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ORIGINAL ARTICLE

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Morphometric analysis of *Pinus banksiana* Lamb. root anatomy during a 3-month field study

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Abstract Tree roots are variable in their growth rates, alternating between periods of elongation and dormancy. This variability may have a strong influence on root anatomy. In the present study, field-grown *Pinus banksiana* Lamb. roots were divided into four distinct anatomical regions (i.e. white without mycorrhizae, white with mycorrhizae, condensed tannin, and cork). Changes in root growth, the proportions of the root system occupied by the various regions, and cortical plasmalemma surface area (CPSA) were determined for 6- to 9-month-old ectomycorrhizal *P. banksiana* seedlings during a 3-month period (August through October) in northern Ontario. The region in which the greatest change in length occurred was the condensed tannin zone, which was also the dominant contributor to root length (up to 74% of total). The roots of seedlings grown under artificial conditions had the same zones but in different proportions compared to roots in the field. A correlation was noted between increased root growth, low metacutization, and high soil water availability. The CPSA data were assumed to be a factor influencing ion uptake capacity in a positive manner. Interestingly, increases in CPSA were not directly correlated with changes in root length for field-grown seedlings. The primary contributor to CPSA in the field-grown roots was the ectomycorrhizal zone (approximately 80%). In comparison, the bulk (85%) of the CPSA in the chamber-grown roots was found in the white root region. The conditions under which the seedlings were grown strongly influenced the anatomy of their roots.

Key words Root anatomy · Metacutization · Plasmalemma · Suberin · *Pinus banksiana*

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Introduction

Roots of pouch-grown *Pinus banksiana* Lamb. were previously divided into three distinct anatomical regions, i.e. the white, tannin, and cork zones (McKenzie and Peterson 1995a,b; Fig. 1). The white zone has viable central cortical cells and passage cells (endodermal cells

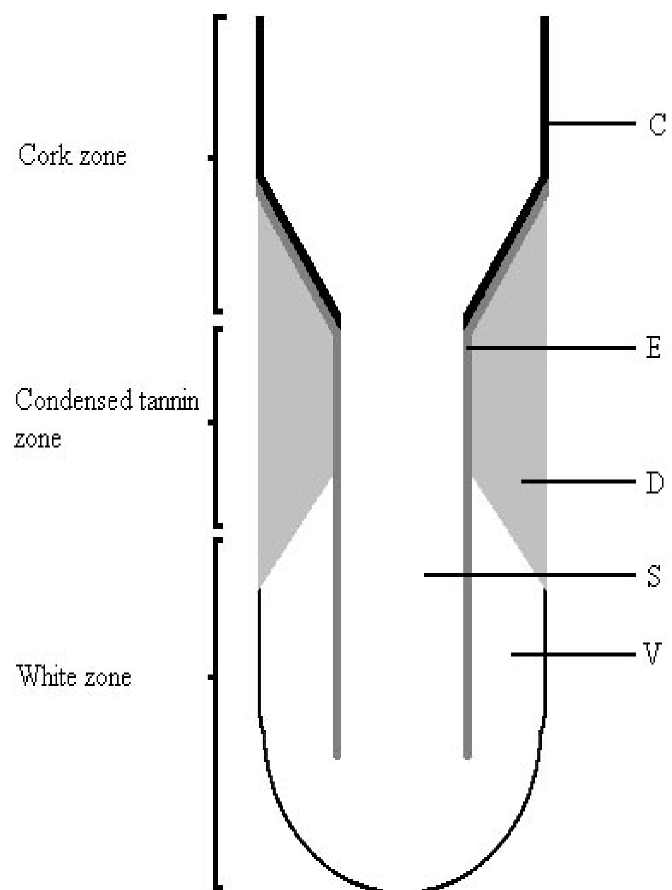

Fig. 1 Representation of three anatomical zones found in a woody root (C=Casparian band, E=endodermis, D=dead cortex, S=stele, V=viable cortex)

without suberin lamellae) in its more mature regions. Unlike the white zone, the cortex of the condensed tannin zone (change in term discussed in Peterson et al. 1999) is dead but the passage cells remain. The cork zone, which is superficially similar to the condensed tannin zone, is characterized by a continuous layer of dead, suberized phellem cells. A number of woody root studies published before the work of McKenzie and Peterson (1995a,b), such as that of Kramer and Bullock (1966), defined roots as either suberized (brown) or non-suberized (white), failing to distinguish between the condensed tannin and cork zones, both of which are brown. For an excellent discussion of root zone terminology, see Richards and Considine (1981).

Ion uptake should vary substantially amongst the root zones identified by McKenzie and Peterson (1995a,b). For a cell to be even potentially capable of taking ions into the symplast, it must be alive and have access to the soil solution. Within the root, access is limited by Casparian bands in the anticlinal walls of the endodermis (Baker 1971; Robards and Robb 1974). Thus, in the white zone, the plasmalemmae accessible to the soil solution are those of the cortex, including the outer membrane of the endodermal passage cells (Peterson and Enstone 1996). In the condensed tannin zone, the relevant plasmalemma would be reduced to the outer tangential faces of the passage cells, and in the cork zone would be reduced to zero. *P. banksiana* roots do not have a coherent epidermis (Wilcox 1954), and the cork zone is so sparingly permeable to ions (unpublished data) that it was treated as impermeable.

In the field, roots commonly undergo changes that influence their anatomy and function. Ectomycorrhizae are a prevalent symbiosis between pine roots and fungi (see Smith and Read 1997). The fungal partner in an ectomycorrhizal symbiosis consists of three components, i.e. the Hartig net, the fungal mantle that ensheathes the white root tip distal to the condensed tannin zone, and the extramatrical hyphae (see Piché et al. 1983). In mycorrhizal associations, especially in infertile soils, the fungus supplies some fraction of certain essential ions to the plant by absorbing them with its extramatrical hyphae and eventually passing the ions to the plant at the Hartig net-cortical cell interface. There is convincing evidence that phosphorus (Melin and Nilsson 1950) and nitrogen (Finlay et al. 1989) are delivered in this manner by ectomycorrhizal fungi. Since these ions are delivered to the cortical apoplast, the cortical plasmalemma surface area (CPSA) is a factor which will influence the plant's capacity for ion uptake from the fungus. Uptake of some other ions may be enhanced by ectomycorrhizae, but the mechanisms by which absorption takes place in the extramatrical hyphae and mycorrhizal root are less clear. These ions may be taken up by plant cells without the active involvement of the fungus. In an ectomycorrhizal root, the possibility for such ion uptake depends, at least in part, on the ion passage through the fungal mantle. At present, there is no consensus regarding the permeability of this structure (Ashford et al. 1988, Behrmann and Heyser 1992).

Another anatomical change roots undergo is metacutization (also referred to as metacutinization), a temporary state in which a layer of cells ensheathing the root apical meristem and connecting to the endodermis deposits suberin in its walls (Wilcox 1954). It is generally accepted that a root with a metacutized tip is non-growing. As the root resumes growth, it will burst through the modified cell layer (Wilcox 1954). Although the metacutis has long been recognized (Mòller 1906; Mager 1913; Plaut 1918), it has received little attention recently.

The growth of tree roots is known to vary over the course of a year. In temperate climactic zones, roots commonly cease growing in summer and winter, with flushes of growth occurring in spring and fall (Wilcox 1962b). The causes of this hiatus are believed to be drought in the summer, and the combination of low water availability and low temperatures in the winter (Wilcox 1968). One impact of lack of root growth is that further soil exploration by roots is prohibited.

In the present work, we examined changes in the anatomy of 6- to 9-month-old, field-grown *P. banksiana* roots during the late summer and autumn in northern Ontario. The observations of McKenzie and Peterson (1995a,b) were extended from soil-free pouches [consisting of a paper backing on which the nutrient solution was held within a flat, plastic pouch (Piché et al. 1983)] to pot-grown and field-grown roots that were in a more natural environment. At each time of sampling, several measurements were made, including the total root length and the length of each root region, as well as the total number of root tips and the number of each type of root tip (growing or metacutized). In addition, dimensions and numbers of cortical cells were determined for each root zone. From these measures, the CPSAs for each root zone and the total root were determined. Growth chamber-grown seedlings were analyzed in the same manner, to extend the observations from the field to seedlings grown under different conditions. Growing seedlings in pots allowed the introduction of soil as a growth medium while retaining growth-chamber conditions. Through this study, we were able to show the contribution of each root zone to mycorrhizal root systems characteristic of soil-grown roots, and how the root growth rate and CPSA changed over the specified time period.

Materials and methods

Plant material

Growth chamber

In early January 1997, *Pinus banksiana* Lamb. seeds were stratified in cold water for 48 h, rinsed, and planted in 0.175-m-deep by 0.0375-m-diameter containers filled with 70% peat moss and 30% medium, horticultural grade vermiculite. The containers were held in a growth chamber at Mikro-Tek (Timmins, Canada) with day conditions 25°C, 35% humidity, light intensity 150–250 μmol/m^2/s, 18 h, and night conditions 18°C, 90% humidity. Seedlings were watered to saturation twice weekly with tap water. Two weeks post-planting, they were inoculated by drenching the soil mix with a concentrated slurry of *Hebeloma cylindrosporum*

(Romagnesi) prepared from solid agar culture plates. Ten weeks later, the seedlings were removed from the containers and the root systems gently rinsed free from soil with a spray of water. The shoot was then removed and the roots were used immediately for the viability, permeability and histochemical tests. Other roots were preserved in a mixture of alcohols (85% ethanol, 15% methanol) until they were analyzed for root zone lengths, root tip type and number, and cellular dimensions.

Field

Seeds of *P. banksiana* were germinated in Mini-Jiffy pouches (mesh cylinders 18 mm in diameter by 83 mm in height packed with finely sifted peat**,** Jiffy Products, Shippagan, Canada) in early February of 1997 at LaFleur Nursery (Timmins, Canada). The seedlings were misted with river water every other day and fertilized (20-20-20 Plant Products, Brampton, Canada) once a week through a boom apparatus. A liquid inoculum of *H. cylindrosporum* was applied through the boom on 18 March. On 10 June, 60 seedlings were transferred to the Kamiskotia Lake flats east of Mt. Rob on a site previously occupied by *P. banksiana*. Once in the field, the roots rapidly penetrated the Mini-Jiffy pouches and entered the surrounding soil. Soil conditions were very sandy, and competition from surrounding plants was minimal.

Soil temperature and moisture were measured every 2–4 weeks from the time of planting to the end of the sampling period. Temperatures were measured at roughly 1000 hours, approximately 0.15 m below the surface by insertion thermometers, at four randomly chosen sites within the plot. Soil moisture was determined gravimetrically, using four 100- to 150-g replicate samples from approximately 0.15 m below the surface. The soil was dried to a constant weight at 95°C in a convection oven.

Root sampling

On the first sampling date (4 August), five seedlings were taken back to the laboratory and used for the viability, permeability and histochemical tests, as in the chamber-grown roots. For the first and remaining sampling dates (8 September, 2 October, 17 October), ten seedlings were randomly selected. After first removing a large quantity of soil to ensure that no roots were severed, the root system was gently shaken to remove excess soil. The seedlings and adhering soil were placed in plastic bags and transported to the laboratory. Within 90 min, the root systems were washed free of soil using a spray of water, the shoots removed, and the roots stored in the alcohol mixture.

Root structure

The anatomy of soil-grown *P. banksiana* was investigated as in the previous study of McKenzie and Peterson (1995a,b). Tests included permeability, vitality and histochemical analyses. On the basis of the results obtained, soil-grown roots could be divided into four zones, i.e. white without mycorrhizae, white with mycorrhizae, condensed tannin, and cork.

The total number of root tips was counted in each of the ten sampled root systems. During this procedure, tips were categorized as brown, white, or mycorrhizal with the aid of a dissecting microscope (Olympus S061). Samples of each root tip type were assessed for metacutization by staining longitudinal sections with Sudan red 7B (Brundrett et al. 1991). The red-stained, metacutized layer was visible under a microscope (Nikon Labophot) using white light.

Root measurements

Total root length was calculated for both the growth chamber- and field-grown seedlings by the gridline-intersect method (Tennant 1975) with a 40×40 mm grid. The length of the white root zones was determined using a ruler. The length of the mycorrhizal zone was determined by first averaging the lengths of 20 fungal mantles and then multiplying this value by the total number of mycorrhizal root tips. Condensed tannin and cork zone lengths were more difficult to establish. For the latter, cross-sections were made basipetally from the distal end of the condensed tannin zone on each root and viewed under a Nikon Labophot microscope with blue light (exciter filter 485–540, barrier filter 515 W, dichroic mirror 400). Autofluorescent cork cells were obvious, when present, as a continuous ring internally adjacent to the endodermis. The cork zone was considered to begin from the first appearance of autofluorescent cork tissue. The total length of the remaining root was measured using a ruler, and the length of the condensed tannin zone was determined by subtracting the mycorrhizal, white, and cork root zone lengths from the total root length.

The CPSA of each root zone was determined by calculating the total plasmalemma surface area of the cells that were alive with access to the soil solution. For the white zone, it was necessary to determine the CPSA contribution for cortical cells including the endodermal passage cells. To determine the plasmalemma surface area of the former, the average diameter and length of the cortical cells external to the endodermis was determined. Each cell was assumed to be a regular cylinder with flat ends, and the plasmalemma surface area was assumed to be equal to the cell surface area. The CPSA contribution of an average cell was then multiplied by the average number of cells in a cross-section. Using the cell length data, the contribution of cortical cells per millimeter of white root length could be determined. The passage cell contribution was determined by multiplying the average width of the outer tangential face of a passage cell in cross-section by the average number of passage cells. This value was multiplied by 1 mm to determine the contribution per millimeter of root length. Refer to Kamula et al. (1994) for more details on these calculations. The mycorrhizal zone CPSA was calculated as in the white zone. Within the condensed tannin zone, only the passage cells could contribute to the CPSA. The cork zone was assumed not to contribute to the CPSA of the root system.

Calculations of CPSA

Cortical cells

In the white and mycorrhizal zones, the central cortical cells contributed to the CPSA. The contribution of the cortical cells was calculated as follows.

The tangential and radial plasmalemma surface area for all cortical cells in a 1 mm length of root, S_1 (mm^2), was determined by Eq. 1. Each cell was treated as if it were 1 mm long, and the slight overestimation this introduces (by ignoring the transverse walls of each cell) was disregarded.

$$S_1=n(h\cdot 2\pi r_c) \quad (1)$$

where h=1 mm (assumed cell height), r_c=average radius of a cortical cell in cross-section (mm) and n=the average number of cortical cells in a cross-section.

The transverse plasmalemmae surface area contribution for a 1 mm length of root, S_2 (mm^2), was determined by Eq. 2.

$$S_2=n(\pi r_c^2)\cdot 2h\cdot h_c^{-1} \quad (2)$$

where h_c=average cortical cell height (mm), and the remainder of the abbreviations are as in Eq. 1. Note that the 2 accounts for the two membranes at the ends of each cell.

Finally, the total contribution of the central cortical cells was determined by adding S_1 to S_2.

Passage cells

The contribution to CPSA of the passage cells per 1 mm of root length, P (mm^2), was determined by Eq. 3.

$$P=n_p\cdot w_p\cdot h \quad (3)$$

where n_p=the average number of passage cells in cross-section, w_p=the average width of the outer tangential face of a passage cell, and h=1 mm.

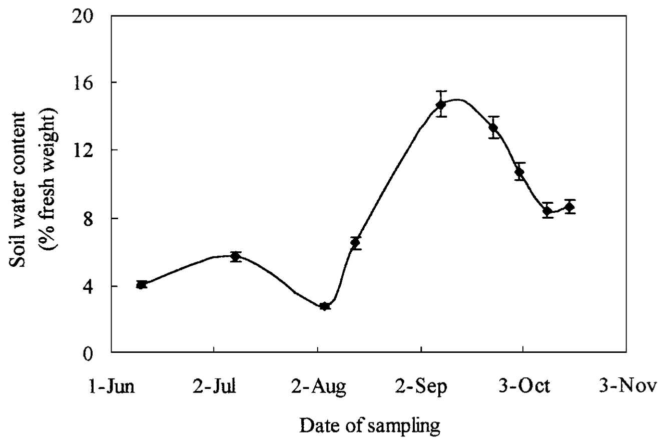

Fig. 2 Percent moisture (±SD) of the soil 0.15 m below the surface at the Kamiskotia Lake site over the time course of the experiment (10 June to 17 October), (*n*=4)

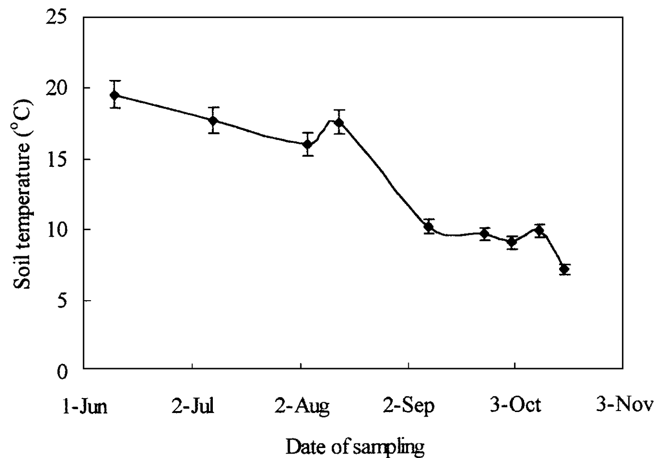

Fig. 3 Soil temperature (±SD) 0.15 m below the surface from the Kamiskotia Lake site over the time course of the experiment (*n*=4)

Results

Field conditions

Soil moisture fluctuated from being very dry (2.8–6.5%) in June, July, and August to wetter in September and October with a maximum of 15% (Fig. 2). It should be noted that northern Ontario experienced a dry summer and fall in 1997, so that soil moisture levels may have been lower than average. Soil temperature declined rather steadily over the measured period, from 19.5°C on 10 June to 7.1°C on 17 October (Fig. 3).

Root anatomy

The three anatomical zones previously described by McKenzie and Peterson (1995a,b) were also present in field-grown roots (data not presented), along with ectomycorrhizal white roots that were not present in their study. Histochemical tests confirmed the presence of condensed tannins in the cortex of the condensed tannin zone. Likewise, brown root tips were found to have condensed tannins in their cortex. Thus, in brown root tips, the condensed tannin zone occupied the root region that was previously white. The white and condensed tannin zones were permeable to berberine (an apoplastic dye) up to the endodermis, while the cork zone was impermeable at its outer surface (data not shown). The only viable cells exposed to the soil solution by the apoplastic continuum were the cortical cells (including the endodermal passage cells) of the white zone and the passage cells of the white and condensed tannin zones.

Contributions of the anatomical zones to total root length

Root growth over the observed time period was variable (Fig. 4). The average total root length per seedling on the first date measured (4 August) was 3.6 m, and it had increased less than 0.5 m by 8 September. However, from 8 September to 2 October a substantial increase in average root length (more than 2 m) was observed. There was little change in the overall root length between 2 October and 17 October.

At all ages, most of the root length consisted of condensed tannin zone, ranging from 72% to 74%. This zone increased substantially in length during the 8 September to 2 October interval. The mycorrhizal zone consistently occupied a lesser fraction of total root length (about 16%), as did the cork zone (about 10%). The white zone represented a very small fraction of the total root length (about 2%).

The total and zonal lengths of the growth-chamber pines differed markedly from those of the field-grown seedlings (Fig. 4). Despite being considerably younger than the field-grown trees (12 weeks compared to 6 months), the root systems of growth-chamber seedlings were over 1.5 m longer than their field-grown counterparts. This may have been a result of the small pouches in which the field-grown seedlings were initially held, as the roots tended not to leave the pouch until they were placed in the field, but were more likely due to the suboptimal environment for tree growth encountered in the field. By the first sampling date, the root system had extended beyond the volume of the Mini-Jiffy pouch. A second striking difference between the field- and chamber-grown pines was that the white zone of the latter roots contributed over 10% of the total root length.

Root tip frequency and structure

The most striking result from an analysis of the field-grown seedling root tips is that a very small fraction (less than 10%) of them were white (Fig. 5). The majority (at least 75% of the total) were mycorrhizal. In fact, it was an increase in the number of mycorrhizal root tips

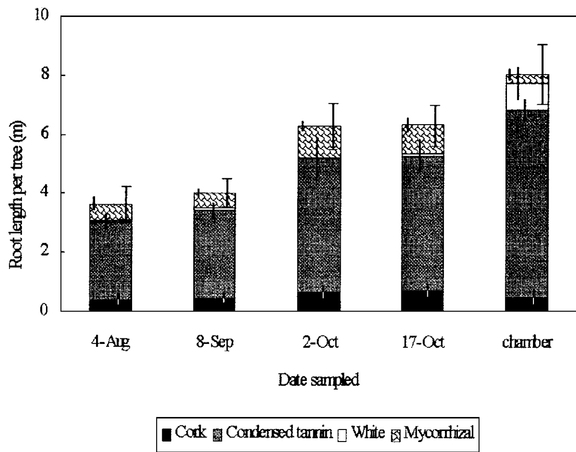

Fig. 4 Lengths of cork, condensed tannin, white, and mycorrhizal zones in roots of field- and chamber-grown seedlings. The *uncapped lines* show the standard deviations of the lengths of the root zones, while the *capped line* shows the standard deviation of the total root length. There is no standard deviation line shown for the white zone of the field-grown roots as it was too small to be visible (n=10)

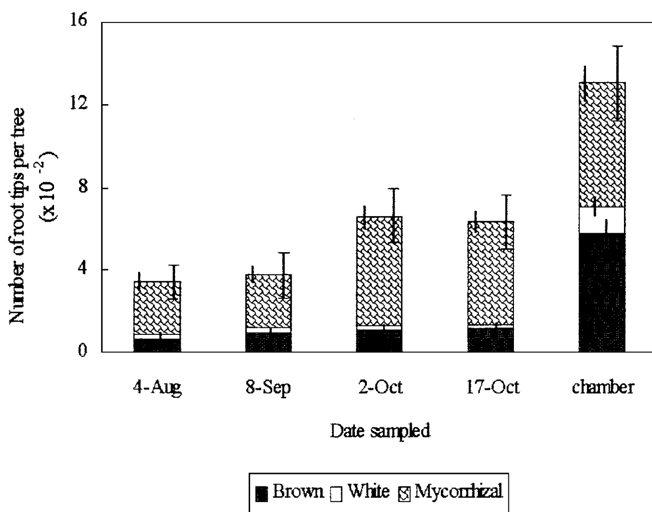

Fig. 5 Numbers of brown, white, and mycorrhizal root tips in field- and chamber-grown seedlings. The *uncapped lines* show the standard deviations of the number for root tip types, while the *capped line* indicates the standard deviation of the total root tip number. There is no standard deviation line shown for the white tips of the field-grown roots as it was too small to be visible (n=10)

that was responsible for the increase in the number of root tips between 8 September and 2 October. This increase was so great largely due to the dichotomous and coralloid pattern of development common in pine mycorrhizae, leading to the production of many tips (see le Page et al. 1997). The remainder of the root tips were brown and had cortical anatomical features of the condensed tannin zone.

The proportion of root tips that were metacutized was lowest (roughly 30%) between September and October

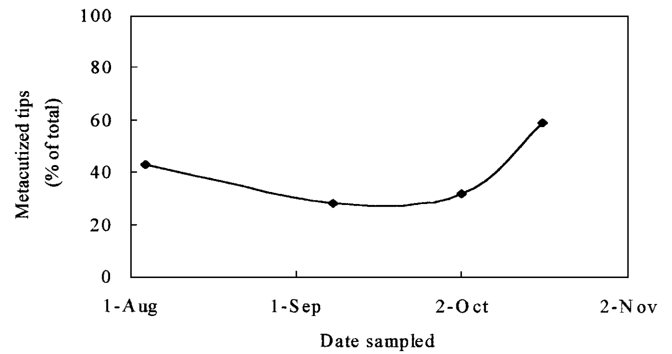

Fig. 6 The percent of metacutized root tips in field-grown seedlings

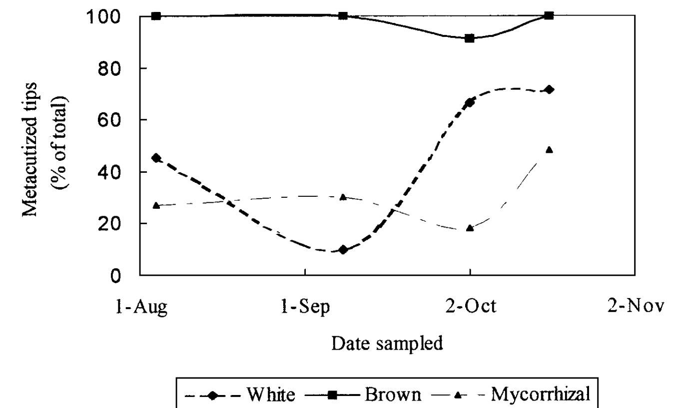

Fig. 7 The percent of metacutized root tips among the three root tip types (white, brown, and mycorrhizal) in field-grown seedlings

(Fig. 6), around the time period of maximal root growth (Fig. 4). Between 2 October and 17 October, the percentage of metacutized tips increased (Fig. 6), indicating a rapid onset of root dormancy or quiescence after the period of elevated root growth, and corresponding with a period of minimal growth (Fig. 4).

The fraction of metacutized tips varied considerably among the three tip types identified macroscopically (white, brown, and mycorrhizal). Brown roots were almost always metacutized (Fig. 7). White roots showed considerably more variability, possessing high numbers of metacutized tips in August and October, and substantially fewer in September. Some mycorrhizal tips were also metacutized (about 25%), increasing to almost 50% by 17 October.

In the growth chamber, there were twice as many root tips as in field-grown seedlings (Fig. 5). The difference was primarily a result of the large number of brown tips on the chamber-grown seedlings.

Cortical plasmalemma surface area

The contribution per unit length of each root zone to CPSA was highly variable (Table 1). The white zone always possessed the greatest CPSA per millimeter, primarily because of its large number of cortical cells. The

Table 1 Average dimensions and numbers of individual living cortical cells (*CC*), passage cells (*PC*), and average calculated CPSA per millimeter root length for each root zone. Standard deviations (SD) and numbers of values (*n*) are included

Sample date	Root zone	Width CC (mm) ±SD (*n*)	Length CC (mm)±SD (*n*)	No. of CC±SD (*n*)	Width PC (mm)±SD (*n*)	No. of PC±SD (*n*)	CPSA (mm^2) per mm root length
4 Aug	White	0.035±0.011 (165)	0.099±0.027 (34)	186±46 (24)	0.029±0.064 (78)	3.5 ±1.8 (24)	24
	Mycorrhizal	0.040±0.014 (76)	0.059±0.017 (34)	49.1±10 (17)	0.036±0.0074 (13)	0.77±0.75 (17)	8.3
	Condensed tannin	–	–	–	0.037±0.0095 (19)	0.63±0.81 (30)	0.023
8 Sep	White	0.034±0.012 (162)	0.106±0.028 (33)	212±73 (19)	0.037±0.014 (59)	3.0 ±1.3 (19)	26
	Mycorrhizal	0.039±0.011 (133)	0.064±0.017 (37)	51.4±12 (19)	0.038±0.0098 (20)	1.3 ±0.95 (19)	8.3
	Condensed tannin	–	–	–	0.034±0.0071 (13)	0.52±0.59 (25)	0.018
2 Oct	White	0.036±0.012 (90)	0.156±0.045 (38)	153.8±42 (12)	0.035±0.012 (42)	3.6 ±1.4 (12)	20
	Mycorrhizal	0.033±0.013 (125)	0.056±0.019 (34)	66.8±38 (12)	0.035±0.015 (16)	1.3 ±1.4 (12)	9.0
	Condensed tannin	–	–	–	0.035±0.013 (24)	1.7 ±1.3 (14)	0.060
17 Oct	White	0.034±0.013 (124)	0.16 ±0.033 (35)	148±39 (22)	0.032±0.0058 (83)	3.2 ±1.5 (22)	17
	Mycorrhizal	0.033±0.011 (105)	0.061±0.017 (35)	70.1±16 (21)	0.035±0.0093 (15)	1.0 ±0.84 (21)	9.0
	Condensed tannin	–	–	–	0.031±0.0063 (20)	1.2 ±1.2 (25)	0.036
Chamber	White	0.022±0.0072 (151)	0.088±0.017 (32)	137±23 (29)	0.029±0.0058 (77)	5.1 ±1.4 (15)	11
	Mycorrhizal	0.026±0.0095 (98)	0.058±0.015 (32)	38.5±8.4 (21)	0.030±0.0031 (23)	1.5 ±0.83 (15)	3.9
	Condensed tannin	–	–	–	0.026±0.0080 (17)	1.1 ±0.99 (15)	0.023

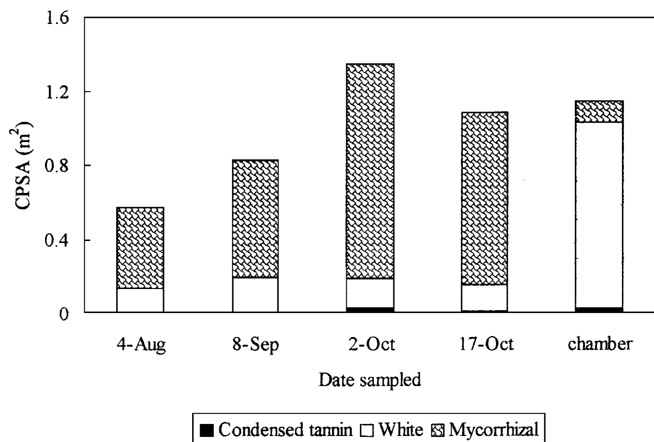

Fig. 8 The average total CPSA (cortical plasmalemma surface area) per seedling root system and the contribution of three root regions (condensed tannin, white, and mycorrhizal) in field- and chamber-grown seedlings

mycorrhizal zone possessed substantially fewer cortical cells so that its CPSA was 33–50% of the non-mycorrhizal white zone CPSA. The contribution of the condensed tannin zone was very small (0.068–0.21% of the non-mycorrhizal white zone), as only the outer membranes of the passage cells of this zone were included.

By 4 August, the average root system of one field-grown seedling possessed 0.57 m^2 of CPSA (Fig. 8). The CPSA more than doubled from 4 August to 2 October (increasing to 1.39 m^2) and had declined somewhat by 17 October (Fig. 8). The largest contributor to CPSA at any time was the root tissue encased in the fungal mantle. In fact, changes in the CPSA contribution by the mycorrhizal roots were primarily responsible for changes in the CPSA of the entire root system. If the mantle is permeable, then the total CPSA would be as presented in Fig. 8. However, if the mantle is impermeable, independent ion uptake by the plant would be impeded for over 81% of the root CPSA, and the total root CPSA would remain relatively constant over the time period observed. In the latter case, the greatest proportion of the CPSA would be contributed by the white zone. The condensed tannin zone, despite its major contribution to total root length (Fig. 4), actually contributed little to CPSA.

In seedlings raised in the growth chamber, by far the largest fraction of CPSA was contributed by the white zone (85%; Fig. 8). Roots ensheathed by mantles contributed significantly less CPSA compared to field-grown plants.

Discussion

Anatomy

The cork, condensed tannin, and white zones described by McKenzie and Peterson (1995a,b) in roots of pouch-grown *P. banksiana* were also found in the ectomycorrhizal, pine seedlings grown in soil both in pots and the field. Earlier anatomical analyses lumped the condensed tannin and cork zones together as "suberized roots" based on their external brown coloration (e.g. Kramer and Bullock 1966; Chung and Kramer 1974). In the present study, the condensed tannin zone constituted the majority of the root length in both mycorrhizal field- and growth chamber-grown pines.

It is important to note that changes in root system CPSA did not correlate with changes in root length because the cork, condensed tannin, white, and mycorrhizal zones all contributed very different CPSAs based on their anatomy. In this research, the longest root region

(the condensed tannin zone) actually contributed very little to the root system CPSA. The only root zone that contributed less was the cork zone, which was considered to be zero in the present work. The region that contributed most to the total root CPSA and was responsible for the change in root CPSA as the season progressed was the mycorrhizal region, and the field-grown seedlings were heavily mycorrhizal. This occurred despite the fact that the mycorrhizal zone contained relatively few cortical cells (on average, 34% as many cells as the non-mycorrhizal white zone). Both the lengths of the white and condensed tannin zones remained rather constant over the 3-month period investigated. Consequently, the change in CPSA of these zones was minimal (Fig. 3).

Root composition and growth

Growth chamber seedlings differed from those grown in the field in several respects. The growth chamber pines had over 80% of their CPSA in the white zone, with the mycorrhizal region making up the bulk of the rest. This occurred despite a high number of mycorrhizal tips, because the length of the average mycorrhizal region on an individual root tip was considerably shorter than in the field (data not shown) and the white zones were considerably longer.

Some of the differences in proportional root anatomy between chamber- and field-grown seedlings were probably related to growth rates. The chamber-grown roots were longer and possessed more root tips than the field-grown seedlings, despite being much younger. Faster root growth by the chamber-grown seedlings undoubtedly reflected better conditions compared to those of the field. Wilcox (1962a) found that with faster growth, maturation of metaxylem, and other structures occurred farther from the root tip. Thus, faster growing roots would be expected to have long white zones. In fact, even the growth of our field-seedlings may have been exceptionally fast as they were initiated under artificial conditions.

The field-grown seedlings exhibited uneven rates of root growth, as described in the earlier literature (Wilcox 1962b). The time periods in which root growth was most rapid, both in regard to new root tips and overall root length, correlated with the times of high soil moisture and not with temperature. The increases in length were primarily due to increases in the condensed tannin zones. These data show that even when the roots were growing relatively quickly in the soil, the condensed tannin zones were maturing shortly behind the tips but the maturation of the cork zone was not similarly accelerated. The majority of the increases in the number of root tips were provided by the mycorrhizal roots. This likely relates to the dichotomous and coralloid growth pattern of mycorrhizal roots in pine.

Metacutization

At all times, a substantial number of root tips were metacutized on the field-grown seedlings. As expected, the fraction of metacutized tips correlated inversely with root growth rate. Roots that were brown to the tip were almost always metacutized (Fig. 7). It is possible that metacutized white and mycorrhizal root tips represent an early stage, and that the cortex of these roots would eventually die. Comparing Fig. 6 to the percent moisture of the soil (Fig. 2) reveals that the time (approximately 12 September) during which a lower fraction of root tips are metacutized coincides with the time of greater water availability. Further, the time interval of maximal increase in root length and tip number corresponded to the time of the lowest fraction of metacutized root tips. The latter correlation supports the earlier conclusion of Wilcox (1968) that metacutization is a feature of non-growing roots.

The function of the metacutized layer is unclear. Originally, it was believed to impede water movement and could, therefore, protect the underlying tissue from drying during drought (Mager 1913). However, Leshem (1970) disproved this idea by establishing that metacutized roots were more permeable to water than white roots. The onset of metacutization seems to be related to both environmental conditions and a natural cycle, neither of which can completely overcome the other (Leshem 1970). We propose that the metacutized layer could protect the underlying viable tissue from attack by microorganisms. Clearly this phenomenon warrants further study.

Ion uptake capacity

The CPSA measured in the present study represented the plasmalemma of living cells which could be contacted by the soil solution. Because ions must access membranes before they can be absorbed into the symplast, the CPSA contributes to the root's potential for ion uptake, along with the activities of transporters on the membranes themselves. The CPSA contribution of the white and condensed tannin zones changed little during the course of the field study (Fig. 8). Instead, the mycorrhizal zone was the dominant contributor both to overall CPSA and changes in CPSA. Conversely, in the chamber-grown seedlings, the mycorrhizal zone contributed much less than the white zone to their CPSA. The difference between the field- and chamber-grown pines may relate to the field trees being much older, differences in soil structure, fungal inoculation, or the more idyllic growth conditions found in the growth chamber.

The large quantity of the root system occupied by the condensed tannin zone could be significant, despite its low contribution to root CPSA. The absorption of water and ions by "suberized roots" was not understood in earlier studies (Crider 1933; Kramer and Bullock 1966; Sougnez-Remy et al. 1993; Van Praag et al. 1993). This

may have been a result of not distinguishing between the condensed tannin zones, which have living endodermal passage cells (Enstone, personal communication), and the cork zones. Similarly, dormant metacutized roots were bordered by a condensed tannin zone with passage cells, which may allow the root to retain some nutrient-acquiring abilities.

The question of ectomycorrhizal mantle permeability is critical for an understanding of the pathway of ion uptake for heavily mycorrhizal woody roots, as the cortical cells of the mycorrhizal zone are separated from the soil solution by the mantle. In this study, the largest amount of root CPSA was located in the mycorrhizal region. While this large CPSA is conducive to enhanced uptake of ions that are delivered by ectomycorrhizae (Smith and Smith 1990), the fungal sheath's permeability to nutrient ions will greatly influence the pathway by which other ions are taken up by the root. If the mantle is permeable to nutrient ions, the CPSA of the root systems would be as shown in Fig. 8. In this case, a minimum of 75% of the root system CPSA is found within the fungal mantle. However, if the fungal mantle is impermeable to nutrient ions, then the underlying root tissue would be more isolated from the soil solution, and most importantly, from those ions that the fungus is not able to absorb and deliver to the root. Based on the present work, elimination of the total mycorrhizal CPSA would reduce the overall CPSA by as much as 85% in the field-grown trees. As well, if the mycorrhizal zone does not contribute to the CPSA for ions not absorbed by ectomycorrhizae, then the change in CPSA for those ions over the course of this study was very small. Clearly the absorptive capabilities of extramatrical hyphae regarding other mineral nutrients and the permeability of the mantle must be clarified.

In summary, this work has established that distinct anatomical zones exist in field-grown seedlings, the anatomy of which should strongly influence ion uptake potential. Among field-grown pines, the majority of the CPSA is located within the ectomycorrhizal mantle and may be somewhat isolated from the soil solution. A significant fraction of root tips were metacutized and contributed little to the root CPSA. It would be interesting to extend this investigation to mature trees. This anatomical analysis stresses the importance of revisiting some basic questions of tree root physiology. Is the cork zone impermeable to nutrient ions and/or water as assumed in this study? Are membrane transporters equally distributed amongst the living plant root cells, or is their prevalence dependent on the location of the cell? What is the purpose of metacutization in root dormancy? These queries will not be easily answered, but must be addressed for a rudimentary understanding of tree root function.

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