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# *Gastrodia minor* Petrie, an Epiparasite of Manuka

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***Summary***

THE non-green orchid, *Gastrodia minor*, obtains its nutrient supplies by the absorption of fungal cytoplasm in the digestive layer of its tubers. The fungus concerned is partially ectotrophic and partially endotrophic on the roots of manuka and behaves, at least in part, as a root-inhabiting parasite.

## OCCURRENCE OF *Gastrodia minor*

*Gastrodia minor* Petrie was first described by Petrie from material collected in the Town Belt, Dunedin, in shady manuka bush (Petrie, 1892), but from this locality it has now disappeared. It is very plentiful at the eastern end of Lake Manapouri growing under the manuka, *Leptospermum scoparium* J. R. and G. Forster, which occurs as a scrub vegetation in clearings in the southern beech forest and as a fringing belt along the shore of the lake. There may be no other plants present except the mosses, *Dicranoloma billardieri* (Schwaegr.) Par. and *Thuidium furfurosum* (H.f. and W.) Jaeg., as an open floor-covering. At the beech-manuka ecotone *G. minor* occurs only 60 cm distant from the larger *G. cunninghamii*, a plant of the *Nothofagus* forests in this area. Neither *Gastrodia* species was found in association with the kanuka, *Leptospermum ericoides* A. Rich., which occurs in groves in some of the clearings. *G. minor* may also be found growing under manuka in the Taihape-Taupo region of the North Island.

## DESCRIPTION OF *Gastrodia minor*

An account of the above-ground portion of the plant has been given by Petrie (Petrie, 1892) and further details have been added by Hatch (Hatch, 1948). There is a slender, umber-brown, flowering stem 8–24 cm high bearing 3–9 flowers. The flowers either do not open at all or open very slightly and were not visited by insects so far as could be determined, but in every case a seed-capsule developed. Hatch has already shown that the structure of the flower is such that self-pollination is possible (Hatch, 1954).

Below ground there is a branching system of 2 to 12 tubers, the largest up to 10 mm in diameter and 40 mm in length (Fig. 1). They lie at a depth of about 70 mm in the soil, the new tubers spreading laterally from the old ones rather than growing to a higher level. Interwoven with the tubers are numerous fine manuka roots not more than 12 mm in diameter and often much less, some

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Fig. 1

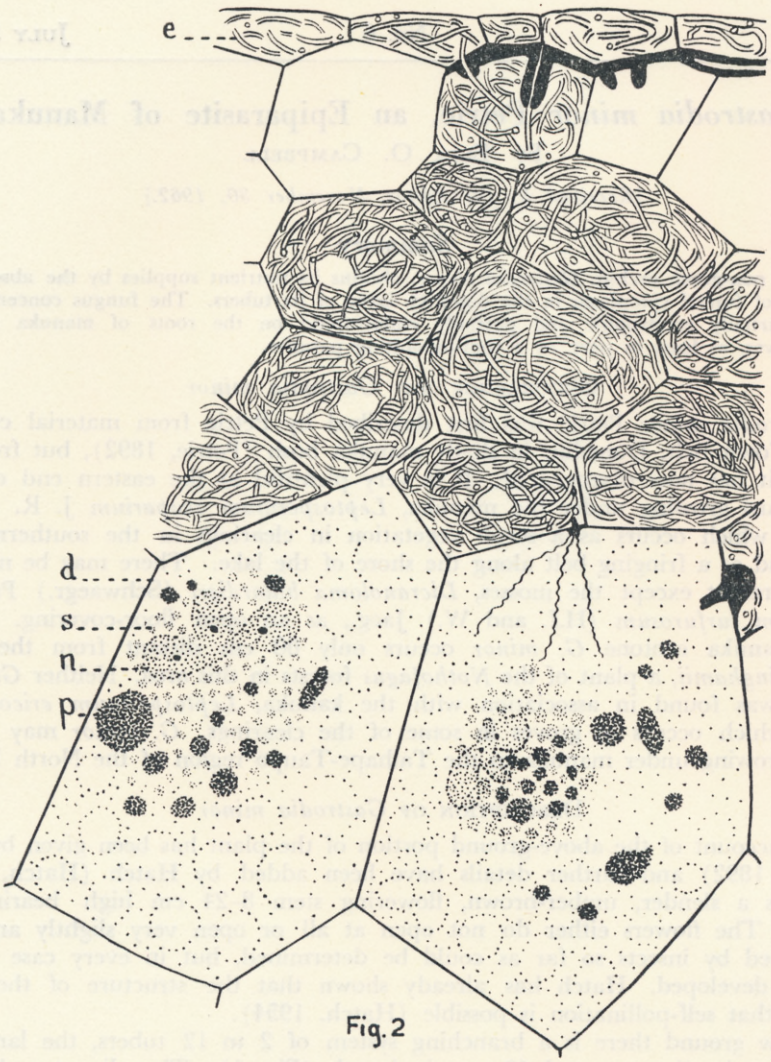


Fig. 2

FIG. 1.—Tuber system of *Gastrodia minor* × 1. r. encircling manuka root, s. scar left by fungal infection.

FIG. 2.—Transverse section of the outer part of a tuber with the second type of infection. × 375. d. digestive layer, e. epidermis, n. nucleus, p. ptyosome, s. starch.

of the finest roots of diameter 0.1 to 0.2 mm lying closely appressed to the tubers and even encircling them. Older rhizomes die away in the autumn, and only those of 2 to 3 seasons persist through the winter, some as vegetative tubers and others with the flower-primordia for next season already present. The tubers lack roots but carry the ragged remnants of scale leaves. Regions of fungal infection are visible as concave, circular marks 2 to 3 mm in diameter or as larger, brown, roughened patches up to 10 mm in length. With a lens it can be seen that in places slender brown hyphae form an open network over the surface of the rhizomes and extend on to the fine roots lying alongside.

## Collection and Treatment of Material

Most of the material was collected in mid-December and early May from the Lake Manapouri site. Here a water-retentive, organic-matter layer, some 70 mm deep, composed of decaying manuka leaves and mosses and penetrated by fine manuka roots is readily lifted from an underlying, morainic gravel or sandy-clay zone. The tubers of *Gastrodia minor* occur at the base of the organic horizon and are readily excavated from the soil.

Immediately following their removal some of the rhizomes and the manuka roots alongside were killed and fixed in formalin-acetic-alcohol. Later these were embedded in paraffin and sectioned at thicknesses of 8$\mu$ to 20$\mu$. For general anatomical study safranin and Delafield's haematoxylin, safranin and fast green, or chrysoidin and fast green proved satisfactory stains. Other rhizomes and roots were brought back to the laboratory and either potted up in the glasshouse or used in an attempt to identify and isolate the fungus as described below.

For comparison of the root structure of manuka in other soils, material was collected from a terrace site in the Manawatu, where the soil type is Tokomaru silt loam with a heavy textured subsoil, and this root material was later sectioned and stained. Also, plants were grown from seed in sterilised potting soil in a glasshouse.

## Anatomy of the *Gastrodia* Rhizome

A transverse section of the rhizome shows the following structure. On the outside is a protective region of suberised cells, consisting of an epidermis of flattened, pavement-like cells and a subepidermal layer of isodiametric cells. The layer immediately below may be suberised also. There is a cortex some 3 cells deep, a few of the cells containing a deposit of calcium oxalate in the form of a bundle of raphides and the rest storing small starch grains. A wide, central zone has vascular bundles scattered throughout the ground tissue of thin-walled cells packed with large, compound, starch grains.

## The Fungal Infection of the Rhizome

There are two types of fungal infection. The first affects the epidermal cells, sometimes the subepidermal cells, and occasionally the outermost cortical cells also. The second affects deeper cells of the tuber.

### *The First Type of Infection*

On the surface of the tubers there are septate hyphae of diameter 6$\mu$ showing clamp connections and having rather thick, brown walls when old (Fig. 8). Usually the hyphae occur singly, but in the confined space of the axils of the scale leaves they may entwine in a loose strand. Infection takes place by a hypha, after first attaching itself, forcing its way along the middle lamella of a radial wall, or occasionally penetrating the outer wall directly, then entering the cell and destroying its contents. Inside the cells the hyphae are usually thin-walled and of diameter 2–2.5$\mu$, but in a few cells there are brown, thick-walled hyphae of diameter 4$\mu$. They lie in a band or loose coil within the cell cavity, and if of

sufficient length show clamp connections. Sometimes they penetrate no further than the epidermis; at other times the subepidermal cells and even the outermost cortical cells are affected also. Entry into a subepidermal cell is effected by hyphae forcing their way along the middle lamella and entering probably through a weakened pit. The hypha grows towards the nucleus which becomes enlarged, then evanescent and finally together with the cytoplasm disappears completely. The hyphae themselves collapse and disappear. Often the penetrated wall is considerably altered, appearing much thicker than previously and, as well as being suberised, giving a slight reaction for lignin. In some cases suberised, cellulosic sheaths which were secreted around the entering hyphae persist as papillose outgrowths (röhrentüpfel) projecting into the cell cavity. In isolated cells where the hyphae have penetrated to the outermost cortex all the walls of the subepidermal cells are thickened. Occasionally röhrentüpfel occur also on the radial walls of the epidermis. The thickened walls and röhrentüpfel appear to seal off areas damaged by the fungus.

*The Second Type of Infection*

This occurs where the tuber lies alongside a young manuka root on which the fungus is well established. Hyphae are densely aggregated between the surfaces of the root and rhizome. Some of the hyphae enter the epidermal cells of the tuber and form a dense coil within each cell over a band some 10 cells wide (Fig. 2). They grow into the cortex through a single passage cell of the subepidermal layer in which a similar, tight coil develops and in some cases röhrentüpfel also. Once within the cortex the hyphae spread tangentially in a zone some 3 cells deep, filling each cell with a tight coil of uniform diameter as they proceed (Fig. 2). The nucleus of each infected cell enlarges, becomes slightly lobed in outline with a conspicuous nucleolus, then becomes evanescent and disappears, leaving a gap in the position it formerly occupied. The cytoplasm disappears also. A single layer of cells on the inner side of the cortex functions as the main digestive layer (Fig. 2). Although not predetermined, the region soon becomes conspicuous by enlargement of the cells in a radial direction and by the course of the infection. The digestive cells are infected by hyphae growing radially inwards from the cortex and not by hyphae travelling tangentially. The entering hyphae branch into many fine threads which become greatly coiled along their length and expanded at their tips as they grow towards the nucleus (Fig. 3). Starch gradually disappears from the cell commencing at the periphery. The enlarged nucleus becomes grossly deformed by constricting into 4 or more portions held together by narrow, isthmus-like regions. Fungal cytoplasm released into the host cell appears as numerous spherical bodies (ptyosomes) which gradually shrink in size as they are slowly absorbed until they disappear completely. In the last stages of absorption the cytoplasm of the digestive cell becomes coarsely granular. The coiled remains of the hyphal walls persist for a time together with a lignified basal sheath at the point of entry. The penetrated wall itself becomes lignified. Gradually the nucleus reassumes a more spherical shape though still enlarged, then eventually loses its stainability and together with the cytoplasm disappears. Usually one or two layers of cells on the inner side of the main digestive cells are also affected by the fungus. Hyphae forcing their way along the middle lamella enter the cells as a fine, coiling, unbranched thread which grows towards the nucleus. Starch gradually disappears from the cells. The nucleus enlarges, sometimes becoming lobed, loses its staining properties, and then disappears together with the cytoplasm and the hyphal thread.

Eventually the whole region of invaded cells below the protective layer collapses, leaving a cavity in the tuber. The protective layer, now unsupported

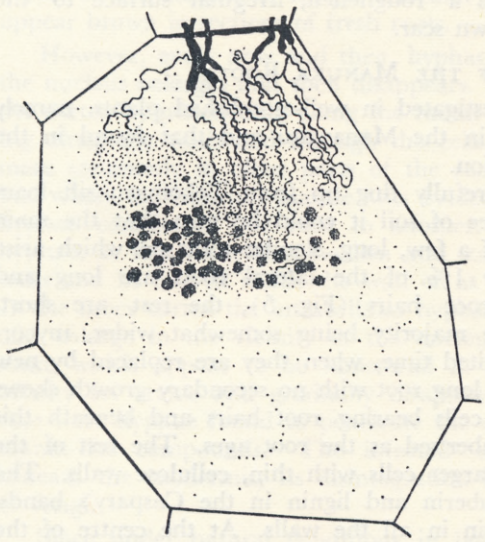

Fig. 3

Fig. 4

Fig. 5

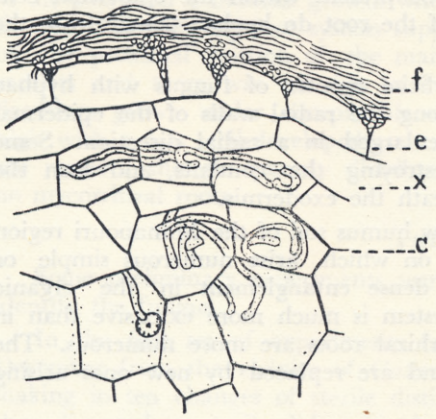


Fig. 6

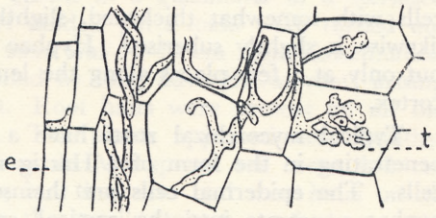


Fig. 7

Fig. 8

FIG. 3.—Transverse section of a digestive cell of a tuber showing numerous coiled hyphae. × 320.

FIG. 4.—Lateral mycorrhizal root of manuka × 5.

FIG. 5.—Lateral non-mycorrhizal root × 5.

FIG. 6.—Transverse section of the outer part of a manuka root with ectotrophic and endotrophic infection × 375. c. cortex, e epidermis, f. fungal mantle, x. exodermis.

FIG. 7.—Longitudinal section of the outer part of a manuka root with endotrophic infection. × 375. e. epidermis, t. swollen ends of hyphae.

FIG. 8.—Single hypha from the surface of a tuber × 400.

from below, sags inwards and presents a roughened, irregular surface to the exterior, or breaks away exposing a brown scar.

### THE MORPHOLOGY OF THE MANUKA ROOTS

Two types of root system were investigated in well-established plants, namely that occurring in Tokomaru silt loam in the Manawatu and that found in the raw humus soil of the Manapouri region.

In plants 1 m high which were carefully dug up from Tokomaru silt loam near Palmerston North and washed free of soil it could be seen that the main framework of the root system consists of a few, long, slender roots on which arise numerous lateral roots. Approximately 1% of the lateral roots are long and slender, with copious, long-persistent, root hairs (Fig. 5), the rest are short, branching roots lacking root hairs, the majority being somewhat wider, mycorrhizal roots which persist for only a limited time, when they are replaced by new ones (Fig. 4). A transverse section of a long root with no secondary growth shows an epidermal layer of rather flattened cells bearing root hairs and beneath this an exodermis which becomes slightly suberised as the root ages. The rest of the cortex some 3–5 cells wide consists of larger cells with thin, cellulose walls. The endodermis at first has a deposit of suberin and lignin in the Caspary's bands, and at a later stage a deposit of suberin in all the walls. At the centre of the root is a small stele usually tetrarch in build.

A few of the short roots have no root hairs, but are not typically mycorrhizal in that the fungal mantle is lacking. They have an epidermal layer of flattened cells with somewhat thickened, slightly suberised walls and an exodermis which likewise is slightly suberised. Hyphae may be present within the epidermal cells but only at a few places along the length of the root do hyphae penetrate to the cortex.

Typical mycorrhizal roots have a superficial mantle of fungus with hyphae penetrating in the form of a Hartig net along the radial walls of the epidermal cells. The epidermal cells are themselves enlarged in a radial direction. Some hyphae penetrate into the cortical cells destroying the contents, and soon the affected cells collapse, leaving a cavity beneath the exodermis.

The root system of the manuka in the raw humus soil of the Manapouri region was found to consist of a few main roots on which arise numerous simple or branched, mycorrhizal roots that form a dense entanglement in the organic matter layer. The branching of the root system is much more extensive than in the Tokomaru silt loam and typical mycorrhizal roots are more numerous. The mycorrhizal roots last for a limited time and are replaced by new ones arising adventitiously.

The apical region of the mycorrhizal root ensheathed by the root cap is loosely enveloped by the fungus. Immediately behind this region the root over a length of some 5 mm has the appearance in transverse section of a typical, ectotrophic mycorrhiza with the fungus forming a dense external mantle some 8 hyphae in thickness and penetrating along the walls of the epidermal cells as a Hartig net (Fig. 6). The epidermal cells become enlarged in a radial direction to as much as three times the original diameter. This enlargement together with the external fungal mantle is responsible for the appearance of stoutness in comparison with a non-infected root. At the stage when the protoxylem is lignified, hyphae may enter the epidermal cells and grow towards the nuclei which enlarge, then become evanescent, and together with the cytoplasm disappear. The hyphae persist in the cells for some time probably in a dormant condition. Further advance is to a large extent barred by the exodermis with its radial walls by now suberised. At a later stage all the walls of the exodermis are suberised and the rest of the

cortex in the absence of fungal infection develops thickened, lignified walls which appear brown in sections of fresh roots.

However, every now and then, hyphae penetrate into an exodermal cell where the nucleus enlarges and then disappears. Occasionally hyphae break through into the cortex by penetrating along the radial walls of the exodermis and then spreading along the walls and through the cells. Cortical infection was found to occur more commonly in older parts of the root where the cortex was still unlignified but where no external mantle was evident, only long, tangled strands of brown hyphae connecting one root to another. In the outer cortex the intracellular hyphae, at first somewhat swollen and staining deeply, soon destroy the cell contents and lie curved inside the wall or as a band running through the cell cavity. In the inner cortex the intracellular hyphae are more slender and their tips branch and enlarge in the vicinity of the nucleus to form coralloid masses retentive of stain, which appear to be haustorial in nature (Fig. 7). The nucleus loses its affinity for stains and gradually disappears together with the cytoplasm. In no case were hyphae found to penetrate the endodermis which gradually accumulates tannin-like compounds. The infected cortex soon collapses leaving a cavity beneath the exodermis, its disintegration hastened by the arrival of other species of fungi.

Since under natural conditions in both the Tokomaru silt loam and in the Manapouri raw humus soil a fungus was found to be closely associated with the roots of the manuka, it was decided to investigate the roots of plants grown from seed in some other type of soil. Seedlings grown in a glasshouse in a sterilised potting soil consisting of 1 part peat:1 part sand:2 parts loam were turned out of the pots after 6 months' growth when the stem was 11–13 cm high. Examination showed a slender tap-root system supplemented by long adventitious roots arising on the proximal portion of the main root. Root hairs were present on all the rootlets and there was no sign of fungus. The plants appeared very healthy. Seedlings grown under comparable conditions in Manapouri soil were 4–8 cm high with a less well-developed root system, but they also had root hairs on all the rootlets, though sparsely distributed on the smaller plants, and there were no mycorrhizal roots.

## THE FUNGUS

Some preliminary experiments were carried out in an attempt to isolate and identify the fungus.

In the first set of experiments manuka roots which had been collected in December from the vicinity of *Gastrodia* rhizomes were washed for 3 hours by shaking in ten changes of sterile distilled water in 500 ml cylindrical flasks and then dissected on sterile slides. Single hyphae were transferred to agar plates and kept in an incubator at 22° C. Either malt agar was used on the plates or medium A, namely:

| | |
|---|---|
| Malt extract | 5.0 gm |
| Peptone | 1.5 gm |
| Agar | 20.0 gm |
| Water | 1,000.0 ml |
| With or without the addition of dextrose | 20.0 gm |

After an interval of either 7 or 14 days portions of any colonies produced were transferred to medium B which was based on the experiments by Garrett in connection with the growth of rhizomorphs of *Armillaria* (Garrett 1953). The plates were examined at intervals over a period of 28 days.

*Medium B*

| | | | |
|---|---|---|---|
| Dextrose | 20.0 gm | Ferric chloride | 2.0 gm per litre |
| Potassium phosphate | 1.0 gm | Thiamin | 0.25 mg per litre |
| Magnesium sulphate | 0.5 gm | Agar | 20.0 gm |
| Peptone | 6.0 gm | Water | 1000.0 ml |

Since the cultures either proved sterile or yielded rapidly growing soil saprophytes it was concluded that single hyphae were too small to be of value as a source of inoculum.

A second set of experiments was set up following the lines of the first except that pieces of rhizomes 2 mm long or of roots 1 mm long were used as a source of inoculum. A drop of sterile 5% lactic acid was added to depress the growth of bacteria. Hyphae of the endophyte spread in the cells of the tuber, but only in 1% of the cases did hyphae grow out into the culture medium and then for only a short distance. When pieces of root were used, soil saprophytes quickly dominated the culture plates. A number of fungi were isolated, but in no instance was *Armillaria* found to be present.

In a third set of experiments rhizomes were collected in May. Immediately following their removal from the soil these were placed in tubes which had previously been partially filled with damp sphagnum moss, plugged with cotton wool and autoclaved. On return to the laboratory these rhizomes were washed by shaking for 3 hours in sterile distilled water in McCartney tubes on an end-over-end shaker (40 r.p.m.), the water being changed at 15 minute intervals. The rhizomes were then cut into pieces 3 mm long, and these after shaking in a fresh lot of sterile distilled water were placed on agar plates and kept at 22° C. in an incubator.

Media used were:

1. Plain agar
2. Medium A as given above
3. Medium B as given above

| | | | | |
|---|---|---|---|---|
| 4. | Dextrose | 20.0 gm | Peptone | 6.0 gm |
| | $KH_2PO_4$ | 1.0 gm | Agar | 20.0 gm |
| | $MgSO_4$ | 0.5 gm | Water | 1,000.0 ml |

In some cases there was added to each 5 ml of the medium, prior to autoclaving, either 1 ml root extract or 1 gm filter paper, but no increased growth of the endophyte was observed as a result.

| | | | | |
|---|---|---|---|---|
| 5. | $NH_4NO_3$ | 10.0 gm | Lactic acid | 2.0 gm |
| | $K_2HPO_4$ | 5.0 gm | Agar | 20.0 gm |
| | $MgSO_4$ | 1.0 gm | Water | 1000.0 ml |

In some cases to each 50 ml solution there was added, prior to autoclaving, either 10 ml root extract or 10 gm filter paper, but no increased growth of the endophyte resulted.

The cultures were examined after 14 days with the following results:

| Culture Medium | 1 | 2 — sugar | 2 + sugar | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Percentage showing slight growth of the endophyte ..... ..... ..... ..... ..... | 0 | 25 | 4 | 4 | 15 | 0 |
| Percentage showing growth of other fungi | 1 | 75 | 100 | 92 | 94 | 88 |

A fourth set of experiments followed the lines of the third set, except that pieces of manuka root growing adjacent to the rhizome were used. After a period of 14 days these yielded the following results:

| Culture Medium | 1 | 2 — sugar | 2 + sugar | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Percentage showing slight growth of the endophyte ..... ..... ..... ..... ..... | 0 | 0 | 0 | 29 | 0 | 0 |
| Percentage showing growth of other fungi | 86 | 100 | 74 | 71 | 100 | 100 |

In a fifth set of experiments pieces of root or rhizome were placed on a medium of sterilised filter paper and root extract, but the cells and the endophyte soon died under these conditions.

One difficulty in interpreting these results arises from the fact that the pieces of inoculum were not equivalent as regards either the presence or the activity of the endophyte. However, it was found that even when the endophyte was alive, any growth on the media used was extremely slow. When no other fungi were present the endophyte grew along the surface of the root or rhizome, but only in two instances did it grow out a short distance into the surrounding medium. It did not attack cellulose and seemed to be able to utilise peptone. The presence of clamp connections and failure to form rhizomorphs indicated that it was not *Armillaria*. It was concluded that the endophyte is a basidial fungus specialised to growth on living manuka roots. Which fungus it might be amongst the vast array of fungi in the manuka scrub could not be determined. All the fructifications which were examined were found to arise from saprophytic mycelia penetrating decaying leaves and twigs of the organic horizon.

## Discussion

Since *Gastrodia minor* lacks both roots and chlorophyll it is fairly obvious that it obtains its nutrient supplies by the process of digestion of fungal cytoplasm within the digestive cells of the tubers. The fungus concerned is present not only within the tubers but also on and within the roots of manuka. Since disintegration of the cortex of the manuka root occurs once the intracellular infection is established, but in its absence only occurs in those roots where secondary growth has commenced and a phellogen has arisen in the pericycle, the fungus in its endotrophic phase is interpreted as being parasitic on the root. What happens during the ectotrophic phase is not clear. The roots have been termed mycorrhizal because of the similarity in appearance to the ectotrophic mycorrhizas of beech and other forest trees. It appears that the fungus is stimulated by substances excreted from the root and waits for the opportunity to penetrate further, but possibly there is some interchange between root and fungus during this phase. Björkman has applied the term epiparasite to *Monotropa hypopitys* which he has shown obtains nutrient material from a fungus ectotrophic on pine and spruce (Björkman, 1960). Although the situation in *Gastrodia minor* differs in details from that in *Monotropa*, the term epiparasite seems appropriate.

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