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The Fungal Association of *Yoania australis*

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Abstract

THE orchid, *Yoania australis,* is parasitic on a fungus, *Lycoperdon perlatum,* which grows in mycorrhizal association with *Beilschmiedia tarairi.*

OCCURRENCE OF *Yoania australis*

THE orchid *Yoania australis* Hatch is locally very abundant under trees of *Beilschmiedia tarairi* Benth. et Hook. f. ex Kirk in the State Forest Reserve and in a forest remnant at Glorit, where it was first noticed by J. and R. Beever, and under taraire in Kirk's Bush, Papakura, where it was first noticed by J. Horsman. Hatch (1963) lists the Waipoua Forest as a further station. More recently it has been found in kauri-taraire forest at Kaukapakapa by Leonie Moore. Apparently it is quite widely distributed under taraire which is a tree species of the Lauraceae endemic to high-fertility mull soils in the forests of New Zealand north of latitude 38°. The principal soil type is Puhoi clay, a weakly leached, northern yellow-brown earth.

COLLECTION AND TREATMENT OF MATERIAL

Material of *Yoania* and of taraire collected from the Glorit area was fixed in formalin-acetic-alcohol, then embedded in paraffin and sectioned at varying thicknesses from 8 to 20μ. As well, material of taraire from Kirk's Bush was examined and some from Karekare in the Waitakeres—an area where so far no *Yoania* has been found. Young seedlings, in some cases still with the large cotyledons as the only leaves, were collected from Kirk's Bush on November 24, 1967, and grown on for four months. An attempt was made, but without success, to germinate in sterilised soil some of the many seeds which still lay on the ground at this time. The fungus has been grown in culture but not as yet to the stage of producing basidiocarps.

MORPHOLOGY OF THE *Yoania*

For most of the year the plant exists as an extensive underground system of fleshy brittle rhizomes, of diameter 2.5 to 4.0mm, which ramify in the soil to a depth of 20cm from the surface amongst the organic matter and the taraire roots. The underground system in flourishing plants is much greater than the drawing by Hatch (1963) indicates. Young rhizomes are white in colour; older regions are brown. On the surface are scars of scale leaves, irregular scars of old infection areas and numerous short conical projections bearing long hairs. From December until

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February the tips of some of the branches grow erect above the ground as pinkish-white flowering stems, up to 12cm high, which later turn brownish. No chlorophyll is formed in any part of the plant.

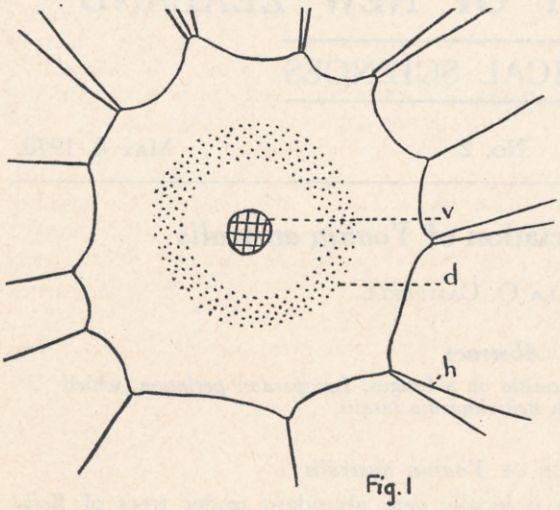
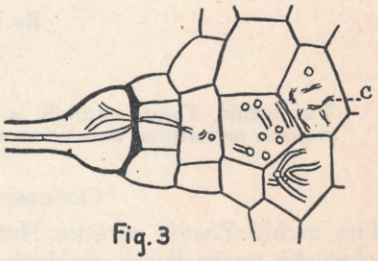


Fig. 1

Fig. 2



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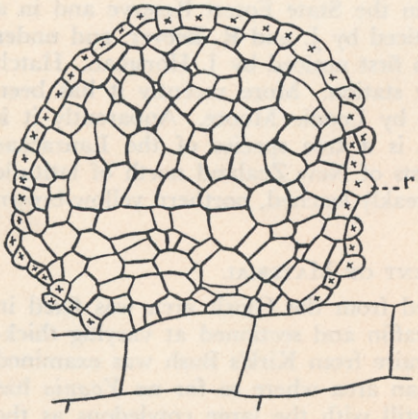

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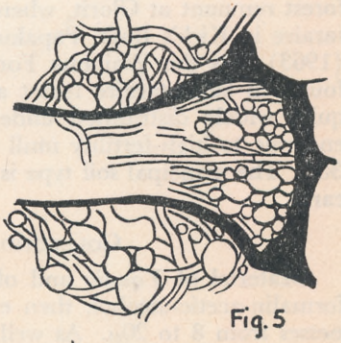

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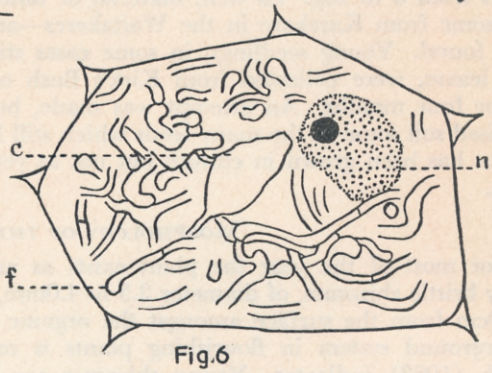


Fig. 6

Fig. 1.—Diagram of a transverse section of a *Yoania* rhizome. × 20. d, fungal digestion zone; h, hairs (full length not shown); v, vascular cylinder. Fig. 2.—Projection bearing a young hair shown in section. × 85. Fig. 3.—Transverse section of the outer part of a rhizome showing an early stage in infection. × 185. c, collapsing hyphae. Fig. 4.—Transverse section of a rhizomorph appressed to the surface of a *Yoania* rhizome. × 800. r, rind. Fig. 5.—Section of the base of a hair showing fungal pseudoparenchyma developing in and around it. × 400. Fig. 6.—Transverse section of a cell of the digestion zone of *Yoania* showing an early stage of digestion. × 400. c, clump at an early stage; f, swollen hyphae; n, nucleus.

The rhizome in transverse section is seen to be irregular in outline due to the conical processes projecting from its surface (Fig. 1). These are 3 to 5 cells in height and at the base up to 6 cells in width (Fig. 2). In the young rhizome the terminal cells of the processes differ from adjacent cells in having abundant, granular, perinuclear cytoplasm and a large nucleus; they also lack the cuticle which is present on the other epidermal cells. Later, these terminal cells produce rather stiff hairs, up to 2.2mm in length and 18 to 24μ in width, composed of a row of as many as six cells. Within the epidermis the rhizome has a parenchymatous cortex, 15 to 21 cells wide, bounded inwards by an endodermis with Casparian bands. Carbohydrate is stored in the cortical cells as granules staining reddish-brown with iodine, except in scattered mucilage cells which contain a bundle of raphides. The central, vascular tissue consists of two to six collateral bundles embedded in a small amount of parenchyma. Each bundle has protoxylem of spiral, annular and occasionally reticulate vessels and tracheids along with protophloem composed of sieve-tubes and parenchyma.

The flowering stem, as seen in sections, differs from the rhizome in that it has 17 to 21 vascular bundles, lacks a recognisable endodermis and cuticle, and is not infected by fungus.

THE MORPHOLOGY OF TARAIRE ROOTS

The root system of taraire consists of long roots which form the main framework of the system and of shorter feeding roots, of a remarkably uniform diameter of 1.0 to 1.5mm, which interweave in the organic-matter layer of the soil. Although there is no sharp line of demarcation between the two types, the general pattern is that of long and short roots. The feeding roots come off from the long roots at a wide angle, in fact almost at right angles. They are either unbranched or else branched racemosely at a wide angle to produce a large-scale, pyramidal outline. They have a limited life-span and are periodically replaced by new ones arising from the long roots.

In order to study the structure of the long roots, sections were cut both of the tap root of seedlings before the fungus had become established on them and of the main roots of older plants taken from the upper soil layers. The deep roots were not excavated, as the trees were growing in reserve areas. In some respects the structure of the long roots is unusual.

While still in the primary condition these roots are up to 4mm in diameter. The vascular cylinder, containing six to 10 protoxylem and protophloem strands, is surrounded by an endodermis in which the cells early develop a suberin lamella on all their walls except for groups of four to five passage cells, opposite the protoxylem poles, in which suberization is restricted to the Casparian band. The cortex is up to 1.0mm in width with many sclereids scattered amongst parenchyma cells and with small, intercellular air-spaces throughout. In seedlings most of the parenchyma cells contain starch-grains but a few contain volatile oil. The cortex is bounded outwards by an exodermis, whose cells are of regular size and of approximately isodiametric shape; in some roots they may contain a crystal of calcium oxalate. The exodermis divides tangentially producing on its inner side an incipient phellogen (cf. Fig. 8) which normally gives rise to a very narrow zone of flattened cork-cells with lignified, suberized walls but forms lenticels in a few places along the root. The exodermis itself becomes suberized and lignified, first on the radial walls, then on all the walls, except in the case of certain cells occurring singly or in pairs; eventually even these become suberized when the cells beneath become corky. At the surface of the root there are no root hairs. Overlying the epidermis is an outer zone, up to 18 cells in width in young regions, and two to four cells in width in older regions, where the cells have brown walls and brown tannin contents (cf. Fig. 8). Longitudinal sections of the apex show that these represent persisting cells of the

root cap (Fig. 11), for only the outermost layers are slowly sloughed off as the root extends.

As the root ages, secondary growth from a vascular cambium adds to the vascular tissue increasing its girth. In the outer pericycle an additional phellogen arises and produces a narrow layer of cork, while in the inner pericycle sclereids develop. The cortex dies off, its old cells persisting for a time as a sheath to the root.

The structure of the short roots in several respects differs from that of the long roots. In seedlings the cortex contains a little starch but in older plants the short roots are early infected by the fungus and do not contain starch. The vascular cylinder is smaller, being only 0.2 to 0.5mm in diameter, and is usually pentarch, occasionally tetrarch. The cortex is narrower, being up to 0.5mm or some 10 to 20 cells wide, and contains fewer or no sclereids. Secondary growth does not occur except for the formation of the incipient phellogen (Fig. 8) resulting in a narrow zone of flattened, suberized cells beneath the exodermis. The brown, outer zone remains as the permanent outer covering of the root.

The Fungus

The fungus occurs in the organic-matter layer of the soil amongst the taraire roots as white rhizomorphs, a few of which are wide and up to 0.5mm in diameter, but thinner ones, of diameter 52μ or less, are found lying externally along the taraire roots, attached at intervals. or penetrating the outer zone. or in the case of some old, dead roots penetrating the cortex. Similar thin rhizomorphs may be found also on *Yoania* rhizomes, appressed to the epidermis (Fig. 4), or to the surface of a hair, lying in the axils of the scales, or even penetrating the epidermis and running tangentially through three or four cells of the cortex. At times the two plants are held together by the rhizomorphs.

From the rhizomorphs finer strands, 10 to 28μ in diameter, arise and single septate hyphae, of diameter 1.7 to 7.0μ, which together form an open network visible with a lens on the surface of the *Yoania* rhizomes, being particularly noticeable in younger parts of them, attached at intervals and closely aggregated around and amongst the hairs. A similar network occurs on the surface of both long and short roots of taraire and was found in material from all the localities in which collections were made, even from the Karekare area where *Yoania* is absent.

The structure of the strands and rhizomorphs varies considerably. The finest strands sometimes consist entirely of fine, thin-walled hyphae of diameter 1.0 to 1.7μ; in other cases the hyphae are of a diameter up to 6.8μ. In the thin, white rhizomorphs there may be a central core of thick-walled hyphae, of diameter up to 14μ, amidst narrower ones with thin walls, and an outer zone of loosely interwoven, thin-walled hyphae; while in those appressed to the surface of the taraire or the *Yoania* there is often a narrow rind of compact, peripheral hyphae, of diameter 3 to 3.4μ, with firmer brown walls (Fig. 4). The wide rhizomorphs show three distinct zones of hyphae (Fig. 7). An outer, cortical zone consists of loosely interwoven, thin-walled hyphae of diameter 1.7 to 3.4μ. It surrounds a subcortical cylinder of rather similar hyphae differing in that they lie more or less parallel lengthwise, are compactly arranged and tend individually to be somewhat wider. In the central core-zone there are narrow, thin-walled hyphae intermixed with thicker-walled ones which are of diameter up to 38μ and have segments up to 900μ long. The wide hyphae and subcortical hyphae often show clamp-connections but those of the outer zone and free hyphae elsewhere are clampless.

The thin strands were found to connect up with rhizomorphs, and the wide rhizomorphs connected up with the basidiocarps of *Lycoperdon perlatum* Pers., which were apparent above the soil at both Glorit and Kirk's Bush on May 26, 1967. Fructifications of many other Basidiomycetes from both localities were examined

but no other one had rhizomorphs of this type attached to its base. Comparable rhizomorphs have been described for other species of *Lycoperdon* such as *L. pyriforme* Pers. and *L. gemmatum* Pers. (Townsend, 1954), also *L. pedicellatum* Pk. (Swartz, 1936).

INFECTION OF THE *Yoania* RHIZOME

Infection in some parts of the rhizome takes place by single hyphae, 2.0–5.2μ in diameter, which come off either from the superficial hyphal network or from thin mycelial strands, and enter the terminal cells of a projection usually through the tip of a hair. Sometimes the hypha proceeds directly to the base of the hair, completely destroying the transverse walls as it passes through; at other times it branches to form a strand which occupies the whole cavity of the hair. The external wall of the hair becomes thickened and lignified so giving support to the delicate fungal thread which traverses it. The cell at the base also develops a thickened lignified wall and here the hyphae may be up to 6.8μ in diameter. Sometimes the basal wall is greatly thickened and may develop short, finger-like projections into the cell below.

From the point of their entry into the rhizome the hyphae spread radially inwards into the cortex (Fig. 3) and then laterally around the rhizome to a varying depth of up to 15 cells from the epidermis, but not as a rule in the epidermis itself. Sometimes they spread completely around the rhizome. The hyphae are constricted as they pass through a wall and within the cells are septate. In the outer cortex they may pass straight through a cell or may branch a few times but do not form dense coils. The finer branches then collapse and disappear but the main branches persist. The infected cells remain alive with walls unaltered chemically. Sometimes the fungus penetrates to a distance of less than six cells from the epidermis and then is difficult to detect.

At other times the fungus penetrates to a much greater depth, even as far as three cells from the endodermis. In the middle region of the cortex it branches freely within the cells. In the innermost region of invaded cells, in a digestion zone two to four cells in depth, the hyphae at first branch and spread throughout the cell, then swell and become deeply staining with such stains as crystal violet (Fig. 6). Eventually most of them appear shrunken and empty, but there remains a conspicuous clump containing amorphous material which stains deeply with Sudan III. The nucleus of each of these digestion cells enlarges up to 2½ times its former diameter and frequently becomes irregular in outline. The size of the nucleolus also increases, to twice its original diameter. As the clumps diminish in size, cells near the endodermis contain many large, compound starch-grains which give a reddish-brown colour with iodine.

Infection in older parts takes place by single hyphae which come off from rhizomorphs, or by rhizomorphs themselves connected directly to the roots of taraire. The infection follows the same course as described above but differs in being more vigorous. Where rhizomorphs lie in the axils of scale leaves, fungal pseudoparenchyma occupies the cavities of many of the superficial cells of both the leaf and the rhizome, and the whole region is enclosed by thickened, lignified walls. Sometimes the rhizomorph attaches itself by an appressorium to either the surface of a hair or the surface of the rhizome, and both narrow and wide hyphae radiating from this point enter hair-cells where they branch repeatedly, filling the base of the hair with pseudoparenchyma (Fig. 5). Sometimes the infection spot is a whole conical projection, a region some six cells in diameter and two to three cells deep, all of whose cells are filled with pseudoparenchyma. Lignified wall-thickening may be present not only around the infection area but collenchyma-like on the angles to a depth of six cells below. At other times the primary infection area is 20 cells in diameter with the rhizomorph penetrating below the surface and digestion taking place in the middle cortex, some 10 cell-layers below, in a zone up to six cells in depth.

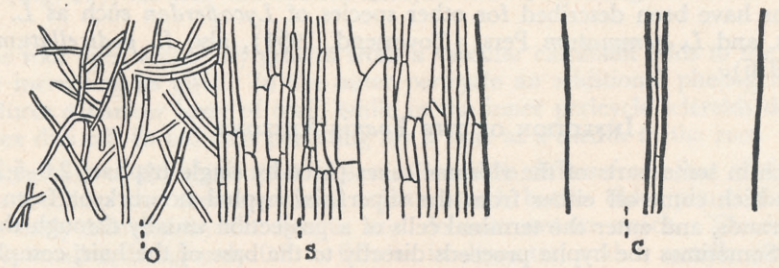


Fig.7

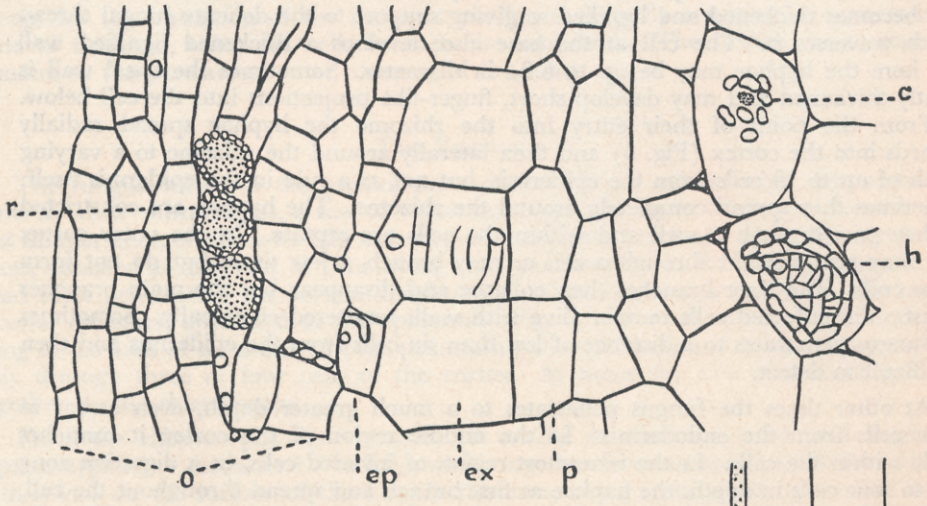
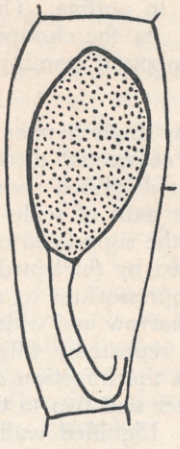


Fig.8

Fig.9



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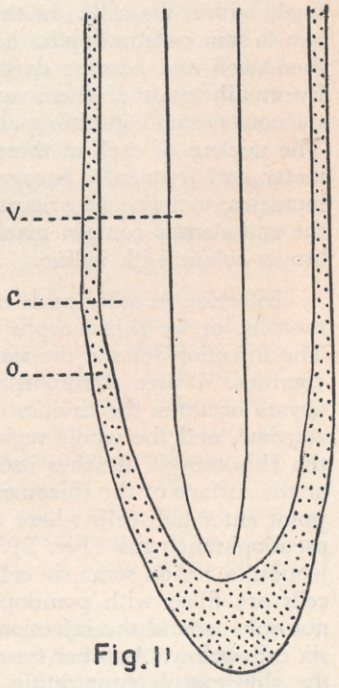

Fig. 11

INFECTION OF THE TARAIRE ROOT

Single hyphae, and sometimes rhizomorphs also, penetrate between the cells of the outer zone in the case of both long and short roots, forcing the layers of cells somewhat apart (Fig. 8). Hyphae then ramify throughout the region, both in and between the cells; in some places near the epidermis they may at times simulate a Hartig net; they are septate but without clamp-connections and if thick-walled may be up to 6.8μ in diameter. The course of the deeper infection varies in different roots according to the vigour of the fungus.

In some cases single hyphae connecting with single hyphae external to the root penetrate into the epidermal cells, where they rarely branch but pass through into a few of the exodermal cells. Here again they rarely branch but usually pass straight through into the cortex, where they occupy the intercellular air-spaces and put out short branches into some of the cells.

If the entering hyphae come off from thin strands, they penetrate the epidermis and the exodermis and then travel through the cortical cells rather than the air-spaces, sometimes branching once within a cell but never filling the cavity. The intracellular hyphae are densely cytoplasmic and can be seen to be septate; their tips sometimes branch and enlarge.

If the entering hyphae come off from rhizomorphs, the attack is more intense. In the exodermis the hyphae are of diameter 5.0 to 6.8μ and often can be seen to be septate, for they tend to branch and coil until they almost fill the cell space. In the cortex they are abundantly present within the cells as well as in the air spaces. Sometimes they coil round inside the cell-wall and are much swollen, with a diameter of 4 to 10μ and granular contents, or there may be thinner branches of diameter 1.7 to 3.4μ. In a few places the hyphal tip may enlarge as a granular vesicle (Figs. 9 and 10) which has a thickened wall and nearly fills the width of a cell, or forms a lobed mass occupying several adjacent cells. At other times the hyphae at first branch repeatedly and occupy most of the cell cavity (Fig. 8); then the central hyphae form coralloid masses which stain deeply (Fig. 8), while the other hyphae within the cell appear empty and collapsed. The cell contents eventually disappear but remnants of the coralloid hyphae persist as unabsorbed material.

Where there are many rhizomorphs on the root, the infection is well established. Hyphae run straight through the cortical cells lengthwise in the root. In a few short roots there were rhizomorphs in the cortex also, but in these cases the cortex was dead and the root itself was dead also. In long roots after secondary growth has commenced, the fungus builds up in the dead cortex and in the deeply seated, new layer of cork which is not present on the short roots.

DISCUSSION

Since the same fungus was found associated with the roots of taraire in all the localities where collections were made, it is regarded as a normal inhabitant of taraire roots, at least in some types of soil. The fungus not only forms a very open, superficial network on the taraire roots but attaches itself firmly by ramification throughout the outer zone and enters the cortical cells where presumably exchange of material with the root occurs. It apparently plays a role similar to that of the

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FIG. 7.—Half the width of a large rhizomorph as seen in longitudinal section. × 400. c, core; o, cortex; s, subcortical zone. Fig. 8.—Transverse section of the outer part of a short root of taraire. × 475. c, coralloid, deeply staining hyphae around the nucleus; ep, epidermis; ex, exodermis; h, hyphae in a cortical cell; o, outer zone; p, incipient phellogen; r, rhizomorph. Fig. 9.—Cell from the cortex of taraire in longitudinal section, showing an early stage in the formation of a vesicle. × 400. Fig. 10.—Vesicle in a cortical cell of taraire in longitudinal section. × 400. Fig. 11.—Root in median longitudinal section. × 30. c, cortex; o, outer zone; v, vascular cylinder.

ectotrophic mycorrhiza of beech or pine, for the taraire roots of themselves are inadequate for absorption.

The fungus is able to exist in part as a saprophyte amidst the organic matter of the soil and builds up on decaying twigs. In pure, or almost pure, stands of taraire, where the fungus is very vigorous as indicated by wide rhizomorphs or by many plants of *Yoania,* the fungus seems to have a harmful effect on the taraire, as many short roots, though not the long ones, are dead.

Yoania so far has been found only under taraire and sometimes when no other species of vascular plant is present. It is considered to be parasitic on the same fungus which lives in association with taraire. The fungus forms an external network on the rhizomes of the *Yoania,* attached at intervals and held in position by being interwoven amongst the hairs. Also, in a few places rhizomorphs are securely attached to the rhizomes and connect directly to taraire roots. The hairs are a striking feature and resemble the mykokleptic hairs described for *Corallorhiza* (Jennings and Hanna, 1899). In the outer cortex of the *Yoania* the fungus in the main exists without damaging the protoplasts but the fine branches disappear and possibly yield up their content to the host cells. In the deeper region of the cortex the shrinkage of the swollen hyphae, on analogy with the interpretation given for other orchids, would result in a greater transfer of material to the *Yoania.*

The attached basidiocarps show the fungus to be a puff-ball, *Lycoperdon perlatum* Pers. This fungus is widely distributed throughout the world (Cunningham, 1927) and according to the herbarium records of Plant Diseases Division, D.S.I.R., Auckland, it occurs among native vegetation throughout New Zealand. It is, then, not confined to taraire which is endemic to northern New Zealand.

Acknowledgments

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Bibliography

Cunningham, G. H., 1927. Lycoperdaceae of New Zealand. *Trans. R. Soc. N.Z.* 57: 187–217.

Hatch, E. D., 1963. Notes on New Zealand Orchids II. *Trans. R. Soc. N.Z.* 2: 185–88.

Jennings, A. V. and Hanna, H., 1899. *Corallorhiza innata* R.Br and its Mycorrhiza. *Sci. Proc. Roy. Dub. Soc.* 9: 1–11.

Swartz, D., 1936. The Development of *Lycoperdon pedicellatum. Amer. Journ. Bot.* 23: 4–7.

Townsend, B. B., 1954. Morphology and Development of Fungal Rhizomorphs. *Trans. Brit. Myc. Soc.* 37: 222–33.

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