

Ectomycorrhizal Fungi of *Salix Rotundifolia* Trautv.

I. Impact of Surface Applied Prudhoe Bay Crude Oil on Mycorrhizal Structure and Composition.

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ABSTRACT. The effects of exposure to crude oil on the structure and quantity of viable mycorrhizae of the dwarf deciduous shrub, *Salix rotundifolia* Trautv., have been investigated at Barrow and Cape Simpson, Alaska over a three year period on experimental plots treated with 5 and 12 l m⁻² of Prudhoe Bay crude oil. *Salix rotundifolia* populations growing adjacent to the Cape Simpson natural oil seep were examined for possible changes which may have occurred as the result of long term exposure to oil. Structural examination of mycorrhizae was accomplished by light and scanning electron microscopy. Structural difference in viable mycorrhizae were observed between control and oil treated plots one year after the application of oil. Ectomycorrhizae with smooth mantle surfaces were found to predominate on the Barrow control plot. The predominant viable mycorrhizae on the oil treated plots demonstrated a marked proliferation of mantle hyphae, presumably a result of the altered soil environment. Prudhoe Bay crude oil applied at 12 l m⁻² caused a large reduction in the number of viable willow mycorrhizae within one week at Barrow. After this rapid initial response, the rate of destruction of mycorrhizae appeared to proceed at a slower rate throughout the remainder of the growing season. The effect of oil in depressing the number of viable mycorrhizae was still apparent three growing seasons after the application of oil. *Salix rotundifolia* growing adjacent to the Cape Simpson oil seep demonstrated greater numbers of viable mycorrhizae and a higher percentage of *Cenococcum graniforme* (Sow.) Ferd and Winge. mycorrhizae than did plants at Barrow.

RÉSUMÉ. On a analysé les effets d'un "brut" répandu, sur la quantité et la structure des parasites mycologiques d *Salix rotundifolia*, en bon état, cela en utilisant des pollutions expérimentales à Barrow, Alaska, et également un suintement naturel de pétrole à Cap Simpson, Alaska. Du "brut" de Prudhoe Bay, répandu sur la base de 12 litres/m², a causé une grande réduction du nombre de parasites de saules, en bon état, cela en une semaine, à Barrow. Après ce résultant initial rapide, il est apparu que le rendement de destruction de ces parasites mycologiques, se stabilisait à un niveau plus lent pendant toute la saison de croissance. Les effets de pétrole étaient encore très visibles, trois saisons de croissance plus tard, après l'épandage du pétrole. At cette époque, il y avait beaucoup moins de parasites mycologiques sains sur les plantes croissant dans des sols traités au pétrole, en comparaison de plantes témoins. On démontrait que *Salix rotundifolia*, grandissant à proximité, sur les suintements naturels de pétrole de Cap Simpson, avait une quantité plus grande de parasites mycologiques, en bon état, et un pourcentage plus élevé de parasitage par "*Cenococcum grainforme*" que les plantes témoins à Barrow. On observait des variations dans les structures, des parasites entre les plantes témoins et les échantillons traités au pétrole, à la lumière et au microscope électronique à balayage. On n'a pas pu déterminer si ces différences étaient le résultat de modifications des parasites, liées à l'épandage du pétrole ou le résultat d'un envahissement par de nouveaux mycetes. Traduit par Alain de Vendigies, Aquitaine Co. of Canada Ltd.

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INTRODUCTION

With the advent of exploitation of Arctic oil reserves, numerous studies have been conducted on the effects of crude petroleum spills on Arctic plant communities. In tundra ecosystems these studies have concentrated primarily on the impact of crude oil on aboveground plant tissues (Freedman and Hutchinson, 1976; Wein and Bliss, 1973). These studies have shown that crude oil significantly reduces the viable aboveground biomass and productivity very soon after contact with vegetation. Recovery time after exposure to oil varies according to season of application and the plant community involved. Freedman and Hutchinson (1976) have reported species of deciduous shrubs, such as *Salix glauca* L. to be the least susceptible to damage by oil. They found that these plants, with lateral buds protected from direct contact with the toxic components of fresh crude oil, were able to quickly restore some plant cover on oil spill areas.

In studies dealing with effects of terrestrial oil spills in the Arctic, root systems have generally received less attention than shoot systems. McCown *et al.* (1973a) indicated that oil spills result in root injury which may lead to winterkill of root systems. These effects may not become apparent until several growing seasons have elapsed. Hutchinson *et al.* (1974) have reported a 30% decrease in belowground biomass of *Carex rostrata* Stokes one year after the application of crude oil at 3 l m⁻². Linkins *et al.* (1977) have described the effects of crude oil spills on the respiration of roots of grasses and sedges from coastal Arctic tundra. During the course of the Arctic summer, after a July 1, 1975, oil spill at levels of 5 or 12 l m⁻², root respiration of grasses and sedges decreased. This decrease was greater and occurred more rapidly in drier soils. To date, however, the effects of crude oil on the root systems of deciduous shrubs remain largely uninvestigated.

Trappe (1962) reported that many species of the Salicaceae form ectomycorrhizae. Katenin (1964) examined members of the genus *Salix* in the tundra of the U.S.S.R. and found ectomycorrhizae associated with all species. Stutz (1972) reported *Salix arctica* Pallas to be mycorrhizal on the Truelove Lowland in the Canadian Arctic, and Miller *et al.*, (1974) reported all four species of *Salix* present at Barrow, Alaska, to be ectomycorrhizal. The adaptive significance of these root fungal symbiotic relationships under conditions of a short growing season, as found in Arctic tundra, has been discussed by Meyer (1973). Ectomycorrhizae greatly increase the functional surface area of the root system available for mineral and nutrient uptake. This increased input apparently enables the plant to complete its annual growth and reproductive cycle in a shorter period of time. According to Harley (1975) the mantle, or fungal sheath, of an ectomycorrhiza plays a significant role in the continued success of plants in areas where climate limits plant growth, or can disrupt growth during a normal growing season. The mantle acts as a specialized storage organ for minerals and nutrients, which can be transported from the mantle to the plant root in times of need, such as the beginning of the growing season. Ling-Lee *et al.*, (1975) have shown the presence of

polyphosphate granules in the fungal mantle, thus lending support to this hypothesized role for ectomycorrhizae.

The role of mycorrhizal associations in ecologically perturbed areas in the temperate zone, such as coal mine spoils or industrial wasteland, is well documented (Daft *et al.*, 1975; Daft and Hacskaylo, 1977; Marx, 1975; Medve *et al.*, 1977; Schramm, 1966). Schramm (1966) demonstrated that mycorrhizal plants growing on anthracite coal spoils exhibited normal growth and physiognomy. Those non-mycorrhizal plants which could survive on the spoils were invariably stunted and showed signs of severe nitrogen deficiency. Representatives of the genus *Salix* were among those plants shown to require ectomycorrhizal associations for successful growth on the coal spoils. To date, no information is available on the occurrence of mycorrhizae or their role in soils perturbed by oil spills. Thus, despite the importance of *Salix* in reestablishing plant cover on oil spill areas and the significance of mycorrhizae to the survival of *Salix* in tundra plant communities, no investigations have been conducted on the effects of oil on *Salix* mycorrhizal associations.

The goals of the present study were to assess the impact of short and long term exposure to oil on the root system of *Salix rotundifolia*. *Salix rotundifolia* was chosen for this study because of its presence at Barrow, Alaska, and its known ability to form ectomycorrhizae (Miller *et al.*, 1974). This species is also known to be present adjacent to the Cape Simpson oil seep which makes it possible to evaluate changes which may have occurred as the result of long term exposure to oil. Impact of oil was monitored by following changes in the structural aspects and quantities of viable mycorrhizae occurring between control and oil treated areas. Structural changes were followed by the use of light and scanning electron microscopy. Quantification of viable mycorrhizae was obtained by root tip counts.

MATERIALS AND METHODS

Site Description:

Plots approximately 5 m² were chosen at the U.S.I.B.P. Tundra Biome Site 1, at Barrow, Alaska, which were of a homogeneous vegetation type dominated by *S. rotundifolia*. Control and oil treated plots were established on 1 July 1975. The oil treated plots received 5 and 12 l m⁻² respectively of surface applied Prudhoe Bay crude oil. A second 12 l m⁻² plot for root tip counts was established in the same area on 4 July 1977. Plots at the Cape Simpson natural oil seep, number 2, were established with *S. rotundifolia* as the dominant vegetation. These areas were as close to the active seep as possible.

Experimental Techniques: Detailed examination of the structure of the *S. rotundifolia* root systems was begun in the summer of 1976. Sampling was initiated on 3 July 1976, and was conducted at weekly intervals until 7 August 1976. The Cape Simpson oil seep was sampled once on 25 July 1976. Samples were taken as cores 5.5 cm in diameter to a depth of 8.0 cm (Laursen, 1975).

By the onset of the 1976 sampling period well over 50% of the original *S. rotundifolia* cover had been destroyed on both oil spill treatments established in 1975. Because structural investigations were concerned only with those roots surviving in the post spill environment sampling on oil treated plots was biased in that cores were taken only from areas containing live *S. rotundifolia* plants.

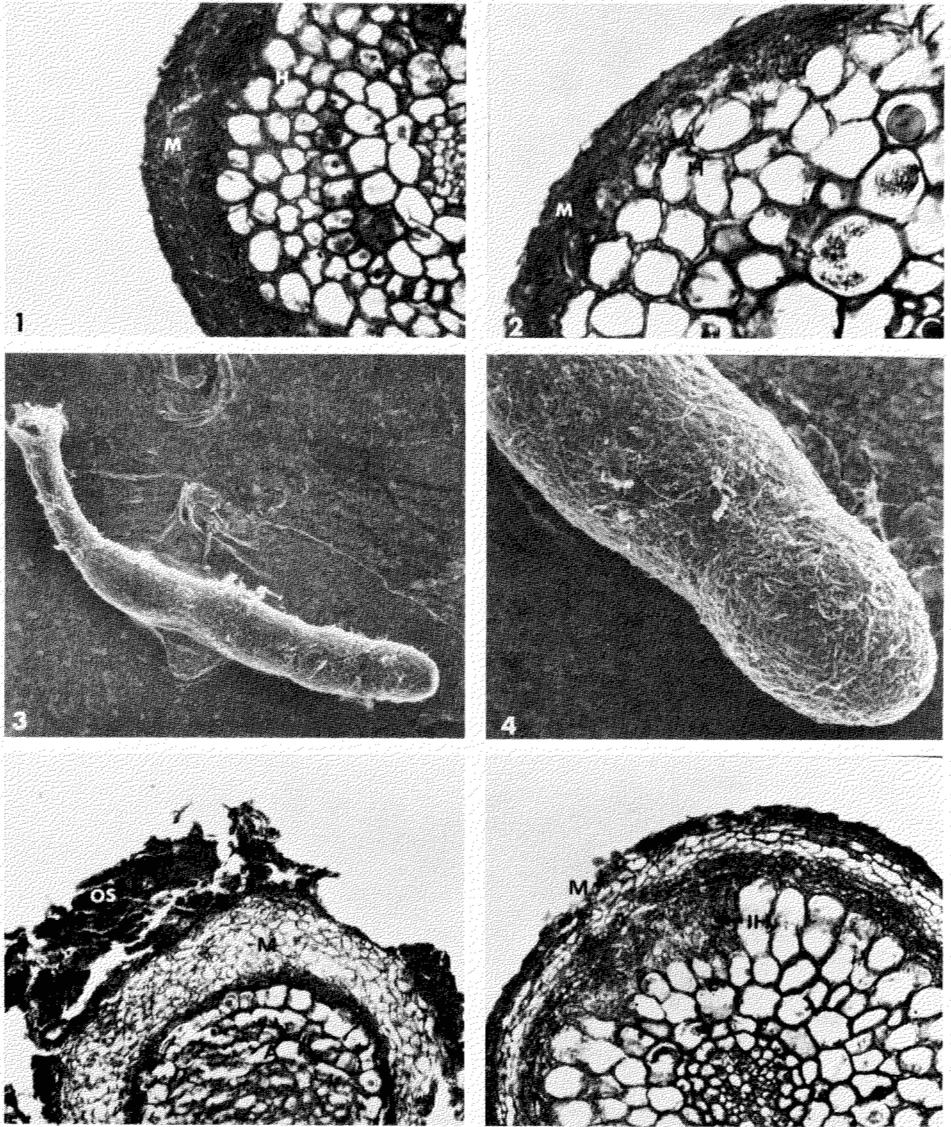
Roots were separated from soil under a stream of cold running water. Roots which demonstrated no obvious signs of damage were numbered and described macroscopically. Roots for light microscopy were fixed in 3% glutaraldehyde in 0.1 M Na_2HPO_4 - NaH_2PO_4 buffer, at pH 7.0, for 3–6 hr at room temperature, then washed and stored in buffer in a refrigerator. Following this, they were dehydrated, embedded in paraffin and sectioned. Sections cut at 5–7 μm were stained according to Gurr (1965). Duplicate samples were prepared for SEM examination according to the technique of Kinden and Brown (1975) and then examined in an AMR-900 scanning electron microscope.

Root tip counts were made using cores 8 cm in length and 2.5 cm in diameter. Generally, five or six cores were taken per plot, with the exception of the 1975 oil spill plots, where the paucity of live plants allowed for only two replicates per sample date. The 1977–12 1 m^2 plot was sampled at weekly intervals during the summer of 1977. The 1975 oil spill plots and the Cape Simpson oil seep were sampled on three separate dates. Roots were separated from cores under a stream of cold running water immediately upon arrival at the laboratory. Viable mycorrhizae were counted according to the criteria of Harvey *et al.*, (1976) and information gained from microscopic examination of roots collected during the 1976 field season.

RESULTS

Two forms of mycorrhizae were commonly found on the control plot at Barrow. The first was a monopodially branched system with the individual branches reaching 1–2 cm in length. The reddish-white mantle was smooth and ranged in diameter from 23–46 μm , with a mean of 35(5.6) μm (Fig. 1). The mantle was formed of tightly interwoven small diameter hyphae (Fig. 1), whereas the hartig net was well developed penetrating to the innermost cortical layer. No intracellular hyphae were observed. The second form had a smooth white mantle (Figs. 3, 4), ranging in thickness from 12–43 μm , with a mean of 22(7.7) μm , and having a well developed hartig net with no intracellular penetration (Fig. 2). Clamped hyphae were occasionally seen attached to this mycorrhiza, indicating that the symbiont was a basidiomycete.

The predominant mycorrhizal type from the 5 1 m^2 plot formed monopodially branched mycorrhizae, the individual branches of which averaged 2–3 cm in length. The smooth reddish-brown mantle ranged from 26–58 μm in thickness, with a mean of 37(7.9) μm . The mantle consisted of



Salix rotundifolia mycorrhizae.

FIG. 1. Cross section of reddish-white ectomycorrhiza from control plot, with thick mantle (M) and Hartig net (H). $\times 400$.

FIG. 2. Cross section of white mycorrhiza from control plot showing thinner mantle (M). $\times 600$.

FIG. 3. SEM preparation of white mycorrhiza from control plot. $\times 50$.

FIG. 4. SEM of the same mycorrhiza showing smooth mantle surface. $\times 200$.

FIG. 5. Cross section of reddish-brown mycorrhiza from 5 l m² treatment with oil impregnated soil (OS), mantle (M), and root apical region (A). $\times 400$.

FIG. 6. Cross section of same mycorrhiza with intracellular hyphae (IH). $\times 400$.

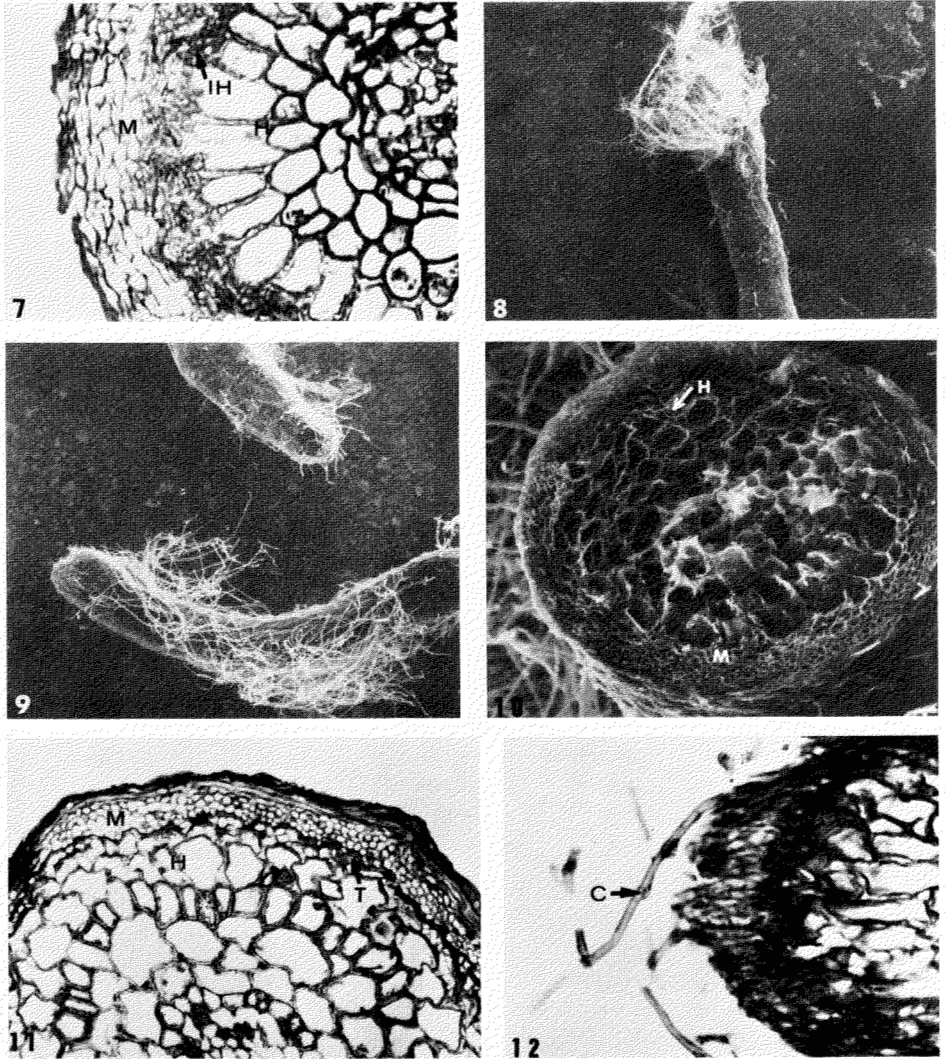
two distinct layers (Figs. 6,7), the outer layer was composed of large pseudoparenchymatous fungal cells whereas the inner layer consisted of extremely small, densely interwoven hyphae. The hyphae of the inner mantle formed a typical hartig net, but also invaded root cap cells and cells of the outer cortex (Figs. 5,6,7). The presence of intact nuclei in the apical region of this type was used as a criterion for establishing its viability (Fig. 5).

The most commonly encountered ectomycorrhiza form on the 12 1 m² plot was a form with numerous branches 1-2 mm long. The yellow-brown mantle gave rise to numerous wefts of clamped mycelium (Figs. 8,9,10,12) indicating a basidiomycete symbiont. The mantle ranged in thickness from 14-40 μm , with a mean of 22.5(7.0) μm . The hyphae of the inner mantle were arranged parallel to the long axis of the root (Figs. 10,11). The hartig net was poorly developed and usually penetrated only one cell layer into the cortex; no intracellular hyphae were observed. A second commonly encountered mycorrhiza from the 12 1 m² treatment formed monopodially branched mycorrhizae with branches 5-7 mm in length. The gray mantle of this mycorrhiza was also characterized by aggregates of attached clamped mycelium (Figs. 13,14).

The most common mycorrhiza of *S. rotundifolia* growing adjacent to the Cape Simpson oil seep was formed by *Cenococcum graniforme*. The fungus formed jet-black ectomycorrhizae identical to those described by Hatch (1934). The enlarged unbranched tips of the mycorrhiza gave rise to stiff black hyphae (Figs. 15, 16). Sections tangential to the mantle surface demonstrated the unique hyphal arrangement described by Hatch (1934) and Trappe (1969) (Fig. 17). The mantle ranged in thickness from 12-35 μm , with a mean of 23(6.6) μm , which is similar to the range reported by Marx and Davey (1969) for the same fungus associated with pine roots. The hartig net penetrated to the inner cortical cells and showed no signs of intracellular penetration (Fig. 18).

During the two study seasons it was observed that all of the *S. rotundifolia* short roots examined from all sites were ectomycorrhizal. Many morphological forms of ectomycorrhizae were observed when conducting root tip counts, but few are as easily recognizable as that formed by *C. graniforme*. Thus root tip counts were broken down into only two categories, those mycorrhizae formed by *C. graniforme* and those formed by fungi other than *C. graniforme*.

Mycorrhizal root tip counts collected from plots at Barrow, following application of oil at 12 1 m² in 1977, showed a rapid decrease in the number of viable mycorrhizae in treatments relative to the control (Fig. 19a) beginning one week after the application of oil and continuing throughout the remainder of the sampling period. On each of the sample dates, except for 2 August, the number of viable willow mycorrhizae in oil treated plots was approximately half the number found in the control. The seasonal mean value for viable mycorrhizae for the 12 1 m² treatment was 55% that of the control plot (Table 1). During the same sampling period the number of viable mycorrhizae in the control plot increased to a peak on 19 July, and then gradually decreased until the final sample on 9 August (Fig. 19a).



Salix rotundifolia mycorrhizae.

FIG. 7. Cross section of reddish-brown mycorrhiza with dimorphic mantle (M) and intracellular hyphae (IH). $\times 400$.

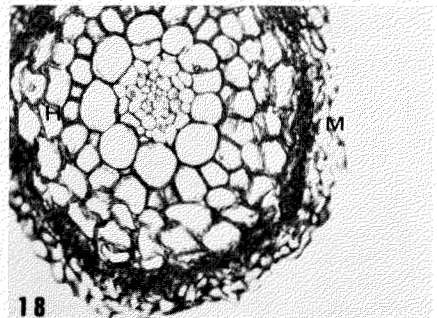
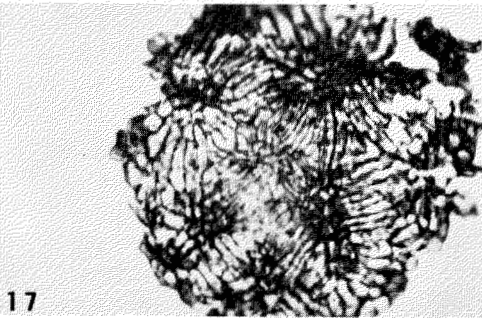
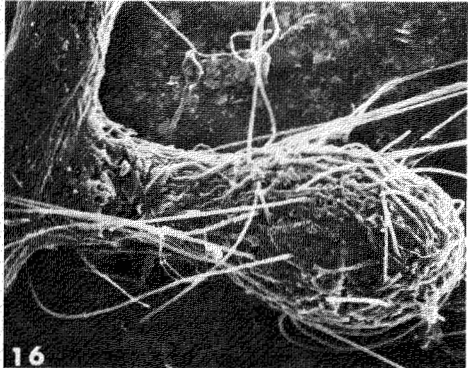
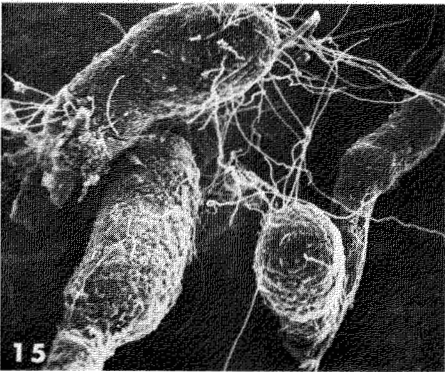
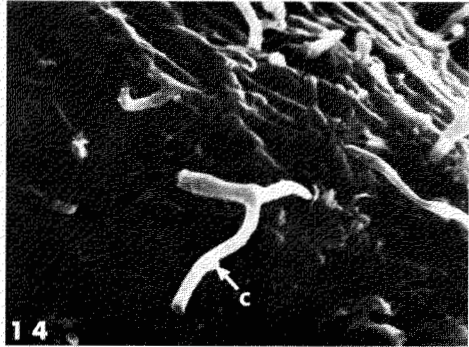
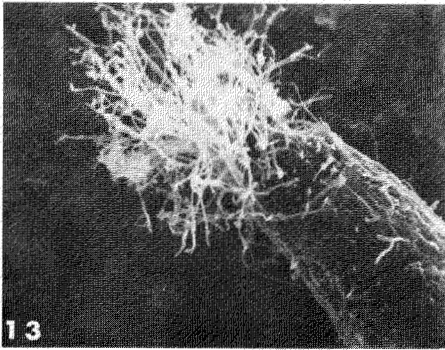
FIG. 8. SEM preparation of yellow brown mycorrhiza from 12 1 m² treatment with hyphae emanating from mantle surface. $\times 100$.

FIG. 9. SEM preparation of the same mycorrhiza. $\times 100$.

FIG. 10. SEM of the same mycorrhiza in cross section with mantle (M) and Hartig net (H) evident. $\times 500$.

FIG. 11. Cross section of the same mycorrhiza with mantle (M), Hartig net (H), and tannin cells (T) evident. $\times 400$.

FIG. 12. Oblique section of the same mycorrhiza showing attached mycelium and clamp connection (C). $\times 450$.



Salix rotundifolia mycorrhizae.

FIG. 13. SEM preparation of gray mycorrhiza from 12 m^2 treatment showing proliferation of mantle hyphae. $\times 200$.

FIG. 14. SEM of the same mycorrhiza showing clamp connection (C). $\times 1000$.

FIG. 15. SEM preparation of *Cenococcum graniforme* mycorrhizae from Cape Simpson, Alaska. $\times 100$.

FIG. 16. SEM of *C. graniforme* from Cape Simpson, Alaska, showing stiff hyphae attached to mantle. $\times 200$.

FIG. 17. Tangential section to mantle of *C. graniforme* from Cape Simpson showing typical hyphal arrangement. $\times 1000$.

FIG. 18. Cross section of the same mycorrhiza showing mantle (M) and hartig net (H). $\times 400$.

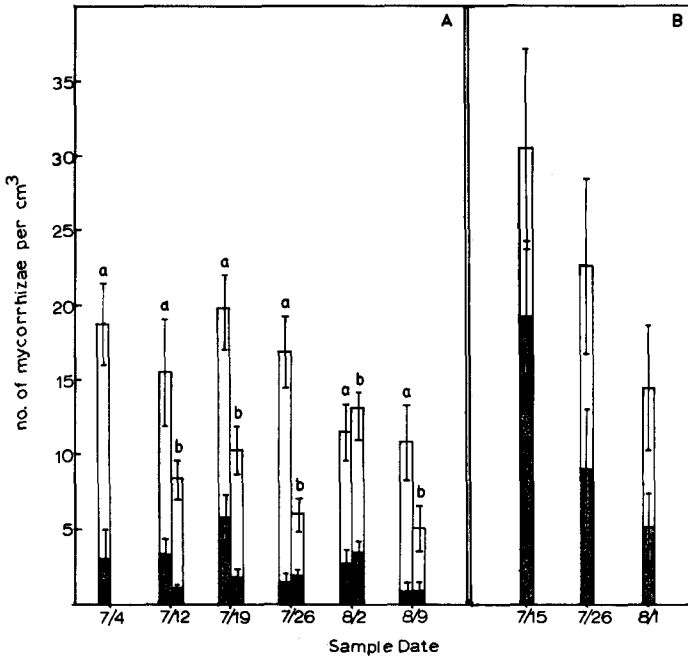


FIG. 19. Results of the 1977 *S. rotundifolia* root tip counts from (A) Barrow, (a) control and (b) 1977 12 l m⁻² plots and from (B) Cape Simpson oil seep. Shaded area indicates the mean number (\pm SE) of mycorrhizae formed by *C. graniforme*. The values of (A) are the average (\pm SE) of six cores, while the values of (B) are the average (\pm SE) of five cores.

The number of mycorrhizae formed by *C. graniforme* relative to the total number of mycorrhizae in controls ranged from 7 to 30% with a mean for the sample period of 18% (Table 1). The percentage of *C. graniforme* mycorrhizae from the 12 l m⁻² plot ranged from 12 to 30% with a mean of 21% (Table 1).

Plots to which oil was applied at 5 and 12 l m⁻² levels in 1975, were sampled on only three occasions in 1977 due to the scarcity of living willows on these sites. The results of the root tip counts from the 1975 oil spill plots are summarized in Table 1. The seasonal mean value of viable mycorrhizae for the 5 l m⁻² plot three years after application of oil was 70% that of the control. Approximately 14% of the viable mycorrhizae from this treatment were formed by *C. graniforme*. The number of viable mycorrhizae from the 12 l m⁻² treatment was 16% that of the control values. Of these, less than 1% were formed by *C. graniforme*.

The results of root tip counts from *S. rotundifolia* growing adjacent to the Cape Simpson oil seep (Fig. 19b) show a decrease in the number of viable mycorrhizae from the first sample date on 16 July to the final sample date on 1 August. The seasonal mean for number of viable mycorrhizae (Table 1) at Cape Simpson is approximately 32% higher than the seasonal mean for the Barrow control. At Cape Simpson the number of mycorrhizae formed by *C. graniforme* relative to the total number of mycorrhizae ranged from 63 to 31%, with a mean for the sample period of 45% (Table 1).

TABLE 1. Annual means for viable mycorrhizal root tip counts of *Salix rotundifolia* for 1977

Site	Sample size (n)	Mean \pm SE (mycorrhizal tips per cm ²)	Range	% <i>C. graniforme</i>
Barrow, Alaska				
Control	36			
Total mycorrhizae		15.4 \pm 1.5	10.7 - 19.3	
<i>C. graniforme</i> mycorrhizae		2.8 \pm 0.7	0.8 - 5.8	18
1977 12 l m ⁻² spill				
Total mycorrhizae	30	8.5 \pm 1.4	5.0 - 13.0	
<i>C. graniforme</i> mycorrhizae		1.8 \pm 0.5	0.9 - 3.4	21
1975 5 l m ⁻² spill				
Total mycorrhizae	6	10.9 \pm 4.4	3.8 - 19.5	
<i>C. graniforme</i> mycorrhizae		1.5 \pm 1.2	0.1 - 3.7	14
1975 12 l m ⁻² spill				
Total mycorrhizae	6	2.5 \pm 0.1	2.3 - 2.7	
<i>C. graniforme</i> mycorrhizae		0.1 \pm 0.1	0.0 - 0.3	0.04
Cape Simpson, Alaska				
Total mycorrhizae	15	22.6 \pm 4.6	14.4 - 30.4	
<i>C. graniforme</i> mycorrhizae		12.2 \pm 3.5	5.1 - 19.2	54

DISCUSSION

From the results of studies on control plots at Barrow, it appears that the dominant mycorrhizal forms of *S. rotundifolia* can be characterized as having smooth mantles with few protuberant hyphae. These forms are true mycorrhizae (Marks and Foster, 1973), in that they form a distinct mantle and hartig net, with no apparent intracellular invasions of root cortical cells. However the most abundant mycorrhizal form on the 5 l m⁻² treatment one season after application of crude oil appears highly modified. The dimorphic nature of the fungal mantle and the intracellular invasion of apparently healthy cortical cells are not typical of ectomycorrhizae. The intracellular penetration of the outer cortical cells gives this association a strong resemblance to certain ectendomycorrhizal associations (Zak, 1976). These associations are thought to be rather rare under natural conditions, but have been reported by Mikola *et al.*, (1964) to occur on the roots of pine seedlings growing in soil exposed to heavy burning. The thick outer mantle described in the present study does not, however, agree with the typical concept of ectendomycorrhizae, which, according to Marks and Foster (1973), has a very thin or nonexistent mantle. It is possible that exposure to oil stressed the root by removing its carbohydrate supply through the destruction of the leaves. Assuming this were to happen, the balanced symbiotic relationship would shift in favor of the fungus thus allowing intracellular invasion of cortical cells to take place. The dimorphic nature of the fungus mantle is possibly the result of the differentiation of the extant symbiont into two distinct cell types. However it might also be the result of the weakening or death of the original symbiont as the result of exposure to oil, and subsequent invasion by a new mycorrhiza forming fungus.

The most interesting aspect of the two most commonly encountered mycorrhizae from the 12 l m⁻² treatment was the very strong development of fungal hyphae from the mantle surface. According to Zak (1973), it is reasonable to assume that mycorrhizae with attached mycelium are more effective at absorbing nutrients than mycorrhizae with smooth mantles as the result of increased surface area. It also seems likely that these mycorrhizae are very effective at absorption of water. The presence of oil at both levels was seen to decrease the amount of soil moisture in these plots and may well have contributed to the abundance of these types of mycorrhizae (Linkins and Antibus, 1978). Skinner and Bowen (1974) were able to stimulate the development of dense masses of fungal mycelium from the mantles of mycorrhizae by means of soil sterilization techniques. They attributed this stimulation to factors such as abatement of competition for available resources or the release of nutrients from organisms destroyed by the sterilization. It seems possible that the application of 12 l m⁻² crude oil could also elicit such a response from certain mycorrhizae which were able to survive its initial impact.

From the results of these studies, it is apparent that structural differences do exist between mycorrhizae of *S. rotundifolia* found in control and oil spill areas within a year after exposure to oil. The noted structural differences, however,

may arise in several different ways. It is possible that a mycorrhizal complex, which survived exposure to oil becomes modified such that it is better adapted to function in the oil impregnated soil environment. The proliferation of mantle hyphae in mycorrhizae exposed to oil may merely be a phenotypic response to a new set of soil conditions. Alternatively, certainly mycorrhizal fungi which are present in low numbers prior to the spill may have spread their infection subsequent to the spill and become the dominant fungal associates in the post spill environment. Finally, totally different symbiotic fungi, not present prior to the spill, may have invaded the spill area and become established in this environment. Which of these possible explanations best describes the findings of this study cannot be determined at this time. The solution to this problem holds potential for future research in this area, and is of importance in understanding revegetation of oil spill areas.

The results of the root tip counts from Barrow control and the Cape Simpson oil seep would indicate that the number of viable willow mycorrhizae has reached a maximum by the middle of the growing season and declines thereafter. Oil applied at 12 l m^{-2} destroys approximately half of the viable mycorrhizae within a week. The response of these root systems to oil appears to closely resemble that of shoot systems where contact with oil causes very rapid destruction of leaves (Freedman and Hutchinson, 1976). After this initial rapid response, the number of viable mycorrhizae continue to decline relative to the control, but at a slower rate. The rate of root death may decline after the first week as the oil becomes less toxic, due to the volatilization of light weight components (Sexstone and Atlas, 1977). It is also possible that this decrease in the rate of root destruction is more apparent than real due to the initiation of new short roots by surviving long roots.

Root tip counts from plots established in 1975 demonstrate that even after three years there is a marked reduction in the number of viable roots due to oil. The reduction of viable roots is even greater than indicated by root tip counts, since these were from cores taken in areas with living plants. The reduction in number of viable mycorrhizae was closely tied to the reduction in living shoot material. In neither plot was there any significant plant cover at the end of three seasons. Death of *S. rotundifolia* plants on these sites appeared to be due to desiccation, as the symptoms were similar in every respect to those described for *Salix glauca* by Hutchinson *et al.*, (1976). They suggested desiccation was the result of the inability of roots to supply shoots with an adequate water supply. The destructive effects of oil appear to have been compounded in the present study by a pair of extremely dry growing seasons in 1976 and 1977.

The seasonal mean value for the number of viable willow mycorrhizae was found to be much higher for Cape Simpson than Barrow. According to Meyer (1964), differences in number of mycorrhizae per volume of soil can be most closely tied to differences in soil properties. The availability of nutrients and water appear to be the key determinants of the number and percentage of roots which develop and become mycorrhizal. Limitations of nutrients or water seems to enhance root development. Meyer (1964) considers the

number of suberized tips per volume of soil to be the best index of a plant's ability to absorb water. Willows growing near the Cape Simpson seep are found in very dense, tarry soils. The hydrophobic nature of these soils may explain why there are more mycorrhizae formed by *C. graniforme* at Cape Simpson relative to Barrow. Meyer (1964) and Worley and Hacskaylo (1959) using potted plants watered at different intervals have been able to demonstrate an increase in the relative frequency of *C. graniforme* mycorrhizae in plants exposed to periodic drought. Katenin (1972) also reported that *C. graniforme* occurred on unfavorable sites in the Russian Arctic. Mexal and Reid (1973) demonstrated that the optimum growth of *C. graniforme* in pure culture occurred at far lower water potentials than the other mycorrhizal fungi they tested. The high percentages of *C. graniforme* mycorrhizae at Cape Simpson are consistent with the idea that this fungus is well suited to sites of low soil moisture. It is interesting to note that Schramm (1966) did not find *C. graniforme* to be important in the colonization of mine spoils in Pennsylvania, however, he did report that water was not a factor restricting the establishment of plants on these sites. Recently, Medve *et al.* (1977), reported that plant growth and invasion onto strip mines areas could be increased by inoculating these sites with *C. graniforme*. Their results point to the possible potential of this fungus in reclaiming ecologically disturbed sites.

It has been suggested by McCown *et al.*, (1973b) that long term exposure to oil may have led to the development of plant populations tolerant of oil at Cape Simpson. The occurrence of high numbers of *C. graniforme* mycorrhizae found in the present study in tarry soils adjacent to the Cape Simpson oil seep may be taken as indication that this fungus has become well adapted to this environment. Current studies utilizing pure cultures of this fungus are aimed at determining how tolerant it is to exposure to drought and hydrocarbons. Should this fungus prove to be highly adapted, it will be of potentially great value in the reclamation of oil spill areas in the Arctic, by virtue of the wide range of hosts it is known to infect (Trappe 1964).

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