occurred externally rather suddenly on most of the remaining cocoons. From the appearance of these fungous growths it was evident that they were formed by the *Isaria*. About two months after beginning the experiments the fungi formed the well-known forked sporophores and the microscopical characters proved the fungus to be *Isaria*. Spores had been produced abundantly at very early stages and no doubt had become disseminated throughout the cage. When about three months

after starting this experiment I examined the interior of the cage again, I found the whole moss superficially and throughout the layer studded with fine whitish colonies of fungi. These were examined and found to be small colonies of *Isaria*. These colonies remained up to date very minute, but never disappeared. New ones constantly appeared and at present the moss is peculiarly studded all over with minute *Isaria* colonies. These colonies having no supply of congenial food remained small and were of course of starved appearance. I next separated a few and transferred them to a petri dish containing nutrient gelatine. Here they made three days' rapid growth and no doubt would have covered in the usual way the whole surface but for the appearance of gelatine liquifying bacteria which put a premature end to my observation. Nevertheless, it was proved repeatedly that the fungous spots consisted of *Isaria farinosa* and no other. It was surprising to me that, never throughout these experiments, I was able to observe other fungi; like *Penicillium* and other common moulds. Several important conclusions may be drawn from these experiments.

1. Granted that the cocoons used in experiments A and B were in equal condition as far as their being alive is concerned, it is shown from the greater number of adults or parasites emerging from cage (experiment A) and from the infection of a large proportion of cocoons in experiment B that the fungus *Isaria farinosa* is truly parasitic on larch sawfly cocoons.

2. It is evident that spore infection of the cocoons had taken place. On no occasion I observed the infection of adults; they died rapidly but remained uninfected.

3. The fungus *Isaria farinosa* is capable of vegetating saprohpytically for a considerable length of time, provided sufficient moisture is available. The conditions under which this mode of life was observed were close to natural conditions.

4. Owing to this saprophytic mode of life there remains little doubt that the pupating larvæ of the larch sawfly infect themselves when taking to the ground for pupation. The colonies observed in the moss appeared about the end of July and continued to show up to the end of September, during which time, of course, the pupation of the larch sawfly takes place in nature.

III.

I have to record some observations on another experiment undertaken to discover whether it is possible to infect larch sawfly adults and cocoons with spores of *Isaria* from pure cultures. For this purpose a flat glass dish containing *Sphagnum* was sterilized on three successive days in an hot air sterilizer. Although the moss became brown in colour

it still retained satisfactorily moisture subsequently introduced. I then placed a number of living adults and cocoons in this apparatus and dusted the whole with spores that had been produced in a pure culture of *Isaria*. The living adults had all died after three days and none of those (11) emerging from the cocoons contracted the fungous disease. After 21 days no more adults emerged, although 13 cocoons remained, which I had evidence to believe contained living adults. Of these, nine eventually developed the typical *Isaria* and the moss also began to be covered with numerous *Isaria* colonies. This experiment confirms my other observations and also indicates that the disease may be artificially introduced even at so late a stage in the development of the larch sawfly. Infection takes place in nature, no doubt, much earlier.

Although none of my experiments were made under strictly natural conditions; that is to say in the open air, yet the observation that the fungus *Isaria* is regularly found year after year under larch trees, when once it has been found, may indicate that the results obtained really closely show what takes place in nature.

In conclusion, I may say that in *Isaria farinosa* we possess one certain factor, by which the increase of the larch sawfly may be controlled. Whether it is necessary to resort to these means in the face of the every year increasing number of insect parasitized larch sawfly cocoons—is somewhat doubtful.