SOME INVESTIGATIONS ON THE MYCORRHIZA OF CALLUNA, ERICA AND VACCINIUM*

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SUMMARY

The symbiosis of endotrophic fungi with *Calluna*, *Erica* and *Vaccinium* species was studied in artificially infected seedlings and cuttings. The fungi investigated are not species-specific, *e.g.*, a fungus isolated from the European *Vaccinium oxycoccus* can serve the North American *V. macrocarpon* as a symbiont. When added to sterile cuttings the fungus stimulates root formation, and both with seedlings and cuttings it enhances growth.

These symbiotic fungi have cellulase and pectinase.

The process of penetration into the epidermis has been studied electron microscopically. The protoplast of an invaded epidermal cell is not killed by the fungus which eventually is digested by the living plant cell. The fungus cannot invade the subepidermal cells, probably because the secondary cell wall of the latter consists of, or contains, multiple layers of suberin. However, break-down products of the digested mycelium may not be prevented by the suberin layer from being assimilated, as radioactive serine was found to be taken up when applied ca. 1 cm from the tip of the root.

1. INTRODUCTION

The anatomy of the tip of the long and slender roots of the *Ericaceae* investigated in the present study is illustrated in *fig. 1*. At the tip, one finds beneath the one-layered epidermis another layer which soon splits up in the subepidermis and the endodermis, both one-layered. The young root is monarchal. The slime envelope is thickest at the tip. It is not a product of disintegrating calyptra cells. Small groups of the latter cells may still be found at some distance from the tip. Moreover, slime is also found on slightly older parts of the root, where rootlets still lacking a calyptra are about to emerge. LEISER (1968) therefore concluded that the slime is secreted by the epidermal cells. The slime is stained by ruthenium red and so proves to contain pectin or pectin-like substances, but it also contains cellulose (BURGEFF 1961; LEISER 1968). Leiser observed pits in the outer cell wall of the epidermis (in roots of *Azalea*) which might play a role in the secretion of slime.

The endotrophic mycorrhiza in *Ericaceae* was first described by FRANK (1887) and signalized as a case of symbiosis, a view shared by JANSE (1897). TERNETZ (1907) isolated a fungus from the root of *Calluna vulgaris* and identified it as a *Phoma* species. Her finding that this isolate, when grown in pure culture on an N-free medium, was able to fix atmospheric nitrogen aroused much interest in this kind of mycorrhiza. According to RAYNER (1922, 1925, 1929a, b) and RAY-

* Dedicated to Professor Dr. G. van Iterson, Jun.

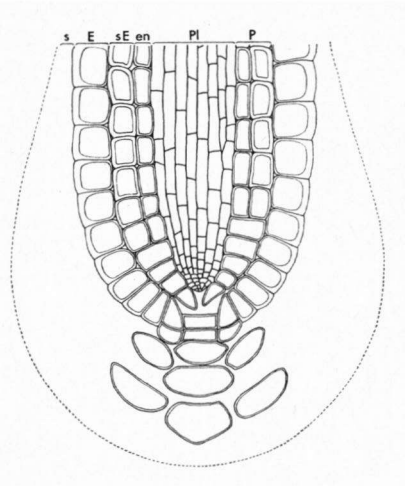

Fig. 1. Schematic drawing of median section of the root of *Vaccinium macrocarpon*.

NER & SMITH (1929) symbiotic fungi supply the plants with a part, at least, of the assimilable nitrogen needed, but this capacity was denied by FREISLEBEN (1933), BAIN (1937) and BURGEFF (1961). According to these authors the symbiotic fungi of the *Ericaceae* do not belong to the genus *Phoma*.

The taxonomic position of these fungi is difficult to establish as most strains have never been observed to sporulate. At the Centraalbureau voor Schimmelcultures in Baarn one strain could be identified as a *Cladosporium* species. Their distribution in peat areas is limited to places inhabited by *Ericaceae* (BURGEFF 1961) as shown by sterile seedlings not developing mycorrhiza if planted elsewhere in peat.

The growth of the plants is distinctly favoured by the presence of the endotrophic fungus as was found by comparing the growth of sterile with that of artificially infected plants (FREISLEBEN 1933, 1934, 1936; BAIN 1937; BURGEFF 1961). These authors also found that a fungus isolated from one species may form a mycorrhiza with plants of another species, even if the latter belongs to a different genus. A further conclusion from these investigations was, that the fungus is only to be found in the epidermis and in the slime layer of the young root. Earlier investigators had reported that it spread through the entire plant (DUFRENOY 1917; RAYNER 1922, 1925, 1929; ADDOMS & MOUNCE 1931, 1932).

The fungi may be attracted to the roots of *Ericaceae* by the tannin which is released by the epidermal cells, as this substance is digested by them (HUFSCHMIDT 1957). Subsequently, they apparently find means to enter the living epidermis somewhat nearer to the apex of the root. To the present author it seems an open question whether they find pits in the cell wall as were seen by BURGEFF (1961), as these pits may have resulted from the action of fungal enzymes.

It is, of course, highly probable that pectinase and cellulase are formed by endotrophic fungi; still, a few experiments on the presence of these enzymes have been made and the results will be reported.

The electron microscopical work was concerned with

1. the factor(s) preventing the passage of the fungus from the epidermal cells into those of the subepidermis, and
2. the submicroscopic image of the fungus in the invaded tissues at various stages in the development of symbiosis.

The necessity of cultivating sterile plants led to some additional observations on the apparent absence of the fungus in cuttings and on the influence of the fungus on the formation of roots.

2. MATERIAL AND METHODS

2.1. Plants:

From Mr. Weber, "Heidetuin", Driebergen: *Calluna vulgaris* cultivar *Beale*, cuttings, *Erica vagans* cultivar *alba*, cuttings, *Vaccinium myrtillus*, cuttings, *Vaccinium macrocarpon*, cuttings.

From Terschelling: *Vaccinium macrocarpon*, seeds.

2.2. Fungi:

Mycelium radicis myrtilli α isolated from *Vaccinium myrtillus*, *Mycelium radicis myrtilli* β isolated from *V. myrtillus*, both obtained from the Centraalbureau voor Schimmelcultures in Baarn, which received them from Freisleben.

Mycorrhiza strain 4318c isolated from *Calluna vulgaris*, Mycorrhiza strain 461 isolated from *Vaccinium oxycoccus*, Mycorrhiza strain 464 isolated from *V. oxycoccus*, Mycorrhiza strain 4714 isolated from *V. oxycoccus*, all from the Centraalbureau voor Schimmelcultures in Baarn, which received them from Burgeff.

The mycelia of all these fungi are black and resemble each other. Strain 464 has been identified with *Cladosporium macrocarpa* Preuss.

2.3. Sterile plants and plants inoculated with a known symbiotic fungus grown from seeds and from cuttings

a. Seeds from ripe berries of *Vaccinium macrocarpon* were shaken for several minutes with 30–40% H_2SO_4 in order to soften the seed-coat, rinsed with water, and sterilized by shaking with 1–2% Ca-hypochlorite for one hour. After rinsing with sterile deionized water the seeds were allowed to germinate.

a.1. The seeds were sown on sterile peat in an Erlenmeyer flask and incubated in darkness at 17°C. Germination took place after 2 weeks, and after another 2 weeks the seedlings were transferred each to a culture tube with the same peat. They were then placed in a glass house, in subdued light. Inoculation with fungi was performed after a few weeks.

a.2. Other seedlings were transferred to tubes with 2% washed agar in deionized water.

b. Twigs were submerged in 0.35% cuprooxycarbonate in plastic bags completely filled with sterilizing solution. After 2 hours the twigs in the bags were washed several times with sterile water. For cuttings, twig ends of 5–6 cm length

were used and care was taken to remove all disinfectant from them. They were planted in pots with sterile peat, or peat inoculated with one of the fungi.

To ensure rapid interaction of the fungus with the cutting, inoculated peat was prepared in the following way. Five ml of a 10% malt extract was added to a layer of peat, 2 cm thick, on the bottom of a 250 ml Erlenmeyer flask. After mixing and sterilizing, the substrate was inoculated with the fungus and incubated at 27°C. When the mycelium had covered nearly the entire surface this batch was used to inoculate several litres of sterile wet peat. After incubation at 27°C for 3 weeks during which the peat was shaken every few days, it was suitable as a substrate for the cuttings, and distributed over pots on a table in an unheated glass house (temp. 4–15°C, very high humidity). The plants were watered with deionized water.

As a rule, *Ericaceae* are propagated in September when the young shoots are well developed. In the present work the cuttings were made in March. This may have accentuated the differences between sterile and fungus-infected cultures.

2.4. Tests for cellulase and pectinase

The fungi were grown in shaking cultures in media of the following composition: 0.75 g KH_2PO_4, 0.25 g $MgSO_4$, H_2O, 0,25 g $CaCl_2$, 0.1 g $FeSO_4$, 1 ml yeast water, 1000 ml water, and either 10 g carboxymethylcellulose or 5 g Na-pectate. After incubation with the former substrate at 27°C for 21 days, the relative content of cellulase was estimated by running the decanted culture liquid through an Oswald viscosimeter at 27°C.

Pectinase activity of cultures with Na-pectate was estimated by adding 1 ml of the filtered culture liquid to 10 ml of a filtered 0.5% Na-pectate solution at pH 5.5, incubating at 27°C, and after 2.5 and 26.5 hours determining the outflow time at 27°C in the Oswald viscosimeter.

2.5. Electron microscopy

Root tips selected under a binocular microscope were cut to a length of ca 5 mm, fixed in 6.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.8 at 4°C for 3 hours or one night. After rinsing with veronalacetate buffer pH 7.2, they were post-fixed in 1% OsO_4 in veronalacetate buffer pH 7.2 at 4°C for several hours, dehydrated in a series of alcohol solutions, and via propylene oxide embedded in either epon or epon-araldite (MOLLENHAUER 1964). Other fixing agents, *e.g.* $KMnO_4$, were less successful.

Sections were cut transversely to the root axis, then post-stained with either uranylacetate (GIBBONS & GRIMSTONE 1960) or leadcitrate (REYNOLDS 1963).

3. RESULTS

3.1. Plants grown from sterile seeds of *Vaccinium macrocarpon*

Seedlings grown in culture tubes on peat if kept sterile grew to a length of only 2–3 cm and died within a few months. However, if the peat was inoculated with

one of the endotrophic fungi, most plants attained a length of 10–15 cm and did not die during the course of the experiment (*table 1*).

Table 1. Influence of strains of symbiotic fungi on growth of seedlings of *Vaccinium macrocarpon* on peat.

Number of plants	Fungus added	Number of healthy plants (10–15 cm long)	
		after 75 days	after 285 days
6	*M. r. myrtilli* α	6	5 + 1 w[1])
6	*M. r. myrtilli* β	6	5 + 1 i[2])
6	Strain 4318c	6	3 + 2 w[1])
6	Strain 461	6	3
6	Strain 464	6	4
6	Strain 4714	6	6
10	none	3 w[1])	–

[1]) w = weak plant, 3–5 cm long, [2]) i = infected by parasitic fungus.

The development of the plants was better the earlier the inoculation with the fungus was effected. Each of the isolates favoured growth of the seedlings, though there may be some difference in the effectiviness of the strains. All plants had long internodes and small leaves, characteristic, according to Freisleben and Burgeff, of lack of nitrogen.

Microscopic examination revealed that all strains had penetrated into the epidermis of the roots.

3.2. Plants germinated on peat, but grown on washed agar in deionized water

These plants developed little and rather slowly, probably because of lack of salts. This applies both to cultures with and without fungus. The roots of plants to which the fungus *Mycelium radicis myrtilli* had been added penetrated the agar and grew reasonably well (even though not all root tips developed mycorrhiza), whereas those of plants to which no symbiotic fungus had been added, though slightly better developed than on sterile peat, did not penetrate into the agar but formed numerous very short rootlets at their tips. Observations were made after 75 and 127 days.

3.3. Plants grown from sterilized cuttings on peat, in pots

Vaccinium myrtillus and *V. macrocarpon* developed much better if the peat had been inoculated with a symbiotic fungus than if it had not *(table 2)*. After one year some of the plants grown in pots with peat to which no fungus had been added were found to have developed mycorrhiza, but these plants were always retarded in comparison to plants in inoculated peat. If the cuttings had carried the fungus one would not expect so great a difference between inoculated and non-inoculated cultures; it is therefore much more probable that the fungus has

Table 2. Cuttings grown in peat with and without fungus added.

Plant species	Fungus added	Number of cuttings	Number of healthy plants	
			after 30 days	after 365 days
Calluna vulgaris	4318c	50	38	38
Calluna vulgaris	none	50	1	–
Erica vagans	461	50	1	1
Erica vagans	464	50	13	13
Erica vagans	4714	50	5	5
Erica vagans	none	50	1	–
Vaccinium myrtillus	*M.r.m.*α	50	43	43
Vaccinium myrtillus	*M.r.m.*β	50	42	42
Vaccinium myrtillus	none	50	35	35[1])
Vaccinium macrocarpon	461	50	50	50
Vaccinium macrocarpon	464	50	50	50
Vaccinium macrocarpon	4714	50	50	50
Vaccinium macrocarpon	none	50	50	50[1])

[1]) A few of these plants had mycorrhiza.

somehow infected the pots in the course of the year though the pots were not in contact with each other.

Calluna and *Erica* plants under the same conditions can only develop in the presence of a symbiotic fungus and therefore can be said to need the symbiosis more than the *Vaccinium* plants.

3.4. Electron microscopy

A transverse section just above the root meristem shows the structure of the root of *Vaccinium macrocarpon (fig. 2a)*. The top-most layer of large cells is the epidermis. The following two layers are the periblem; the outer of these two is the subepidermis; the inner might be called the endodermis. The plerome consists of thin-walled cells with large nuclei. Vessels have not yet been formed.

The epidermal cells are practically empty but for the densely stained tannin which has been precipitated during the embedding process. Precipitated tannin is also found in the two layers of the periblem, and in the slime layer, a small part of which is visible in the upper right-hand corner. The tannin in the slime layer has been released from dying epidermal cells. The precipitated tannin being much harder than the rest of the preparation, sectioning results in some parts of the material being compressed and other parts torn. In this picture, a large crack has developed between the epidermal cells and the slime layer; another crack between the tertiary wall (consisting of cellulose and therefore unstained and electron-transparent) and the thin protoplasmic layer of a subepidermal cell, the vacuole of which contains much precipitated tannin. In the plerome only a minority of the cells contain tannin. In this picture no mycorrhiza is visible except one transversely cut hypha in the slime layer, the position of which suggests that it has digested part of the tannin.

The inset *(fig. 2b)* is a magnification of a very small part of *fig. 2a*, indicated

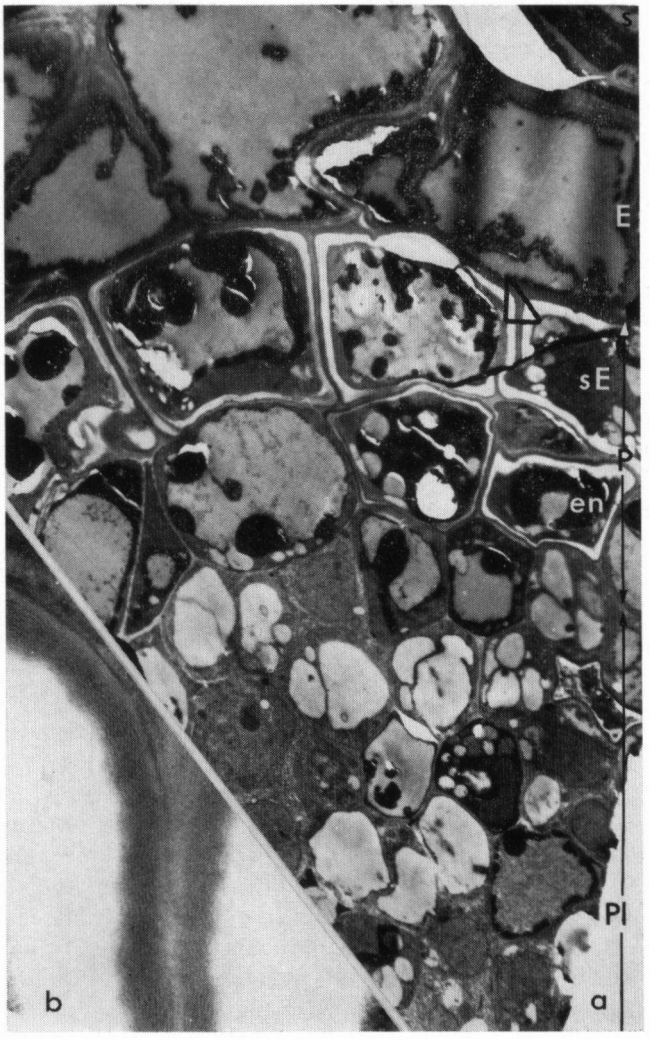

Fig. 2a. *Vaccinium macrocarpon*. Part of transverse section close to the tip of the root. Topmost are the epidermal cells (E). The periblem (P) consists of the subepidermis (sE) and the endodermis (en). The central part of the root is the plerome (Pl). Fixation: glutaraldehyde and OsO_4; post-stained with uranylacetate. Magnification 2,400×.

Fig. 2b. Part of the same section, see triangle in *fig. 2a*. The secondary wall of the subepidermal cell consists of stained layers of suberin, the tertiary wall of unstained cellulose. Magn. 24,000×.

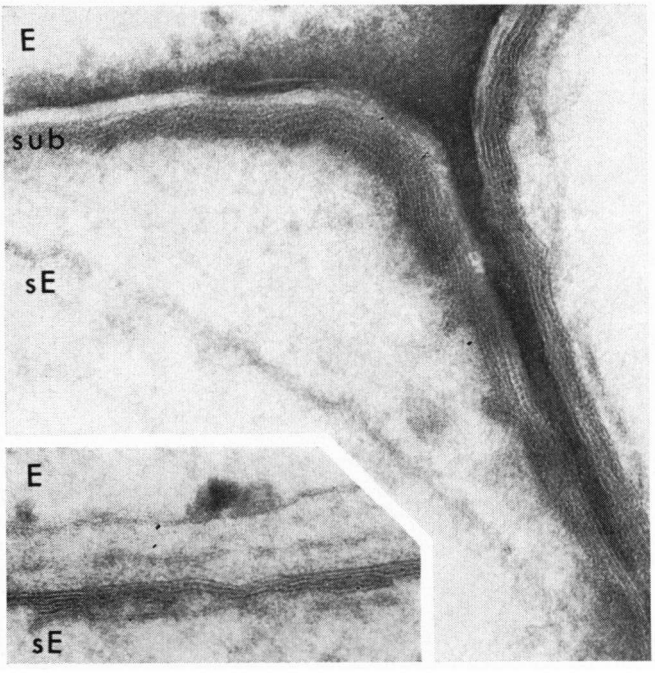

Fig. 3. In a young subepidermal cell (sE) the protoplast deposits suberin layers onto the primary wall. E = epidermal cell. Fixation $KMnO_4$. Magn. 128,000 ×.

by a small triangle. It shows the primary wall between two subepidermal cells. The pectin is slightly stained. The secondary wall consists of multiple layers which have been found to characterize suberin (FALK & EL-HADIDI 1961). The tertiary wall, quite white in this print, consists of cellulose. Closer examination of *fig. 2a* now reveals that all the subepidermal cells have suberinized walls, and that so do some of the cells of the next layer, whereas others in this stage have only a primary wall like the smaller cells of the plerome. In the young subepidermal cell these suberin layers are deposited onto the primary wall *(fig. 3)*.

The slime layer enveloping the young root often contains a few thick layers of precipitated tannin; in *fig. 4* there are two such layers. Tannin is formed inside living cells, but after the death of the epidermal cells it diffuses from the latter into the slime layer. Electron micrographs of sections always show it in the form of a precipitate; in the slime layer it presents itself as distinct layers embedded in electron-transparent material. This suggests that the tannin has never spread evenly through the slime layer.

Cross-sectioned hyphae may contain much protoplasm, but some are nearly completely empty and have become very flat. The epidermal cell in *fig. 4* still lacks the endotrophic fungus.

Epidermal cells invaded by the fungus are usually quite full of hyphae.

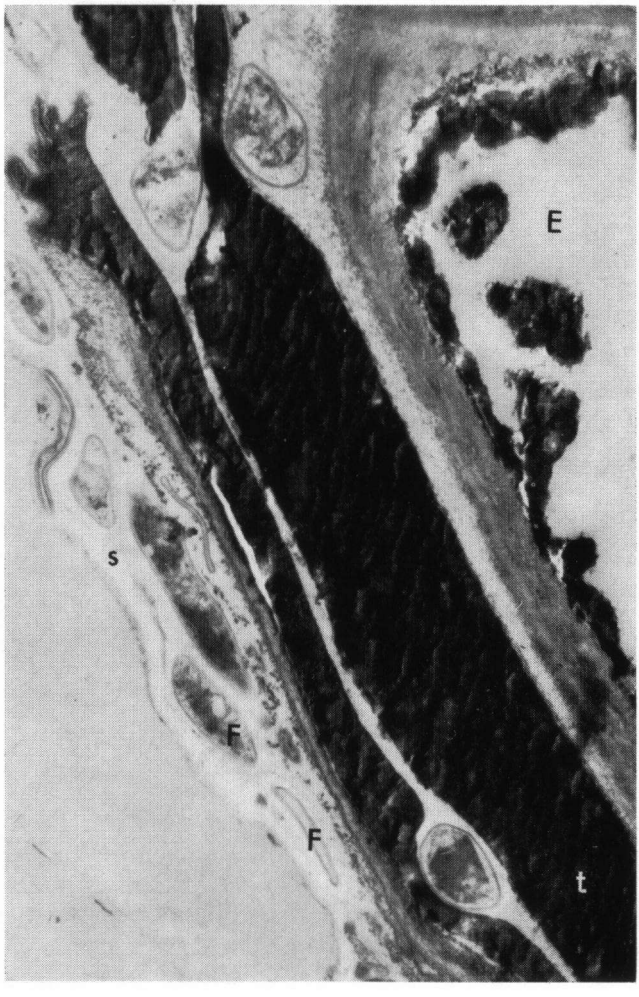

Fig. 4. *V. macrocarpon*. Part of an epidermal cell (E) with precipitated tannin (t). Thick layers of tannin and cross-sectioned fungal hyphae (F) in the slime layer (s). Some hyphae quite empty and flat. Fixation: glutaraldehyde and OsO_4; post-stained with uranylacetate. Magn. 11,200×.

Besides hyphae, *fig. 5* shows a few hardly discernable plant mitochondria. A hypha inside an outer cell wall layer seems to have digested some wall material since its immediate surroundings are more electron-transparent than the rest of this layer. Another hypha apparently caused the inner layer of the wall to swell considerably (Sw), maybe due to attack by the enzymes of the fungus.

Of the epidermal cell to the right only part of the cell wall – probably a transverse wall – appears in this section. One sees a very fine fibrillar structure but no cell contents whatever.

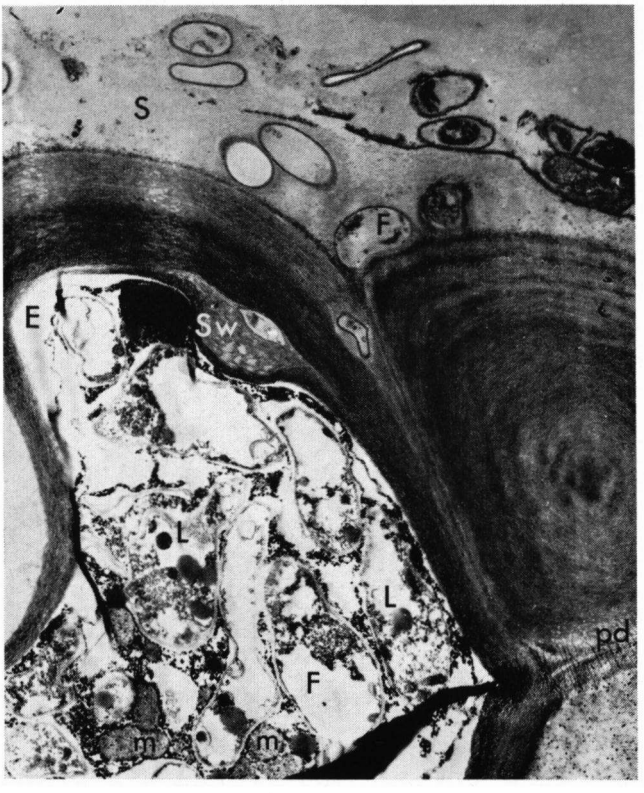

Fig. 5. *V. macrocarpon* + *M. r. myrtilli* α. Slightly oblique section farther from the tip than *figs. 2* and *3*. Epidermal cell full of young hyphae (F) enveloped by plant protoplast with tannin (black patches) and mitochondria (m). Hyphae contain lipoid droplets (L). To the right an epidermal cell the wall of which was sectioned tangentially. The cell wall between this cell and the bordering subepidermal cell shows plasmodesms (pd). Fixation: glutaraldehyde and OsO_4; post-stained with uranylacetate. Magn. 6,700×.

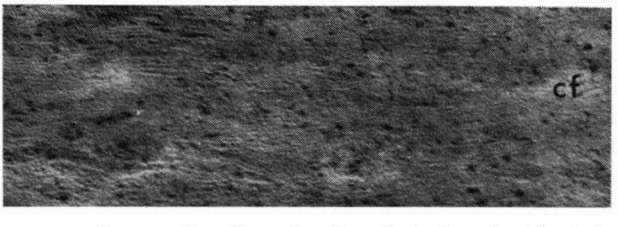

Fig. 6. *V. macrocarpon*. Cross-section through cell wall shadowed with platinum. Cellulose fibrils (cf) protruding from matrix. Fixation: Glutaraldehyde and OsO_4. Magn. 144,000 ×.

When growing inside the cell wall, the fungus tends to creep between the successive cellulose layers. This is demonstrated in *fig. 7*, where cellulose fibrils show up as thin white lines in a darker matrix, the electron-density of which is due to post-staining, probably of pectin, with uranylacetate. The fungus cell has stretched parallel to these fibrils. This does not apply to the cell of which a part is seen at the bottom of the micrograph. The disarrangement of the cellulose

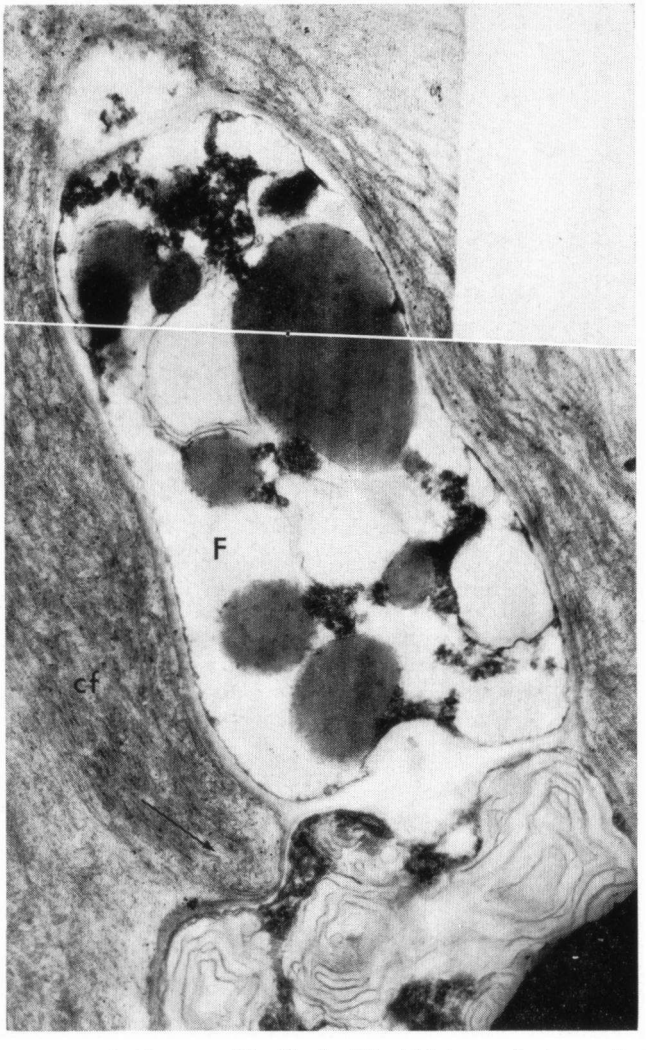

Fig. 7. *V. macrocarpon* + *M. r. myrtilli* α. Hypha (F) which has pushed apart the layers of the cell wall of an epidermal cell. Cellulose fibrils (cf) show up as white lines; at → the hypha has broken its way through a cellulose layer. Fixation: Glutaraldehyde and OsO_4; post-stained with uranylacetate. Magn. 35,000 ×.

fibrils (arrow) suggests that, at this location, the fungus has broken a way through some of the cellulose fibril layers of the cell wall.

The fungus may also press apart the primary walls of the epidermal cells and the subepidermal cells. The subepidermal cell in *fig. 8* is characterized by the multiple suberin layers in its secondary wall. As in the preceding micrograph the cellulose fibrils in the wall of the epidermis cells show up as white lines, but here the matrix is more heavily stained (with Pb-citrate). To ascertain that the white lines are fibrils, a similar section was not post-stained, but shadowed with platinum; the fibrils are now visible as low ridges *(fig. 6)*. Apparently, on the

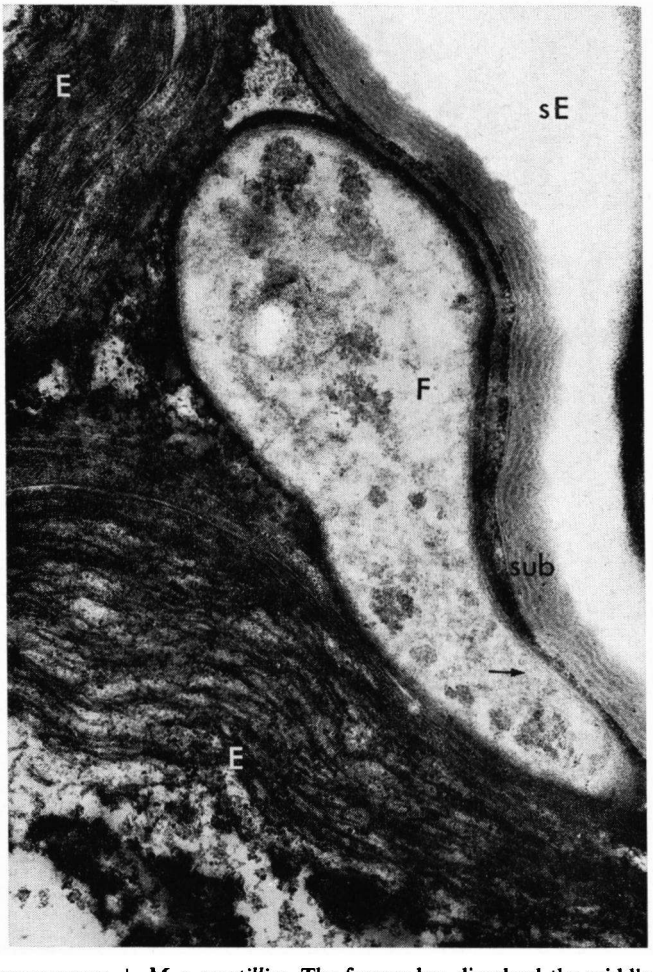

Fig. 8. *V. macrocarpon* + *M. r. myrtilli* α. The fungus has dissolved the middle lamella between epidermal cells (E) and a subepidermal cell (sE). At → also part of the primary cell wall of the latter has been dissolved, but the suberin (sub) has not been attacked. Fixation: Glutaraldehyde and OsO_4; post-stained with Pb-citrate. Magn. 52,000 ×.

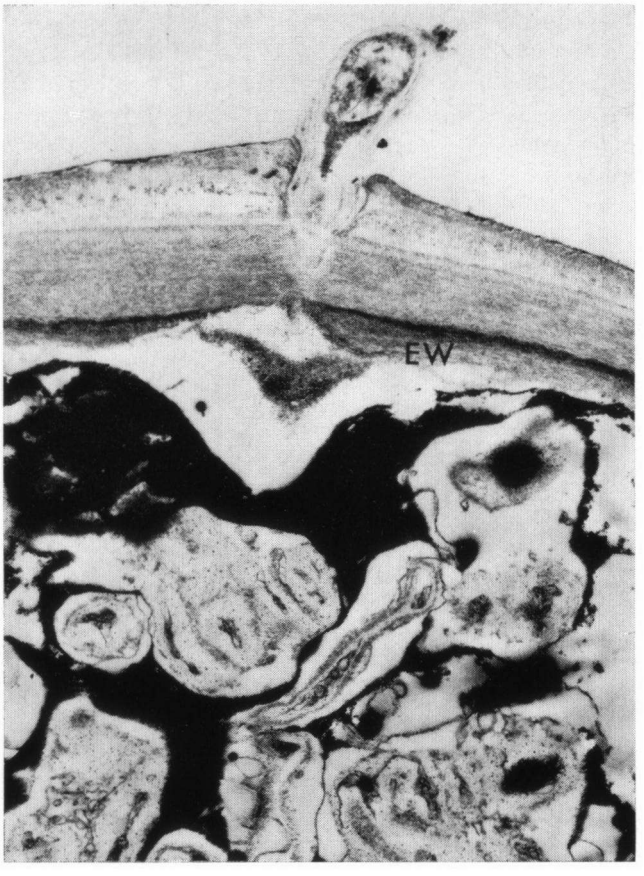

Fig. 9. *V. myrtillus* + *M. r. myrtilli* α. Oblique section through fungal hypha entering an epidermal cell. EW = extra cell wall layer. Fixation: Glutaraldehyde and OsO_4; post-stained with uranylacetate. Magn. 9,600 ×.

surface of the section, the cellulose fibrils protrude a little above the matrix (compare MASER, O'BRIEN & MCCULLY 1967). Also the closer packed cellulose fibrils of the tertiary wall of the subepidermis can be demonstrated with this technique.

The chance that a section will show the place where the fungus has penetrated the cell wall is very small, but *fig. 9* shows a probably oblique section through a pore in the wall completely filled with a fungal hypha. The rounded forms of the edges of the outermost layer of the cell wall suggest that the fungus has made a pore by dissolving the cellulose and pectin of the wall. The epidermal cell in this micrograph is nearly filled up with mycelium and dark masses of precipitated tannin.

The mycelium within the epidermal cells is eventually digested by the plant protoplasm. Fibrillar rests of a hyphal cell wall are shown in *fig. 10.*

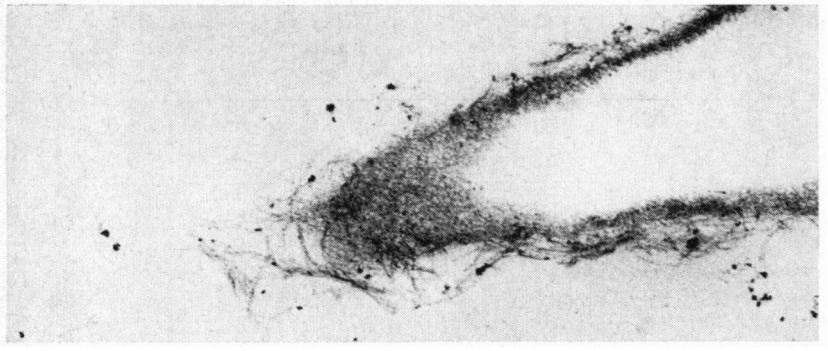

Fig. 10. *V. macrocarpon.* Fibrillar rests of digested hyphal cell wall in epidermal cell. Fixation: Glutaraldehyde and OsO_4; post-stained with uranylacetate. Magn. 44,000 ×.

3.5. The presence of cellulase and pectinase

Since symbiotic endotrophic fungi obviously must have means for entering the cells – in this case only the epidermal cells – they may be expected to possess enzymes for dissolving the cell wall. The latter consisting mainly of cellulose and pectin, these enzymes would be cellulase and pectinase. A few experiments have been made to ascertain the presence of these enzymes in carboxymethylcellulose- or Na-pectate-grown cultures of the symbiotic fungi used in the present work. The tests for cellulase and pectinase have been described under "Material and Methods". The results are given in *tables 3* and *4*.

The results of these tests show that all the symbiotic fungi obtained from the C.B.S. have cellulase and pectinase. This does not prove, in itself, that they are symbionts, but a fungus lacking cellulase and pectinase is unlikely to form an endotrophic mycorrhiza. During the present work three isolates were made from roots of *Vaccinium macrocarpon* from Terschelling; only one of these had cellulase and pectinase and therefore could be a symbiont, but experiments with sterile seedlings grown in peat infected with this strain proved that it was not, since none of the plants developed well.

Table 3. Break-down of carboxy-methylcellulose by symbiotic fungi of *Ericaceae* in 21 days at 27 °C, as evidenced by decreased viscosity of decanted culture liquid estimated with an Oswald viscosimeter.

Fungus		Time of outflow in seconds
Mycelium radicis myrtilli α		26
Mycelium radicis myrtilli β		22.8
Mycorrhiza from *Calluna vulgaris*	4318c	36.8
Mycorrhiza from *Vaccinium oxycoccus*	461	26.5
Mycorrhiza from *Vaccinium oxycoccus*	464	35.5
Mycorrhiza from *Vaccinium oxycoccus*	4714	23.9
No fungus added		65.5
Time of outflow of pure water		19.2

Table 4. Break-down of Na-pectate by enzymes in filtered culture liquid from Na-pectate-grown symbiotic fungi of *Ericaceae*, as evidenced by decreased viscosity of 0.5% Na-pectate solution estimated with an Oswald viscosimeter.

Culture liquid of:	Time of outflow in seconds	
	after 2.5 hours	after 26.5 hours
Mycelium radicis myrtilli α	23	
Mycelium radicis myrtilli β	23.5	
Mycorrhiza from *Calluna vulgaris*	161	44.5
Mycorrhiza from *Vaccinium oxycoccus* 461	30	
Mycorrhiza from *Vaccinium oxycoccus* 464	58	23
Mycorrhiza from *Vaccinium oxycoccus* 4714	24	
Culture liquid heated to 100 °C	240	

3.6. Consequences of the walls of the subepidermal cells being suberinized

The finding reported above that the walls of the subepidermal cells are suberinized raised the question whether parts of the roots with such cells are still able to take up dissolved substances, *e.g.* break-down products of hyphae in epidermal cells. If not, this task could only be performed by the root tips.

Young plants of *Vaccinium macrocarpon* from culture tubes were carefully cleaned of adhering peat. The roots were spread on the bottom of a large petri dish. From one plant (A) the tips of some of the roots were fixed between a cover glass and a piece of filter paper *(fig. 11)*. From another plant (B), older

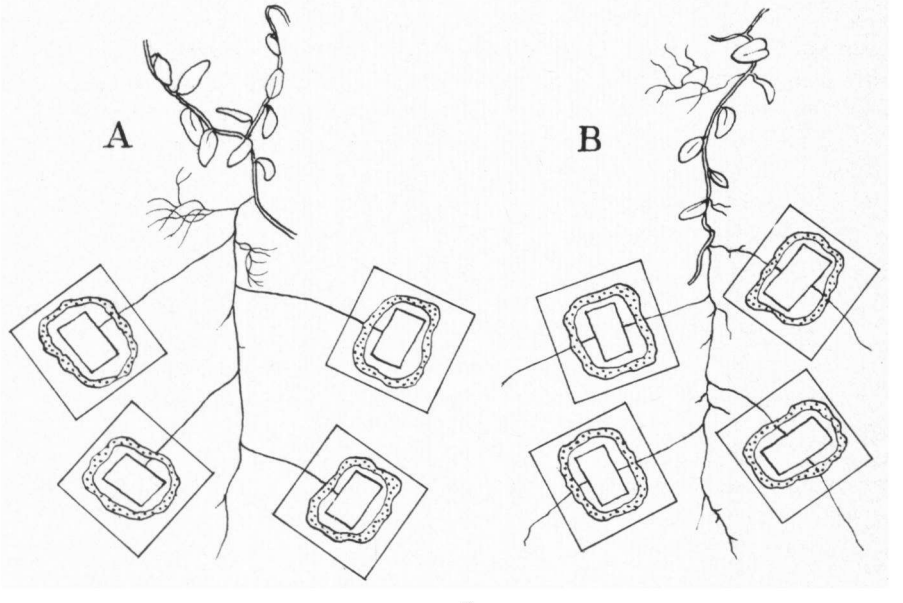

Fig. 11. Schematic representation of experiment with DL-serine-l-^{14}C.

parts of the roots where the subepidermal cells might be expected to have suberinized cell walls were used. A paraffin ridge prevented moisture applied to the filter paper from reaching any part of the root outside the enclosed area. After administering the test liquid (10 µl of 0.3% DL-serine-l-^{14}C), the top leaf was picked immediately, the next leaf after 30 minutes, etc. The leaves were dried under an infra red lamp, then glued onto a glass strip, and the latter placed on photographic film and left there for one month. After development of the film, leaves containing some serine-l-^{14}C showed up on the film. With plant A, the first leaf visible had been picked 90 minutes after the experiment started, and with plant B, this period had been 165 minutes. So one may conclude that assimilation of the amino acid had taken place in a part of the root with suberinized subepidermal cell walls, but it was also found that assimilation at the root tip had been faster. No subepidermal cells without suberin, nor pits in the suberinized walls were found which might have suggested how transport of dissolved substances through the subepidermis is effected.

4. DISCUSSION

The positive results of the culture experiments with seedlings and cuttings of the North American *Vaccinium macrocarpon* and the fungus of the European *V. oxycoccus* agree with the conclusions of FREISLEBEN (1933, 1934), BAIN (1937) and BURGEFF (1961) that the symbiotic fungi of the *Ericaceae* are not species-specific. The results also corroborate these authors' finding that the fungus only occurs in the roots.

Although a great many preparations were examined in the electron microscope, no fungal hyphae have ever been found in the subepidermis. Probably this layer keeps free of fungi by means of the suberin layers which were never found to have been attacked by the fungus. The only place open to further invasion by the fungi is the root apex where no suberinized cell walls occur. This way the fungus may infect the plerome, the root tip dies, and no mycorrhiza is formed.

The results of the cellulase and pectinase tests do not agree with those of KOX (1954). This may be due to the latter author using filter paper as a source of cellulose instead of the less resistant carboxymethylcellulose, and probably to lack of aeration of the nutrient media in the test for pectinase.

Key to labelling

cf	cellulose fibrils	L	lipoid	s	slime
E	epidermis	m	mitochondrion	sE	subepidermis
en	endodermis	P	periblem	sub	suberin
EW	extra cell wall thickening	pd	plasmodesmata	Sw	cell wall swelling
F	fungus	Pl	plerome	t	tannin

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