Arbuscular mycorrhizae of American ginseng (*Panax quinquefolius*) in cultivated field plots: plant age affects the development of a colonization lag phase[1]

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Abstract: Temporal patterns of colonization of roots of perennials by arbuscular mycorrhizal fungi are poorly understood because annual crops are more often studied. The objective was to monitor in detail the growth and mycorrhizae of the perennial American ginseng (*Panax quinquefolius* L.) over the first 2 years after seeding. In particular, the extent of carry-over of colonization into the second year was examined. Delayed germination of some seeds caused by chance-driven variability in the time needed for seed maturation after seed collection provided an opportunity to compare first- and second-year plants under identical conditions in the second year. For all plants, development of arbuscules rose to a maximum in mid-August, falling partially thereafter. Production of hyphal coils proceeded more slowly but steadily, so that end-of-season colonization was composed equally of arbuscules and hyphal coils. Between seasons, taproot dry mass was reduced by 18%, whereas lateral root length increased by 15%. These changes were probably caused by root turnover, which left little initial colonization of roots at the start of the second year. Strikingly, second-year plants did not exhibit a colonization lag phase, whereas a lag of 43 days was seen for the first-year plants alongside. Possible reasons for this difference are discussed.

Key words: perennial, arbuscules, hyphal coils, taproot, Ontario.

Résumé : Les patrons de colonisation dans le temps des racines des plantes pérennes par les champignons arbusculaires sont peu connus parce qu'on étudie plus volontier les plantes annuelles. L'objectif était de suivre en détails la croissance et la mycorhization du ginseng américain (*Panax quinquefolia* L.), une plante pérenne, pendant les deux années suivant la germination. On a accordé une attention particulière au passage de la colonisation dans la seconde année. Le délai de germination de quelques graines, provenant d'une variabilité aléatoire du temps nécessaire pour la maturation des graines après leur récolte, a fourni l'opportunité de comparer des plantes d'une et de deux années sous des conditions identiques, au cours de la seconde année. Pour toutes les plantes, le développement des arbuscules atteint un maximum à la mi-août, diminuant partiellement par la suite. La production des pelotons mycéliens s'effectue plus lentement mais régulièrement, de sortesque la colonisation à la fin de la saison est composée également de pelotons et d'arbuscules. Entre les saisons, la masse sèche de la racine principale diminue de 18%, alors que la longueur des racines latérales augmente de 15%. Ces changements proviennent probablement d'un recyclage des racines, ne laissant que très peu de la colonisation initiale des racines au début de la seconde année. Il est clair que les plantes de deuxième année ne subissent pas de délai de colonisation, alors qu'on observe un délai de 43 jours chez les plantes de première année voisines. Les auteurs discutent les raisons possibles de cette différence.

Mots clés : plante pérenne, arbuscules, pelotons mycéliens, racine principale, Ontario.

[Traduit par la Rédaction]

Introduction

The fleshy taproot of the herbaceous perennial American ginseng (*Panax quinquefolius* L.) is eaten regularly by millions of people because of the health-giving properties that are claimed for the ginsenosides it contains. These ginsenosides are triterpene saponins (Smith et al. 1996). Natural populations of this species in the eastern part of North America have been decimated by overharvesting (Charron and Gagnon 1991), and in recent times, American ginseng cultivation has developed extensively in Canada and the United States (Proctor and Bailey 1987). In Canada, 2000 and 700 ha of American ginseng were being cultivated in the mid-1990s in the Provinces of Ontario and British Columbia, respectively (Whitbread et al. 1995).

Naturally occurring American ginseng lives up to 60 years and produces one or more taproots from a horizontal perennial rhizome (Charron and Gagnon 1991). One aerial shoot

Received February 2, 1999.

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is produced from the rhizome each year (Lewis and Zenger 1982). Similar to the storage root of the carrot (*Daucus carota* L.), the taproot of American ginseng quickly looses its cortex and undergoes secondary thickening as it develops, and from it are produced a number of root axes with laterals (Peterson et al. 1998). Under commercial production, American ginseng typically produces one taproot per plant. The rhizome of plants harvested after 3–5 years of cultivation is small compared with the taproot (Proctor and Bailey 1987).

Arbuscular mycorrhizae of American ginseng are of the *Paris*-type (Smith and Smith 1997) morphology, forming hyphal coils that sometimes develop intercalary arbuscules, with vesicles formed sparsely, and with colonization proceeding in a cell-to-cell manner within localized root regions (Whitbread et al. 1996). Mean colonization of first-year plants by arbusculate coils reached a seasonal maximum of 57% of root length at a well-fertilized Ontario farm in September 1994; the corresponding maximum for third-year plants at a nearby similar farm was 45% (Whitbread et al. 1996). The functional capacity of mycorrhizae with *Paris*-type morphology is not known to be different from those of the more extensively studied *Arum*-type (Smith and Smith 1997). Indeed, growth responses relative to non-mycorrhizal controls were seen after inoculation of pot-grown American ginseng with various arbuscular mycorrhizal fungi (Li 1995).

Most crop species are annuals, and so data for mycorrhizae of herbaceous perennials in agricultural systems are few. Roots of the perennial crop species asparagus (*Asparagus officinalis* L.) were collected simultaneously at the end of a single growth season from various fields in Michigan (Wacker et al. 1990). Plant stands varying in age from 1 to 15 years were all colonized with arbuscular mycorrhizal fungi, and using a rating system of 0–4 that they defined, the mean colonization values were between 1.7 and 2.8 for the different crop ages (Wacker et al. 1990). American ginseng is a perennial, which leads us to pose questions about the development of its mycorrhizae. Particularly, there is no information on the carry-over of colonization from one year to the next. Persistence of colonization through the winter in the root systems of temperate woodland perennials has been observed (Merryweather and Fitter 1998).

The objective of the present study was to determine the temporal patterns of mycorrhizal associations of American ginseng cultivated in a well-fertilized experimental garden typical of commercial production in Ontario. Given the potential of mycorrhizae for disease control with ginseng (Han et al. 1997), colonization data are functionally meaningful even under the high-fertility conditions typical of commercial gardens. In particular, the aim was to follow in detail the colonization of roots of simultaneously germinating plants through 2 years and, thereby, investigate the relationship between colonization in the late part of the first-year and that in the following year. Although our purpose was not to relate colonization and plant growth in terms of cause and effect, we assessed shoot and root growth for all samples to interpret the patterns of colonization more easily. There was a fortuitous occurrence of first-year plants in the second-year seedbed that was caused by delayed germination of some seeds. American ginseng seeds exhibit innate dormancy by incomplete development, as described by Harper (1977). Thus, American ginseng seeds are dormant when collected and need variable lengths of time for subsequent maturation (Proctor and Bailey 1987). The time needed depends on the length of time they had to mature while attached, which is variable among seeds on a single plant, and also on the microsite temperature and moisture conditions during the period of up to 22 months used for stratification (Oliver et al. 1992). Germination of seeds between the two years was therefore determined by chance. Delayed germination was not localized, and so it did not cause any differences in plant density. The delayed germination was used to evaluate the effects of plant age and seedbed age on mycorrhizal development in the second year.

Materials and methods

Field work was done in a ginseng garden at the Pest Management Research Centre of Agriculture and Agri-food Canada, Delhi, Ont., Canada. This garden was located on a sandy loam in the Fox soil series with 89% sand, 4% silt, and 7% clay. Organic matter for this soil is 1.1%, and the cation exchange capacity is 4.1 mequiv.·100 g^{-1} dry soil. The range used had a history of rye (*Secale cereale* L.) and tobacco (*Nicotiana tabacum* L.) cultivation prior to its use for American ginseng. A rate of 90 $t \cdot ha^{-1}$ fresh mass of solid cattle manure was applied in early August 1994 and incorporated using a rototiller to a depth of 20 cm. The garden was fumigated in September 1994 using 74 $g \cdot ha^{-1}$ Vorlex® injected at a depth of 25 cm in the soil. To maximize contact between the soil and the fumes from the Vorlex®, the soil surface was sealed by dragging a roller across the site to compress the upper 4 cm. Even though soils used for tobacco in this region invariably have high fertility because of previous applications of fertilizer, the soil was fertilized with 11.3 kg $P \cdot ha^{-1}$ and 28.2 kg $K \cdot ha^{-1}$ for maintenance. Two weeks after fumigation, raised seedbeds were prepared so that each seedbed could accommodate 12 contiguous 10-cm wide rows. Seedbeds were shaped by removing of soil from up to a depth of 25 cm from either side of the strips of soil that were to form the seedbeds, and depositing the removed soil towards the centre of each strip. Thereby each plant across the study area experienced the same conditions for natural inoculum persisting after fumigation. Each row was seeded mechanically with between 33 and 40 seeds·m^{-1} in October, 1994. Following planting, a mulch of oat (*Avena sativa* L.) straw of 8-cm depth was laid over the seedbed surface. In April 1995, NH_4SO_4 fertilizer at a rate of 11 kg $N \cdot ha^{-1}$ was applied. Soil test data for a composite sample taken on November 10, 1994, across the range used were as follows. Soil acidity was pH 5.8 for a saturated soil–water paste. On the basis of dry mass, soil P extracted by 0.5 M $NaHCO_3$ at pH 8.5 was 100 $mg \cdot kg^{-1}$, and following extraction with neutral 1.0 M ammonium acetate, macronutrient cation concentrations were 357 mg $K \cdot kg^{-1}$, 118 mg $Mg \cdot kg^{-1}$, and 613 mg $Ca \cdot kg^{-1}$.

Plant samples were taken from six separate plots within a single seedbed. Plots were 137 cm wide, 122 cm long, and arranged contiguously in a line through a 732-cm long section. For each collection date, a sample from a plot comprised five randomly selected plants, for which pooled measurements were made. The same plots were used in both 1995 and 1996 for first-year and second-year plants, respectively. In addition, a second and similar set of samples was taken in 1996 for first-year plants resulting from delayed germination of seeds that had been planted the previous year. Keeping the shoots and root systems as intact as possible, the plants were carefully removed from the plot using a hand trowel and returned to the laboratory. Beginning on May 31 in both 1995 and 1996, plant samples were collected nine times with approxi-

mately even spacing in each year, with the final samples taken 117 and 114 days later, which were on 25 and 22 September in 1995 and 1996, respectively.

In the laboratory, shoots and root systems were gently washed clean under running water. At their bases, the lateral roots were cut away from the taproots using a scalpel. The rhizomes were small and not distinguished from the taproot. Dry mass of the taproots and shoots was determined after drying to constant weight in a fan-ventilated oven at 60°C for 48 h. Lateral roots were cut into segments approximately 2 cm long, and their length was measured directly. For each sample, a subsample of about 20 root segments was taken at random. The subsamples were fixed overnight in 50% ethanol, rinsed three times with distilled water, cleared in boiling 5% KOH for 1 h, similarly rinsed again three times, and then stained with chlorazol black E (Brundrett et al. 1984). Percentage of root length colonized by arbusculate hyphal coils, and by non-arbusculate hyphal coils, was determined using a magnified intersection method at 200× (McGonigle et al. 1990); these measurements are referred to below as arbuscular colonization (AC) and hyphal-coil colonization (HCC), respectively. In the rare cases where both arbusculate and non-arbusculate hyphal coils were present across the same intersection of hairline to root, only the count for AC was incremented. Thereby, AC and HCC can be added to give percentage root length colonized (%RLC).

Temporal patterns for changes in shoot dry mass, taproot dry mass, and lateral root length were summarized using curve fitting. In most cases, a quadratic model was used:

$$[1] \qquad y = a + bx + cx^2$$

but in two cases, a linear model was used instead, because it gave a better fit as determined by the r^2 value. No attempt was made to use curve fitting for colonization data.

Results

Shoot dry mass followed a convex quadratic pattern for the first-year plants in 1995 (Fig. 1*a*), the second-year plants in 1996 (Fig. 2*a*), and for the first-year plants in the second-year seedbed in 1996 (Fig. 3*a*). Shoot dry mass rose from 22 mg·plant^{-1} on May 31, 1995 (Table 1), to a maximum of 100 mg·plant^{-1} on September 14 in the same year, with little change over the following 11 days (Fig. 1*a*). In 1996, the second-year plants had a shoot dry mass of 274 mg·plant^{-1} on May 31 (Table 1), revealing that significant shoot production had occurred over the 8 months of the late autumn, winter, and early spring. Through the 1996 summer growth period, shoot dry mass of the second-year plants rose to a maximum of 483 mg·plant^{-1} on August 30, falling slightly to 471 mg·plant^{-1} at the final collection on September 22 (Fig. 2*a*). The shoot dry mass produced by the first-year plants in the second-year seedbed in 1996 was lower (Fig. 3*a*) compared with that seen for the first-year plants during the previous year (Fig. 1*a*). The shoot dry mass of the first-year plants in 1996 had a maximum of 58 mg·plant^{-1} on August 16, and the final value on September 22 was 48 mg·plant^{-1} (Fig. 3*a*).

For the first-year plants in 1995 and 1996, taproot dry mass increased similarly (Fig. 1*b*, 3*b*), in spite of the differences in the shoot production of these plants (Figs. 1*a* and 3*a*). From little initial mass (Table 1), tap root dry mass rose to final values of 211 mg·plant^{-1} and of 212 mg·plant^{-1} for the first-year plants on September 25, 1995 (Fig. 1*b*), and September 22, 1996 (Fig. 3*b*), respectively. The second-year plants in 1996 had linear increases in taproot dry mass

Fig 1. Growth and mycorrhizal development of first-year plants in a first-year plot in 1995: shoot dry mass (*a*), taproot dry mass (*b*), lateral root length (*c*), and mycorrhizal colonization (*d*). Details for fitted curves are given in Table 1. Values are means ± SE (n = 6).

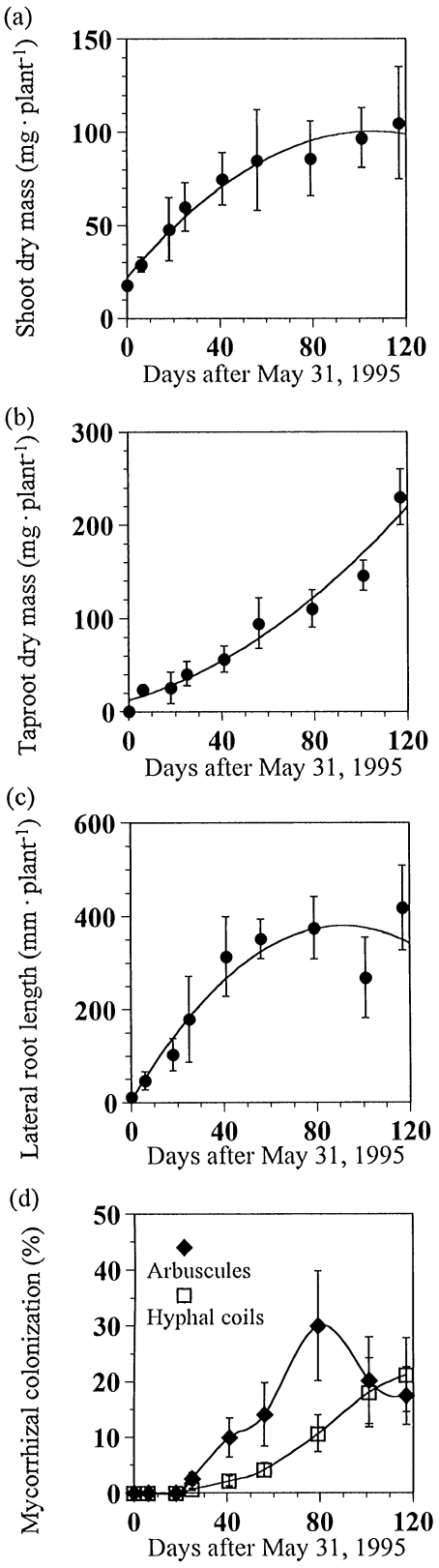

Fig 2. Growth and mycorrhizal development of second-year plants in a second-year plot in 1996: shoot dry mass (*a*), taproot dry mass (*b*), lateral root length (*c*), and mycorrhizal colonization (*d*). Details for fitted curves are given in Table 1. Values are means ± SE (n = 6).

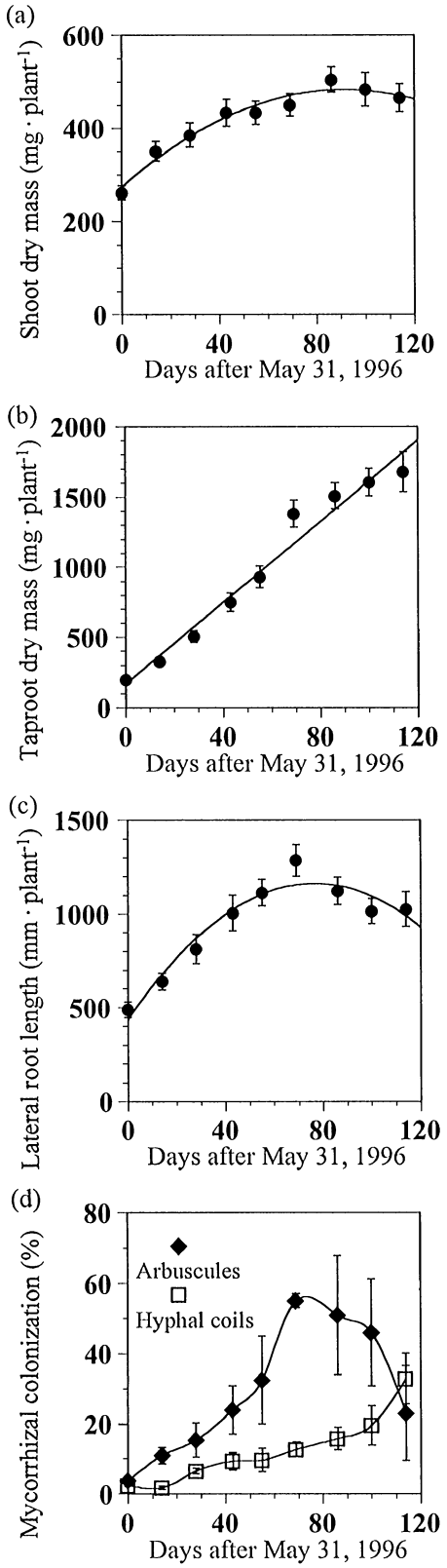

Fig 3. Growth and mycorrhizal development of first-year plants in a second-year plot in 1996: shoot dry mass (*a*), taproot dry mass (*b*), lateral root length (*c*), and mycorrhizal colonization (*d*). Details for fitted curves are given in Table 1. Values are means ± SE (n = 6).

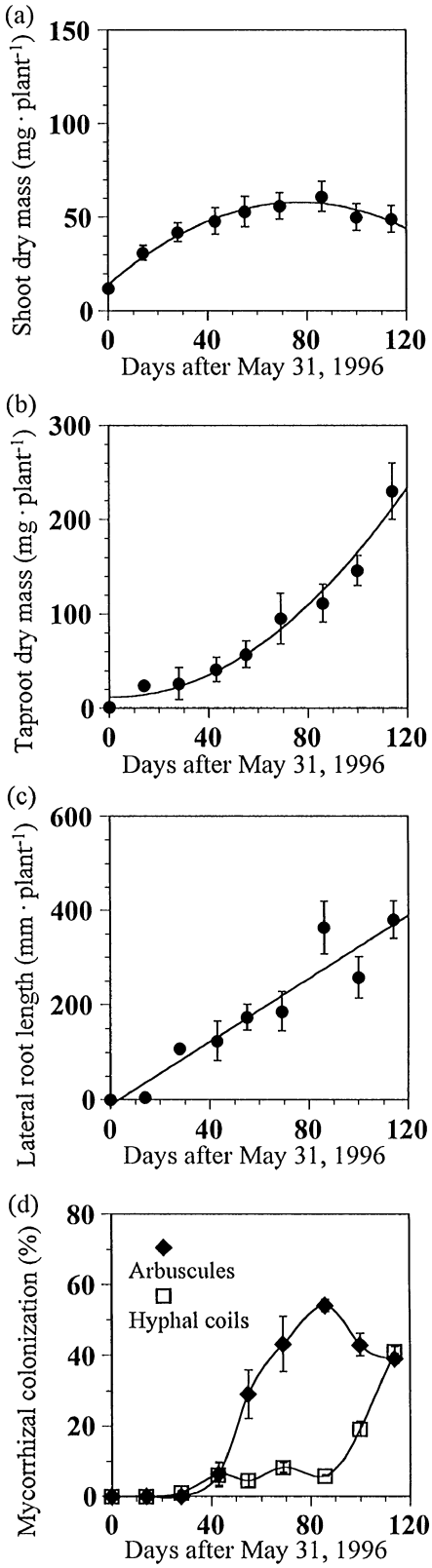

Table 1. Regression coefficients for the fitted quadratic curves for growth measurements (y) against time (x) from Figs. 1–3.

Figure	Plant year	Plot year	Growth measurement	a	b	c	r^2
1*a*	First	First	Shoot dry mass	22.08	1.479	–0.0070	0.973
1*b*	First	First	Taproot dry mass	12.34	0.724	0.0083	0.964
1*c*	First	First	Lateral root length	5.075	8.221	–0.0451	0.886
2*a*	Second	Second	Shoot dry mass	274.4	4.577	–0.0250	0.962
2*c*	Second	Second	Lateral root length	438.0	18.92	–0.1235	0.934
3*a*	First	Second	Shoot dry mass	14.09	1.137	–0.0074	0.972
3*b*	First	Second	Taproot dry mass	11.57	–0.035	0.0157	0.971

Note: Curves are in the form $y = a + bx + cx^2$. Values for coefficients a, b, and c are given in the units that correspond to those in the graphs. For Figs. 2*b* and 3*c*, respectively, the following linear functions gave the best fit: taproot dry mass (mg) = 171.7 + 14.44 × time in days, $r^2 = 0.968$; and lateral root length (mm) = 3.34 × time in days – 11.04, $r^2 = 0.902$.

(Fig. 2*b*), rising from 172 mg·plant^{-1} (Table 1) to 1818 mg·plant^{-1} over the summer season. Evidently, a taproot mass loss of approximately 18% occurred over the period between September 25, 1995, and May 31, 1996.

The temporal patterns of change for length of lateral roots corresponded reasonably well to convex quadratic fitted functions for both the first- and second-year plants in 1995 and 1996, respectively (Figs. 1*c* and 2*c*). From close to zero initially (Table 1), root length rose to a maximum of 380 mm·plant^{-1} on August 30, 1995 (Fig. 1*c*). On May 31, 1996, root length of the second-year plants was 438 mm·plant^{-1} (Table 1), revealing a net gain of root length over the period since the end of the previous summer growth season. For the second-year plants, maximum root length was 1163 mm·plant^{-1} on August 16, 1996, with a decrease to 990 mm·plant^{-1} for the final sample on September 22, 1996 (Fig. 2*c*). Length of lateral roots for the first-year plants in the second-year plot rose in a linear manner (Fig. 3*c*) to a final value of 369 mm·plant^{-1} on September 22, 1996. This end-of-season value for the lateral root length in 1996 was close to the corresponding value of 350 mm·plant^{-1} for the first-year plants in the previous year (Fig. 1*c*).

Mycorrhizal colonization followed a similar overall temporal pattern for all plants. This pattern consisted of a rise in AC that subsequently declined, finishing close to the final value for HCC, which rose more slowly but continually over the summer growth season (Figs. 1*d*, 2*d*, and 3*d*). However, a lag period was seen for the development of colonization for the first-year plants in both years (Figs. 1*d*, 3*d*), but not for the second-year plants in 1996 (Fig. 2*d*). To allow comparison among the present data, we arbitrarily defined the lag phase as existing where %RLC was 5% or less. On the basis of this definition, the lag period for the first-year plants was not broken until after the fourth sample had been taken on June 25, 1995 (Fig. 1*d*), and not until after the third sample on June 28, 1996 (Fig. 3*d*). In contrast, for the second-year plants, total colonization was already at 6% on May 31, 1996, and it had increased to 22% by June 28, 1996 (Fig. 2*d*).

The AC was higher for both the first- and second-year plants in 1996 (Figs. 2*d* and 3*d*) compared with that seen for the first-year plants in 1995 (Fig. 1*d*). The AC for the first-year plants had a maximum of 30% on August 27, 1995, falling subsequently to 18% on September 25 (Fig. 1*d*). However, AC reached maximum values of 54% on August 25, 1996 (Fig. 3*d*), and 55% on August 8, 1996 (Fig. 2*d*), for the first- and second-year plants, respectively; corresponding final values for AC were 39 (Fig. 3*d*) and 23% (Fig. 2*d*) on September 22, 1996. The final value for HCC was 21% for the first-year plants on September 25, 1995 (Fig. 1*d*). The HCC was also higher in 1996, reaching 41 (Fig. 3*d*) and 33% (Fig. 2*d*) on September 22, 1996, for the first- and second-year plants, respectively.

Discussion

Year-end root production found here was similar to that reported previously for first- and second-year plants (Proctor and Bailey 1987). The shoot growth of first-year plants in 1996 was about one half of that seen in the previous year, but the taproot mass and length of lateral roots were similar for the first-year plants in both years. The reduced shoot growth of first-year plants in 1996 compared with 1995 cannot have been due to differences in weather between the two years, because the second-year shoots grew well in 1996. The shading necessary for growth of American ginseng reduces photon flux density by about 70% relative to that in the open (Proctor and Bailey 1987), but further shading of first-year plants by their larger second-year neighbours in 1996 must have occurred. Deletion competition for nutrients is unlikely given the high soil fertility. The investment in roots by the first-year plants in 1996 is a typical response of plants of stable communities like woodland. To resist competition, such plants occupy space by investing in structural tissues and maintaining root activity (Fitter and Hay 1987).

Taproot mass at May 31, 1996, showed an 18% reduction compared with the final harvest in 1995, but there was a 15% increase in lateral root length over the same period. These changes suggest that carbohydrate resources were mobilized within the plant for root production during this period. The gain in shoot dry mass between the first- and second-years may also have used taproot resources. The complete loss of colonization over the same period suggests that a significant degree of turnover of lateral root did occur, because it is unlikely that these roots persisted while the colonization within them disappeared. Persistence of arbuscular mycorrhizal fungi in long-lived roots is known from woodland systems (Brundrett and Kendrick 1988; Merryweather and Fitter 1998).

The most striking feature of the data herein is the lack of a lag phase for the colonization of the second-year plants, even though one is evident for the first-year plants growing alongside them. The pattern of development of %RLC was summarized by Mosse et al. (1981) as sigmoid and com-

posed of three phases. First, a lag phase when little or no colonization develops in the young root system. Second, a period of rapidly increasing colonization, for which the spread of the fungus out paces that of root extension. Third, a plateau typically located at less than 100% colonization. The lag phase lasts longer with reduced inoculum density in pot soil (data of Smith and Smith 1981, as re-calculated by Smith and Read 1997), with increased P fertilizer (Rosewarne et al. 1997), and with plowing as compared with no tilling (McGonigle and Miller 1993).

Environmental conditions can be discounted as reasons for the absence of a lag phase for the second-year plants, because a lag phase was seen for the first-year plants growing next to them simultaneously. It is unlikely that the lag phase of the sigmoid curve is a fixed property of mycorrhizae of annuals and of perennials in their first year. A lag phase was absent for seedlings of sugar maple (*Acer saccharum* Marsh.) emerging naturally in established woodland (Brundrett and Kendrick 1988).

The lack of a lag phase in the second-year plants could be explained in theory by fine-scale variations in inoculum density that may have developed over the first year. If inoculum was the limiting factor that caused the lag phase in the first-year plants in both years, then locally high concentrations of inoculum in close proximity to the roots of the first-year plants in 1995 could have lead to the swift colonization of the second-year plants. Roots of the first-year plants in 1996 would not have been in contact this enriched inoculum, which would probably have been in the form of extraradical mycelium. Mycorrhizal hyphae have been shown to be capable of surviving winter conditions in Ontario soil (Addy et al. 1994; McGonigle and Miller 1999).

Changes in plant carbon resources have been shown to affect colonization (Merryweather and Fitter 1998; Pearson et al. 1994; Amijee et al. 1993). Elevated soluble-carbohydrate availability in the roots of second-year ginseng plants, which could have been present relative to first-year plants establishing from seed, may have contributed to the absence of a lag phase for the second-year plants.

In summary, for American ginseng in cultivated field plots in Ontario, the transition from the late September of the first year to the beginning of June of the second year was marked by several changes. Taproot dry mass decreased, whereas length of lateral roots increased. These changes probably reflect use of taproot resources for root turnover, which is consistent with colonization being scarce at the start of the second-year growth season. A colonization lag phase was absent for the second-year plants, but not for first-year plants alongside. This lack of a lag phase probably reflected fine-scale changes in inoculum density across the plots that were developed in the previous year, or it was possibly caused by greater availability of carbohydrate resources for second-year plants. Further studies will be needed to evaluate these two possible explanations.

Acknowledgements

This work formed part of an M.Sc. study by J.P. Hovius in the Department of Botany at the University of Guelph. Financial support from the Natural Sciences and Engineering Research Council of Canada and from Agriculture and Agri-food Canada is gratefully acknowledged. Thanks to Dr. Roger Koide for helpful comments on this work, and thanks to Dr. Ron Beyaert and others at the Pest Management Research Centre of Agriculture and Agri-food Canada, Delhi, Ont., for their cooperation in this project.

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