MYCORRHIZAL DEVELOPMENT ON CONTAINERIZED TREE SEEDLINGS

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Abstract.--Beneficial effects of ectomycorrhizal infection are demonstrated. At present the ability to increase production of mycorrhizal containerized tree seedlings depends on inoculum availability and effective control of cultural conditions. Efforts in these directions should lead to the production of plantable seedlings equipped with absorbing rootlets that are asssociated with selected fungi, thereby contributing to morpho-physiological quality and survival rate.

Résumé.--Cette communication fait ressortir les effets bénéfiques de l'infection ectomycorhizienne. À l'heure actuelle, la capacité d'accroître la production de semis mycorhizés d'arbres en mottes emballées dépend de la disponibilité de l'inoculum et de la maîtrise des conditions de culture. Les efforts en ce sens devraient aboutir à la production de semis plantables dont les radicelles absorbantes sont associées à des champignons sélectionnés, ce qui améliorera leur qualité morphophysiologique et augmentera leur taux de survie.

INTRODUCTION

The use of containerized tree seedlings is evolving rapidly and their production presents few problems when they are given optimal growth conditions, generously fertilized and cautiously protected by biocide treatments (Waldron 1972, Tinus et al. 1974, Tinus and McDonald 1979, Anon. 1980).

Although it is possible to surpass nature in the initial greenhouse growth period, it is after outplanting that seedlings find themselves in ecologically different environments to which they have to adapt if they are to survive (Baker 1972, Tinus 1974, Sutton 1979, van den Driessche 1980).

Field survival rates of tree seedlings are frequently lower than expected. It has been suggested that this may be due to the non-mycorrhizal nature of outplanted seedlings (Mikola 1973, Marx 1977, Cordell and Marx 1980).

The first objective of this paper is to describe the nature of the ectomycorrhizal relationship under natural conditions. The difference between the feeder roots of forest-grown and container-grown seedlings will be described, and the advantages of ectomycorrhizae will be explained briefly by an examination of their structure.

Secondly, the feasibility of producing healthy containerized seedlings bearing mycorrhizae will be illustrated and the results of successful experiments with different tree species and ectomycorrhizal strains will be reported. Desirable cultural conditions will be presented, the use of biocides commented on and the importance of strain selection and type of inoculum underlined.

Experiments on jack pine *(Pinus banksiana* Lamb.) conducted by the authors will be reported, and the importance of fertilizer balance during seedling production will be stressed. Special consideration will be given to the description and measurement of

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nutrient availability in the substrate as well as to the addition of fertilizers.

Finally, considerations of container size, species, phenology and mycorrhization will be discussed along with an example showing that the precise qualification of fertilizer added can be achieved even on a production scale.

NATURE OF ECTOMYCORRHIZAE

Ecological Considerations

In nature, newly emerging roots become living elements among beneficial, neutral and detrimental organisms in the soil. They participate in soil dynamics and are influenced to a great degree by their environment (Dommergues and Krupa 1978, Krupa and Dommergues 1979).

Ecological studies of the root systems of a number of tree species indicate that the absorbing roots are associated with soil bacteria and fungi (Imshenetskii 1955, Shemakhanova 1962, Riedacker and Gagnaire-Michaud 1978). If the bacteria and fungi are virulently pathogenic, the root will struggle until senescence or death. On the other hand, if they are of a symbiotic nature, as are the mycorrhizal fungi, the root will live in harmony and remain functional for some time. The lifespan of an ectomycorrhiza may be one or two years, depending on environmental conditions (Meyer 1973, Harvey et al. 1980). Consequently, the balance between favorable and unfavorable associations of seedling rootlets greatly influences the establishment, growth, and development of trees in the field.

When the radicle emerges in an artificial substrate, such as is used in container production, it develops under privileged conditions. It successively produces roots of the second and third order, which support their uninfected, absorbing short roots and very often bear root hairs. These tender roots, succulent and unprotected, are very efficient in this particular environment. However, they are very different, both morphologically and physiologically, from those of seedlings developing under natural conditions (Boullard 1968, Harley 1969, Marks and Kozlowski 1973, Smith 1980).

During cultivation of seedlings, fertility levels are often optimized, biocides are regularly used and the substrates contain virtually no ectomycorrhizal fungi. As a result, the root systems of containerized seedlings are, ecologically speaking, deficient in ectomycorrhizae (Fortin 1972, Marx and Barnett 1974, Zak 1975) (Fig. 1).

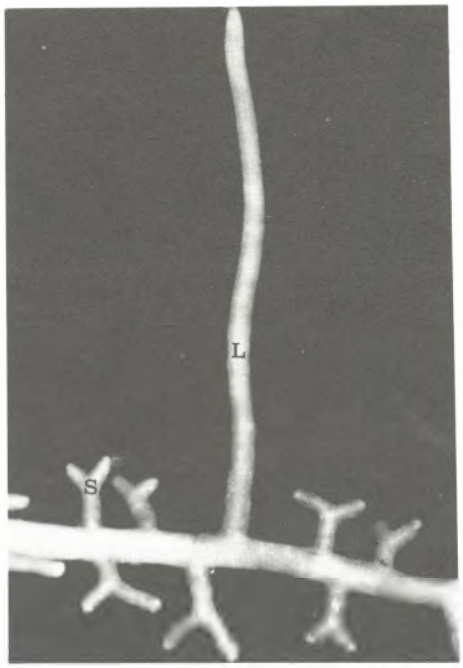

Figure 1. Long lateral roots (L) and non-mycorrhizal dichotomous short roots (S) of jack pine seedlings grown in containers.

At the forest seedling production level, it seems desirable to imitate nature as much as possible, especially if the seedlings are to be planted on all kinds of sites, more or less disturbed, for which it is difficult to predict the survival rate.

For the shoot, it is possible and even desirable to induce bud formation during the last weeks of the growth period (Tinus 1974, Sutton 1979). This is achieved by modifying fertilization regime, reducing watering and, when feasible, shortening the photoperiod and decreasing the temperature.

Similarly, it is possible and desirable to fit the root system with efficient absorbing structures, i.e., with ectomycorrhizae (Mikola 1973).

Advantages to Seedlings

Nutrient absorption and photosynthesis are interrelated processes necessary to maintain seedling growth. Consequently, soon after outplanting, the plant roots must explore the soil in search of mineral nutrients. If the absorbing roots are associated

with ectomycorrhizal fungi the seedling benefits from nutritional, metabolic and prophylactic advantages (see below) (Harley 1969, Bowen 1973, Marks and Kozlowski 1973, Mark 1978) which will be reflected by increased survival rates and earlier growth responses.

Nutritional advantages

- Increased absorbing area of roots
- Availability of nutrients from a larger soil volume
- Improved competitiveness with soil microorganisms and other plants for water and nutrients
- Presence of phosphatase and/or nitrate reductase (in some ectomycorrhizal fungi)
- Improved absorption of $PO4^{--}$, Ca^{++}, le, Rb^{+}, Cl^{-}, $SO4^{--}$, Na^{t}, NO_3^{-}, $NH4^{++}$, Mg^{+}, Fe^{++}, Zn^{++}.

Metabolic advantages

- Provision of growth-regulating substances, such as vitamins and hormones, to the plant
- Excretion of many substances to create a selective microenvironment
- Assistance to the fungus in its metabolic functions by organisms living in the mycorrhizosphere.

Prophylactic advantages

- Excretion of volatile substances which select the microflora by their toxicity
- Production of antibiotics by some ectomycorrhizal fungi, which adds to the selective pressure in their vicinity
- Presence of a fungal mantle that acts as a protective barrier against some pathogenic infection
- Utilization of root exudates by the fungal associate and the mycorrhizospheric microflora, which limits their availability to soil pathogens
- Detoxification by some ectomycorrhizal fungi of the phytotoxins often present in soils
- A decrease in the detrimental effects of soil nematodes and other root pathogens in the presence of some ectomycorrhizal fungi.

Field studies conducted throughout the world have demonstrated the superiority of mycorrhizal over non-mycorrhizal seedlings (Anon. 1981, Furlan and Fortin 1981, Hacskaylo and Tompkins 1973). In North America, the Institute for Mycorrhizal Research and Development at Athens, Georgia, has a staff of experts who have evaluated field performance of mycorrhizal seedlings for many years. They state that "increases in tree survival and growth of over 25 percent have been obtained on a variety of forestation sites... routine, coal, and kaolin spoils, etc. in scattered locations in U.S.A." (Cordell and Marx 1980).

Structure and Function

An examination of the structure of an ectomycorrhiza (Fig. 2 and 3) indicates how a fungus associated with the feeder roots may help the root system to establish itself and to function more efficiently. When the hyphae of the fungal sheath of each ectomycorrhiza radiate into the soil, the surface contact area is increased considerably and a much larger soil volume is utilized. By thus increasing the absorbing area and reducing the space between the roots, the fungal hyphae are able to compete successfully with microorganisms and other plants for the available water and nutrients (Bowen 1973, Rambelli 1973), even if expansion of the root system is restricted.

Ectomycorrhizal fungi conduct toward the root system the products of their absorption (Harley 1969) and also some metabolites (Slankis 1973), by means of a network of mycelial strands extended throughout the soil (Fig. 4 and 5). Consequently, if one wishes to maximize the beneficial effects of ectomycorrhization, it is desirable to colonize the seedling feeder roots with fungal strains selected for their nutritional and metabolic characteristics (Trappe 1977).

It is also a great advantage for the feeder roots to be protected by the fungal sheath of the mycorrhizal fungus before outplanting (Zak 1964, Marx 1973). Once in the field, uninfected feeder roots soon encounter the native flora of the soil, among which many kinds of fungi are more or less pathogenic and can certainly retard the establishment of the seedling.

Rapid colonization of the substrate by the hyphae of the mycorrhizal fungus, increased efficiency and absorbing area, protection of the feeder roots and other advantages explain to a large degree why mycorrhizal seedlings should be outplanted if higher survival rates and more rapid growth responses are to be achieved.

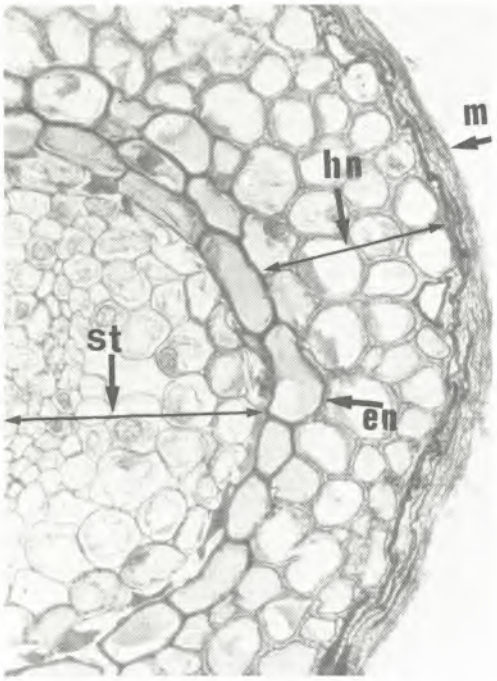

Figure 2. Transverse section showing the stele (st), the endoderma (en), the hartig net (hn) and the fungal mantle (m) of an ectomycorrhiza on balsam fir.

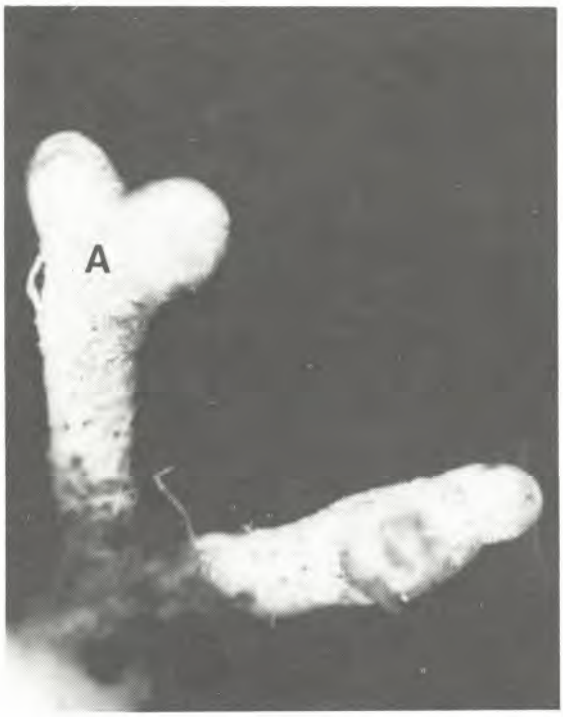

Figure 3. Dichotomous ectomycorrhizae produced in container after inoculating with *Pisolithus tinctorius* (A).

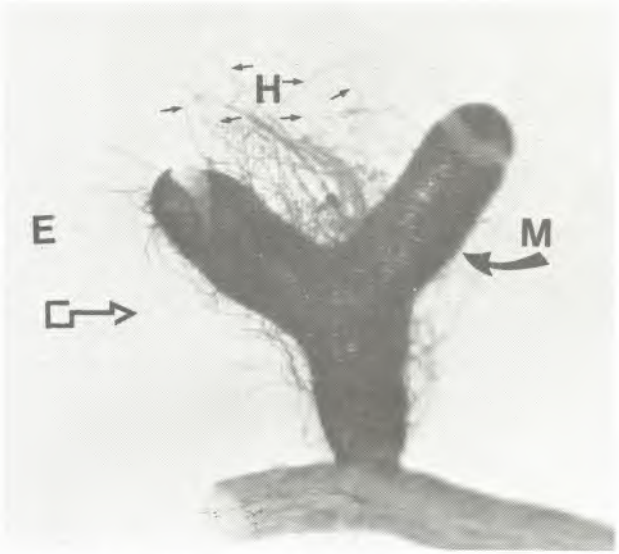

Figure 4. The feeder roots of jack pine showing dichotomous ectomycorrhizae (E), fungal mantle (M) and extramatrical hyphae (H).

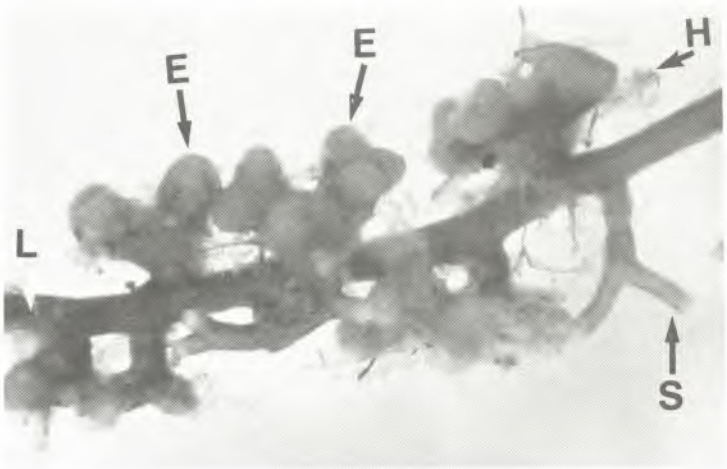

Figure 5. Segment of long lateral root (L) of jack pine bearing coralloid ectomycorrhizae (E) with extramatrical hyphae (H) and also non-mycorrhizal short roots (S).

FEASIBILITY OF MYCORRHIZATION IN CONTAINERS

Tree Species and Fungal Strains Tested

It has been adequately demonstrated that mycorrhization can be induced in containers (Marx and Barnett 1974, Richard 1975, Zak 1975, Landis and Gillman 1976, Ruehle and Marx 1977, Navratil 1978, Cordell and Marx 1979, 1980, Maronek and Hendrix 1979, 1980, Molina 1979, 1980, Ruehle 1980a, b, Anon.

1981, Ruehle et al. 1981). Mycorrhizae have been successfully induced on the following tree species:

- *Cedrus atlantica* Manetti
- *Liquidambar styraciflua L.*
- *Picea abies* (L.)Karst.; *engelmannii* Parry; *glauca* (Moench) Voss; *mariana* (Mill.) B.S.P.; *sitchensis* (Bong.) Carr.
- *Pinus aristata* Engelm.; *banksiana* (Lamb.); *caribaea* Morelet; *clausa* (Chapm.) Vasey; *contorta* Dougl.; *echinata Mill.; elliottii* Little and Dorman; *flexilis* James; *halepensis Mill.; kesya* Royle and Gordon; *merkusii* Jungh and De Vries; *nigra* Arnold; *oocarpa* Schiede; *palustris* Mill.; *pinaster* Soland.; *ponderosa* Laws.; *rigida Mill.; Mill.; sylvestris* L.; *taeda* L.
- *Pseudotsuga menziesii* (Mirb.) Franco
- *Quercus alba L.; macrocarpa* Michx.; *palustris* Muenchh.; *robur* L.; *rubra* L.; *velutina* Lam.
- *Tsuga heterophylla* (Raf.) Sarg.

Cultural Conditions

Unfortunately, most of the experiments on ectomycorrhizae performed in various parts of the world were not conducted under standard conditions. It is understandable that investigators interested in different aspects of mycorrhization worked with different tree species and fungal strains, but the cultural procedures also varied according to container size, substrate composition, fertilization regimes, etc. (Waldron 1972, Tinus et al. 1974, Carlson 1979, Tinus and McDonald 1979); consequently, it is difficult, if not impossible, to determine from the literature the proper methods for producing mycorrhizal containerized seedlings on a large scale.

The following fungal strains have been tested (asterisks indicate relative frequency of use):

Agaricus sylvaticus (Fr.) Secr.; *Amanita pantherina* (D.C. ex Fr.) Schumm.
Astraeus pteridis (Shear) Zeller; *Boletinus cavipes* Kalch
*** *Cenococcum geophilum* (Sowerby) Ferd and Winge
** *Hebeloma crustuliniforme* (Bull. ex Saint-Amans) Quel.
Hebeloma cylindrosporum
*** *Laccaria Zaccata* (Fr.) Berk. and Br.
Lycoperdon gemmatum Batsh.
** *Pisolithus arhizus* Pers.
**** *Pisolithus tinctorius* Pers. (Coker and Couch)
** *Rhizopogon luteolus; Rhizopogon roseolus* (Corda Insturm.) Fr.
Sphaerosporella brunnea (Alb. and Schw. ex Fr.)
Suillus bovinus; Suillus columnare
** *Suillus granulatus* (Fr.) Kuntze Surcek and Kub.
Suillus luteus (Fr.) S.F. Gray; *Suillus tomentosus* (Kauf.) Snell, Singer and Dick.
*** *Thelephora terrestris* Ehrhart and Fr.

Of these, *Pisolithus tinctorius, Cenococcum geophilum, Laccaria laccata* and *Thelephora terrestris* are the best known and are frequently used to form ectomycorrhizae on a variety of host species.

However, the information available permits us to indicate the range of cultural conditions favorable for mycorrhizal inoculation. Interested parties must determine, in their own facilities, the optimal conditions for the species to be cultivated. This should ensure that every inoculated seedling will become mycorrhizal, with a high proportion of ectomycorrhizal feeder roots, and that the seedlings will be in good morpho-physiological condition, suitable for outplanting, at the end of the production period.

The main physical and biological factors related to seedling production that promote mycorrhizal formulation have been assessed by a number of workers (Hatch 1937; Bjorkman 1942; Bowen 1973; Slankis 1973, 1974; Marx et al. 1977; Reid 1978; France and Reid 1981).

It has been shown that mycorrhizal development is dependent on the amount of light received by seedlings, and that most species are negatively affected by an illumination of less than 50% daylight.

The fertility level is important in relation to the extent of mycorrhization; excessive fertilization has been shown to decrease the infection. However, we think that the precise quantification of the appropriate fertility level for each cultivated species needs further investigation.

Most fungal strains have their maximal growth at pH 4-5, and no particular problems are expected if the substrate acidity can be maintained in that range during the incubation period.

The same observation applies to the admissible temperature, most fungal strains growing well between 15 °C and 30 °C.

Substrate aeration is an important factor. For instance, below 5% exorption of nutrients from roots can occur. However, the hydraulic conductivity is generally sufficient to insure a good aeration level and 15%

aeration is adequate for most physiological activities, including mycorrhization.

The water available in the substrate greatly influences the gaseous exchanges and the availability of nutrients. Hydration of the substrate to 40-50% of its maximum retention capacity will generally yield good results.

Use of Biocides

Biocides are frequently used during stock production to protect seedlings from pathogens and insects and to prevent the growth of fungi and weeds in the substrate.

Available data (Iloba 1978, Pawuk et al. 1980, Marx and Rowan 1981) on some of these biocides (Table 1) indicate that their influence on mycorrhiza development ranges from total inhibition to definite stimulation depending on the product used and on cultural conditions.

The effectiveness of these biocides is related to the buffer capacity of the substrate, the dosage used, the sensitivity of the ectomycorrhizal fungus and that of the cultivated host. Where possible, it is preferable to fumigate the substrate (Mulder 1979) in order to eliminate the competition and increase the efficiency of mycorrhizal infection.

Table 1. Noted effects of some biocides on mycorrhiza development.

1. Fumigants	
- Methyl bromide	(0)[a]
- Vapam	(-)
2. Fungicides	
- Arasan	(+)
- Banrot	(+)
- Bayleton	(+)
- Benlate (benomyl)	(+)
- Benodanil	(-)
- Captan	(+)
- Dexon (fenaminosulf)	(-)
- Mertect (thiabendazole)	(0)
- Terrachlor (quintozene)	(-)
- Truban (ethazole)	(0)
3. Insecticides	
- Lindane	(-)
- Toxaphene	(-)
4. Herbicides	
- 2-4-D, Simazine	(-)
- Others (under study at International Paper Co.)	
5. Repellents	
- Anthraquinone, endrin IMRD (under study at Institute for Mycorrhizal Research and Development)	

[a](0) = effect noted
(-) = negative effects
(+) = positive effects

Inoculum

Different types of inoculum

In containerized seedling production the promotion of mycorrhizal feeder development necessitates the introduction of a source of ectomycorrhizal fungus into the substrate. Up to now different types of inocula have been used[3], each having its advantages and disadvantages (Mikola 1973, Trappe 1977).

Actually, the shift from experimental to production level is achieved mainly by using pure culture inoculum aseptically produced in enriched peat-vermiculite mixture. This procedure permits efficient quality control and favors the fungus to be introduced rather than the many potentially harmful microorganisms that would be favored if forest humus were used.

Many research laboratories, including industrial laboratories, are working to upgrade inoculum quality, to increase the scale of production and to develop efficient methods of incorporation into the substrate. Pure mycelial culture in peat-vermiculite and encapsulation of seeds with basidiospores have commercial potential. However, even if the results obtained with these seem encouraging, the commercial availability of mycorrhizal inocula is dependent on the interest of potential users in improving the quality of the absorbing roots of their seedlings (Kenney 1980).

In our laboratory in Quebec we produce enough inoculum for our nursery and greenhouse experiments. It is produced in two ways, either in peat-vermiculite mix or in liquid medium. Our fungal strains are grown

[3]Main inoculum sources tested (asterisks indicate relative frequency of use):
* Soil and humus
mycorrhizal plant root system
** Spores and sporocarps
pure culture
*** mycelium in solid substrate
* mycelium in liquid suspension
(condensed mycelium) (Sclerotia)

in a nutritive solution similar to that defined by Marx and Bryan (1975) either in autoclavable bags filled with the peat-vermiculite mix (Fig. 6) or in flasks. We intend to inoculuate 100,000 seedlings this fall, but this number is relatively small in comparison with the current production of 3.5 million containerized seedlings in East Angus and the anticipated production in Quebec for the coming year (Dancause 1982).

Figure 6. Laboratory production of ectomycorrhizal fungi for inoculation purposes.

We are confident that, when larger quantities of inoculum are needed for large-scale inoculation, there will be companies able to provide them.

Selection of the fungal strains

In view of the many different ectomycorrhizal fungi (Trappe 1962, Smith 1974, Malloch et al. 1980, Miller 1981) which exhibit varying degrees of efficiency depending on the parameter considered (Bowen 1973, Marx 1973, Slankis 1973, Trappe 1977), studies must be carried out to select the most efficient fungal strains for each tree species and planting site.

Generally, the inoculum is incorporated into the substrate before sowing. Peat-vermiculite inoculum is either uniformly mixed or side dressed in containers while liquid suspensions are usually uniformly distributed in the substrate during irrigation.

Different factors have to be considered and each is of particular importance (Trappe 1977). For example, ease of handling with particular strains, infectivity toward some tree species, rate of spread in the substrate, potential benefits for the seedlings, and persistence and competitiveness in the soil after outplanting all have to be considered. According to Trappe (1977), "the more completely we learn the autecology of ectomycorrhizal fungi, the more intelligently we can select the species for inoculation".

Depending on the substrate used and the location of production equipment, it is possible to observe "spontaneous" mycorrhization in some cultures (Mikola 1973, Tinus and McDonald 1979, Cordell and Marx 1980). However, the extent of mycorrhization is generally low. The main sources of "spontaneous" mycorrhization are ectomycorrhizal fungus propagules in the substrate and, particularly during good sporulation seasons, airborne spores. These two types of natural inoculum can develop a mycelial phase and infect the root systems of seedlings to some degree, under appropriate conditions. The generally low degree of mycorrhization observed, the irregular distribution over the culture and the year to year variability suggest that improvement should be possible if the cultural conditions are adjusted. However, natural infection should not be considered a dependable means of producing mycorrhizal seedlings.

The fact that seedlings may sometimes become mycorrhizal after outplanting is closely related to the status of the planting site (Whitney et al. 1972, Meyer 1973, Mikola 1973, Harvey et al. 1980). This type of mycorrhization probably does not influence seedling establishment, especially since ectomycorrhizal fungus populations decline shortly after deforestation.

Consequently, if there appears to be room for improvement in present production methods and if the aim is to produce the best possible root systems, equipped with adapted absorbing roots, it seems desirable to inoculate the substrate with ecologically adapted fungi (Marx 1977, Trappe and Fogel 1977, Kormanik 1979). Even if inoculum availability at present restricts the mycorrhization of all seedlings now being produced, we are convinced that any grower can successfully produce mycorrhizae on a more modest scale (10,000 to 30,000 seedlings, for example), by using commercial inoculum or by having the microbiology laboratory of a local university produce some inoculum for his needs. Special attention must be paid to the fertility level during production of mycorrhizae, as will be stressed later. At present, information is

available about many ectomycorrhizal fungi which have been used to synthesize ectomycorrhizae in containers on a variety of tree species. The decision to go ahead with the mycorrhization of tree seedlings on an operational scale is now in the hands of growers.

ECTOMYCORRHIZATION OF JACK PINE WITH REGARD TO FERTILIZATION

Fertilization is certainly one of the most important factors to consider when producing forest seedlings (Swan 1960, Ingestad 1967, Brix and van den Driessche 1974, Morrison 1974, van den Driessche 1980, Sheedy 1981), even more so when trying to induce mycorrhiza formation. The degree of mycorrhization is related to the fertility of the soil (Hatch 1937, Bjorkman 1942, Bowen 1973, Marx et al. 1977), and the relative value of the latter is a function of the species considered.

Some Experimental Results

On the basis of previous experiments with growing seedlings, we calculated that a single jack pine seedling could be grown in a container with the application of 3 to 12 mg of nitrogen, 1 to 10 mg of phosphorus and 1 to 13 mg of potassium over a 16-week growth period.

Over a period of several years, eight experiments were conducted at the Research Laboratory on Root Symbiosis at Laval University in Quebec City and at the provincial centre for containerized seedling production at East Angus to quantify the needs of jack pine seedlings grown in containers, both in the greenhouse and in growth cabinets, and to improve our understanding of the relationship between soil fertility and mycorrhization. Most of these experiments were conducted in styroblock-8s or Spencer-Lemaire "Hillson" containers.

In five of these studies, each cavity was individually fertilized, generally every other week, a peristaltic pump being used to standardize as much as possible the components of the prescribed nutrient solution with the relative amounts of nutrients recommended by Ingestad (1967). Substrate inoculation was conducted with inocula produced in our laboratory, either in solid or in liquid form.

Table 2 represents the nine fertility treatments of the inoculated section of a factorial design of 18 treatments each in four replicates of 40 seedlings. It indicates that variation in the addition of elementary phosphorus from 3.3 to 30 mg per cavity together with variation in elemental nitrogen from 3 to 12 mg per cavity promoted the height growth of jack pine from 7.2 cm to 12.1 cm. Dry weight increased from 106.4 mg to 359.7 mg for the shoots and from 70.4 mg to 162.6 mg for the roots. The shoot:root ratio also increased from 1.5 to 2.2.

Although we expected a positive effect from the addition of higher quantities of fertilizers on the growth of jack pine, the results showed increased mycorrhization also. However, comparison of inoculated with uninoculated treatments showed that inoculated seedlings were always smaller than uninoculated ones. The negative effects of inoculation were reduced with increased levels of nitrogen fertilization. For example, in the P2 regime, increasing nitrogen fertilization from N1 to N3 reduced the shortfall (compared with the control) in root dry weight from 27.1% to 4.6% and in the P2 regime from 21.8% to 5.5% (Table 3).

Although the seedlings from the N3P2 and N3P3 regimes were smaller than their inoculated controls (Table 3), they were larger than the seedlings produced "normally", which were fertilized in a different manner. Here also, the dry weights of the inoculated seedlings produced under "normal" conditions were significantly reduced (Table 4).

The production of mycorrhizal jack pine seedlings smaller than the non-mycorrhizal controls indicates that seedlings have to give up a portion of their photosynthesized sugars to sustain fungal development in the substrate (Melin 1956, Meyer 1974, France and Reid 1981). However, if the seedling and the fungus live in a substrate well supplied with nutrients, an increase in absorption, due in large measure to the considerable increase of the mycelial absorbing area (Harley 1969, Bowen 1973, Langlois and Fortin 1978), leads to more photosynthesis (Kidd and Reid 1979), which in turn is reflected in an increase in total dry mass of inoculated seedlings over the controls (Fig. 7).

In another experiment conducted in a growth cabinet, seven inoculation treatments were replicated three times on 32 seedlings each; 12 mg of nitrogen and 3.3 mg of phosphorus were given to each seedling during the 16-week growth period. Seedlings were inoculated with different fungi and their influence on seedling growth varied significantly. For instance, *Cenococcum* was shown to enhance shoot height very significantly in comparison with other treatments, while shoot dry

Table 2. Mycorrhization and growth of jack pine in styroblock-8s, under nine fertility regimes, in the greenhouse.

Fertility regime	(Inoculated) mg/seedling^{-1} /16 weeks^{-1}			Shoot height (cm)	Dry weight		Shoot:root ratio	Mycorrhization (%)
					Shoot (mg)	Root (mg)		
1		N_1	3.3	7.20_a*	106.46_a	$70.45_{a,b}$	1.50_a	0-5
2	P_1 3.3	N_2	6.0	8.41_b	171.71_b	89.39_b	$1.92_{a,b,c}$	0-5
3		N_3	12.0	10.60_c	282.71_c	143.13_c	$1.97_{b,c}$	5-25
4		N_1	3.0	7.25_a	103.87_a	65.67_a	$1.59_{a,b}$	25-50
5	P_2 10.0	N_2	6.0	$7.68_{a,b}$	$156.46_{a,b}$	90.39_b	$1.74_{a,b}$	5-25
6		N_3	12.0	11.54_d	342.53_d	156.66_c	2.18_c	25-50
7		N_1	3.0	6.94_a	113.26_a	$72.37_{a,b}$	$1.58_{a,b}$	50+
8	P_3 30.0	N_2	6.0	8.60_b	175.94_b	102.25_b	$1.72_{a,b}$	50+
9		N_3	12.0	12.11_d	359.78_d	162.65_c	2.22_c	50+

*Vertically, values with different letters are significantly different at the 99% level.

weight, root dry weight and root collar diameter were unaffected. A similar response was found in seedlings inoculated with *Suillus* or *Laccaria*. On the other hand, seedlings inoculated with *Hebeloma*, *Pisolithus* or *Thelephora* showed significant decreases in shoot and root dry weights and in root collar diameter (Table 5).

Hence, it appears that, under a given set of conditions, these fungi differ in their nutritional needs, and this is reflected by the different sizes of the seedlings. Although the extent of mycorrhization varied depending on the fungus used, *Cenococcum* and *Hebeloma* inoculation produced seedlings which differed significantly in size while exhibiting a comparable degree of mycorrhization.

In this last experiment, where inoculation with three of the fungal strains did not reduce seedling dry weight significantly, better control of fertility with appropriate fertilization can enhance the advantages of ectomycorrhizal infection during the initial growth period.

Significance of Fertility and Fertilization

The mycorrhization of containerized jack pine seedlings is shown to be feasible with

Table 3. Effect of substrate inoculation on the growth of jack pine at different fertility levels.

	Difference from control (%)		
	N_1	N_2	N_3
	P_1: 3.3 mg		
Total length	- 6.33	- 5.94	- 4.84
Shoot height	- 8.58	- 10.14	- 10.55
Total dry weight	- 23.71	- 23.72	- 16.81
Shoot dry weight	- 28.06	- 23.45	- 21.58
Root dry weight	- 16.09	- 24.25	- 5.41
	P_2: 10 mg		
Total length	- 2.27	- 5.46	- 9.20
Shoot height	- 7.99	- 17.41	- 19.95
Total dry weight	- 31.18	- 28.23	- 18.39
Shoot dry weight	- 33.54	- 31.52	- 23.44
Root dry weight	- 27.11	- 21.81	- 4.57
	P_3: 30 mg		
Total length	- 4.68	- 4.79	- 2.31
Shoot height	- 14.21	- 9.95	- 8.18
Total dry weight	- 28.11	- 30.38	- 15.48
Shoot dry weight	- 28.67	- 35.40	- 19.31
Root dry weight	- 21.83	- 18.59	- 5.57

Figure 7. Mycorrhizal induction and mycelium development in the substrate inoculated with *Hebeloma cylindrosporum*.

Table 4. Growth of jack pine with and without inoculum (n = 30).

	With inoculum	Without inoculum[a]
Shoot height (cm)	10.56	11.28*
Root length (cm)	15.15	15.03*
Total dry weight (mg)	398.36	487.65***
Shoot dry weight (mg)	261.17	309.78**
Root dry weight (mg)	137.19	177.86***
Shoot:root ratio	1.95	1.75*

No significant difference (*); different at 95% level (**); different at 99% level (***).

[a]With vermiculite added.

different fungi in the greenhouse as well as in growth cabinets. However, seedlings produced so far are small in comparison with MERQ specifications for reforestation. In addition, the low percentage of mycorrhization observed and the difficulty of reproducing these results from one experiment to another when the substrate is different or when containers of different sizes are used persuaded us to investigate the significance of the usual expressions of fertility and fertilization.

Table 6 shows that the distribution of 100 g of fertilizer in a 251.52 m^2 greenhouse corresponds to 3.975 kg per ha; however, the actual quantity received by each cavity depends on the area of the container. In addition to this, even for a given container, the concentration of the added fertilizer is a function of substrate dry weight, and thus of the substrate density. There will be a variation of 4 to 57 in ppm added if substrate densities are comparable with those of nursery soils (1.3) or peat moss (0.09).

The quantity of nutrients available to the seedling will vary, at a given fertility level, according to container volume and substrate density. Variations from 0.05 to 3.31 mg per cavity are expected with different combinations of container size and substrate density (Table 7).

Even if the concentration of the fertilizing solution and the area to be fertilized are known, the quantity applied, the frequency of application and the area of the container must be taken into account in order to evaluate the quantity received in each cavity. For example, a variation from 5 to 31 mg of nitrogen per cavity can be induced by combining these three factors (Table 8).

In order to define as precisely as possible the fertilizer regime used in the aforementioned growth cabinet experiment, five different expressions of the nutrient regime are given in Table 9. Thus, 3.16 mg of phosphorus were given to each seedling during the 16-week growth period, although each fertilization treatment did not necessarily contain the same quantity of the three major elements. Each treatment contributed to an increase in the concentration of a given element, which was quantified as ppm added to the substrate. The concentration of the fertilizing solution was calculated, on the basis of the addition of 5 ml of solution to each seedling at each fertilization. Note that the concentration of the fertilizing solution is not related to the concentration added to the substance. Finally, an equivalent in kg per ha per fertilization treatment was calculated; it varied from 1.3 to 3.9 for elemental phosphorus, depending on the intensity of the fertilization.

The greenhouse fertilization treatment can be evaluated in the same way. Jack pine seedlings in the greenhouse can be colonized

Table 5. Effect of inoculation with liquid suspensions of different fungi on the growth of jack pine in a growth cabinet, in Spencer-Lemaire "Hinson" containers.

	Fungus inoculum[a]						
	C	Hc	Pt	Cg	St	Lacc.	Tt
Shoot height (cm)	4.4	4.12	5.15	6.8	5.3	5.1	4.55
	a,b	a	b,c	d	c	b,c	b,c
Shoot dry weight (mg)	796	441	514	786	873	848	512
	b	a	a	b	b	b	a
Root dry weight (mg)	312	148	197	297	283	317	159
	b	a	a	b	b	b	a,b
Root-collar diameter (mm)	1.7	1.3	1.56	1.75	1.7	1.7	1.46
	c,d	a	b,c	d	c,d	c,d	a,b
% mycorrhization	0	51-75	5-10	51-65	0-5	10-15	20-25
Quality index (Dickson et al. 1960)	0.216	0.102	0.115	0.141	0.173	0.192	0.106

Horizontally, values with different letters are significantly different at the 99% level.
[a] Control Hc = *Hebeloma cylindrosporum*, 75.1; Pt = *Pisolithus tinctorius*, 76.1; Cg = *Cenococcum geophilum*, XX.50; St = *Suillus tomentosus*, 78.1; Lacc. = *Laccaria spp.*, B79.7; Tt = *Thelephora terrestris*, XX.36.

Table 6. Significance of applying 100 g of fertilizer over a 251.52 m^2 area as a function of container type and substrate density.

	Volume (cm^3)	Surface area (cm^2)	Substrate density (g/cm^{-3})	Substrate dry weight (g)	Fertilizer		
					kg/ha^{-1}	$mg/cavity^{-1}$	ppm added
Styroblock-2A	35	4.52	0.09	3.15	3.975	0.179	56.82
			0.60	21.00	3.975	0.179	8.52
			1.30	45.50	3.975	0.179	3.93
MERQ container	95	10.75	0.09	8.55	3.975	0.427	49.70
			0.60	57.00	3.975	0.427	7.49
			1.30	123.50	3.975	0.427	3.45
Spencer-Lemaire "Hillson"	150	14.44	0.09	13.50	3.975	0.574	42.51
			0.60	90.00	3.975	0.574	6.37
			1.30	195.00	3.975	0.574	2.94

Table 7. Significance of a fertility level of 17 ppm as a function of container type and substrate density.

Container	Volume (cm^3)	Surface area (cm^2)	Substrate density (g/cm^{-3})	Substrate dry weight (g)	Fertility	
					ppm	$mg/cavity^{-1}$
Styroblock-2A	35	4.52	0.09	3.15	17	0.05
			0.60	21.00	17	0.36
			1.30	45.50	17	0.77
MERQ container	95	10.75	0.09	8.55	17	0.14
			0.60	57.00	17	0.97
			1.30	123.50	17	2.10
Spencer-Lemaire "Hillson"	150	14.44	0.09	13.50	17	0.22
			0.60	90.00	17	1.53
			1.30	195.00	17	3.31

Table 8. Significance of applying 150 ppm N solution over an area of 251.52 m^2 as a function of container type and quantity applied.

Container	Quantity per pass (L)	Number of passes	Surface area per cavity (cm^2)	Fertilizer received	
				$ml/cavity^{-1}$	mg $N/cavity^{-1}$
Styroblock-2A	373	50	4.52	33.51	5.02
		70		46.92	7.03
		90		60.32	9.04
	532	50		47.80	7.17
		70		66.92	10.03
		90		86.04	12.90
MERQ container	373	50	10.75	79.71	11.95
		70		111.59	16.73
		90		143.47	21.52
	532	50		113.68	17.05
		70		159.16	23.87
		90		204.63	30.69

by ectomycorrhizal fungi when fertilized with 3.3 to 30 mg of elemental phosphorus during the 16-week growing period in a manner such that each fertilization increased the concentration of phosphorus in the substrate by 12 to 107 ppm (Table 10).

Three conclusions can be drawn from our studies with jack pine seedlings:

1) Mycorrhizal infection of containerized jack pine seedlings is feasible if specific growing conditions are met and specific fungi are used.

2) Different fungi have different effects on seedling growth.

3) It is necessary to quantify fertility and fertilization simultaneously, with different expressions, in order to optimize mycorrhizal infection and the morphological quality of the seedling in different containers and substrates.

Practical Considerations

Container size

Container size must be considered when establishing fertilization regime. This became clear to us when we used styroblock-2As in a joint experiment with the Canadian International Paper Company. The precise quantity of nutrients needed for each jack pine seedling during the growing period was determined previously. It is probable that, when this quantity of fertilizer was applied weekly to these smaller containers, the concentration of fertilizer in the substrate after each application was too high since mycorrhization was inhibited. Therefore, the fertilization regime should be adjusted to satisfy the needs of the seedlings without impeding mycorrhizal development, i.e., by repeated additions of low concentrations of fertilizer to ensure that the concentration of mineral in the substrate is compatible with mycorrhiza formation.

Species, phenology and mycorrhization

Our unpublished results indicate that nursery-grown jack pine, red pine *(Pinus resinosa* Ait.), white spruce *(Picea glauca* [Moench] Voss), black spruce *(P. mariana* [Mill.] B.S.P.) and balsam fir *(Abies balsamea* [L.] Mill.) possess particular growth patterns during the season. From this it can be inferred that these species differ in both timing and intensity of nutrient absorption.

Thus, the metabolism of these species is not synchronized, and this is probably the case with their receptivity to mycorrhizal infection as well. For example, nursery-grown jack pine and black spruce seedlings showed strong correlations between bud set, root activity, phosphate absorption and increase in trehalose content of their root systems, which are indicative of the extent of the ectomycorrhizal infection. However, these activities did not occur at the same time or with the same intensity in the two species; for example, natural ectomycorrhizal infection occurred earlier on black spruce than on jack pine.

In containers, it is possible to observe, after the reduction of fertilization and during the hardening period, some sporophores of ectomycorrhizal fungi in the cavity or even under the containers, near draining holes; generally this happens with both *Laccaria* sp. and/or *Thelephora terrestris.* Their presence, however, is not necessarily related to a high level of mycorrhizal infection. It is generally accepted that only seedlings bearing more than 50% mycorrhizal absorbing roots can be considered fully mycorrhizal. Consequently, even if standard cultural practices permit a certain degree of ectomycorrhization, closer monitoring will be necessary to maximize mycorrhizal colonization. Such monitoring will also facilitate the introduction of selected fungal inocula.

It is even probable that the overall benefits of seedling inoculation will be greater for very demanding species or for seedlings which are to be outplanted in soils of low fertility levels.

INCREASING PRODUCTION

The worksheet in the Appendix illustrates how fertilizer requirements may be precisely determined. If one knows the exact amount of fertilizer to give to each seedling during the growing period, the amount to be applied during the production period can be calculated; alternatively, if the overall production regime is known, the quantity of nutrients received by each seedling may be calculated.

In our view, quantification of the nutrients given to seedlings during culture could prove very useful, as it would permit producers to control the growth of seedlings more efficiently and to cope better with any problems which might arise if procedures were changed.

Table 9. Evaluation of fertilization treatments in the growth cabinet experiment.

Expression of fertilization		N	P	K
1. mg/seedling^{-1} /(16 weeks)$^{-1}$		11.35	3.16	4.55
2. mg/ seedling^{-1} /fertilization^{-1}	fac. 1	0.71	0.18	0.28
	fac. 2	1.42	0.37	0.57
	fac. 3	2.13	0.56	0.85
3. ppm added to the substrate /fertilization^{-1}	fac. 1	47.29	12.39	18.96
	fac. 2	94.58	24.78	37.92
	fac. 3	141.87	37.18	56.87
4. ppm in the fertilizing solution	fac. 1	141.87	37.18	56.87
	fac. 2	283.75	74.35	113.75
	fac. 3	425.62	113.53	170.62
5. kg/ha^{-1} /fertilization^{-1}	fac. 1	4.91	1.29	1.97
	fac.2	9.82	2.57	3.98
	fac. 3	14.74	3.86	5.91

Table 10. Evaluation of fertilization treatments in the greenhouse experiment.

Expression of fertilization		N	P	K
1. mg/seedling^{-1} /(16 weeks)$^{-1}$	1	3.00	3.30	7.00
	2	6.00	10.00	
	3	12.00	30.00	
2. mg/seedling^{-1} /fertilization^{-1}	1	0.43	0.47	1.00
	2	0.86	1.43	
	3	1.72	4.28	
3. ppm added to the substrate /fertilization	1	10.71	11.78	25.00
	2	21.43	35.72	
	3	42.86	107.14	
4. ppm in the fertilizing solution	1	85.71	94.28	200.00
	2	171.43	285.72	
	3	342.85	857.14	
5. kg/ha^{-1} /fertilization	1	3.78	4.15	8.82
	2	7.56	12.60	
	3	15.12	37.78	

It may be difficult to specify quantities of commercial fertilizer, especially if two or three different formulations are used at different times or simultaneously during the growth period. To facilitate computation, a worksheet was constructed (Appendix) for use in determining the ratio of different formulations to be used when a particular quantity of fertilizer is needed. In this way it is possible to know precisely what the seedlings receive each time they are fertilized, and the formulation can easily be modified when necessary. This procedure was tested successfully in the spring of 1981, when 3.5 million containerized seedlings were produced according to a precise nutrient schedule.

CONCLUSION

The presence of ectomycorrhizae on the root systems of seedlings has been shown by several workers to increase survival rate and hasten growth of the seedlings after outplanting (Shemakhanova 1962; Mikola 1973, Kormanik 1979, Ruehle 1980c, Ruehle et al. 1981).

The production of containerized mycorrhizal seedlings necessitates close monitoring of cultural conditions, if good-sized seedlings bearing numerous ectomycorrhizae are to be obtained. These seedlings will possess highly effective absorbing organs, especially when inoculated with carefully selected fungal strains (Mikola 1973, Marx 1977, Trappe 1977).

We believe that, by controlling the concentration of nutrients in the substrate, and by adjusting the frequency of fertilization to the growth pattern of the species involved, it should be possible to satisfy the needs of the seedlings even if they are grown in a substrate of low nutrient concentration, and to establish a good mycorrhizal root system.

As growers, we are at the initial stage in the development of a multi-operational process aimed at the production of new forests. Should our responsibility be limited to delivering the seedlings or should

we bear part of the responsibility for mortality rate as well, because we do not yet have a precise understanding of the necessary morpho-physiological qualities of seedlings (Russell 1977, Van Eerden and Kinghorn 1978, Harley and Russell 1979, Sutton 1979)? Research and development in plant biology should be encouraged and the findings used to improve our understanding of the many factors that contribute to seedling quality and performance.

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Appendix overleaf

APPENDIX

Calculation of fertilizer requirements for jack pine production

A. WHAT TO PROVIDE

1. Evaluation of seedlings' requirements for phosphorus:

1,000 mg X 0.25% (P) = 2.5 mg (P)
(a) (b)

(a) - desired dry weight
(b) - phosphorus content in tissue

2. Cultural need:

$$2.5 \text{ mg (P)} \times \frac{100\%}{20\% \text{ (a)}} = 12.5 \text{ mg (P)}$$

(a) - takes into account the usual availability factor for phosphates.

3. Substrate analysis:

$$\frac{17 \text{ mg (P)}}{10^6 \text{ mg (a)}} \times 13{,}500 \text{ mg (b)} = 0.229 \text{ mg (P)}$$

(a) - example of 17 ppm (P)
(b) - dry weight of substrate

4. Amount to supply:

12.5 mg (P) - 0.229 mg (P) = 12.271 mg (P)

5. "ppm added" per application:

$$\frac{12.271 \text{ mg (P)}}{13{,}500 \text{ mg}} \times 10^6 = 908.96 \text{ ppm}$$

6. Number of fertilizer applications:

$$\frac{908.96 \text{ ppm}}{50 \text{ ppm (a)}} = 18.179 \text{ or } 18$$

(a) - if we want to add about 50 ppm at each fertilizer application

7. Quantity of fertilizer per seedling per fertilizer application:

$$\frac{12.271 \text{ mg (P)}}{18 \text{ fertilizations}} = 0.681 \text{ mg (P) per fertilization}$$

8. If fertilizing one greenhouse:

$$0.681 \text{ mg (P)} \times \frac{251.44 \times 10^4 \text{ cm}^2 \text{ (a)}}{14.44 \text{ cm}^2 \text{ (b)}} = 118.58 \text{ g (P)}$$

(a) - area under the spray system
(b) - area of each cavity

9. If using commercial fertilizer:

$$\frac{118.58 \text{ g (P)}}{8.7\% \text{ (a)}} = 1{,}362.99 \text{ g (20-20-20)}$$

(a) - phosphorus content for 20-20-20

B. HOW TO SUPPLY IT

10. Volume distributed per cavity per pass:

$$117 \text{ gal (a)} \times 4.546 \text{ L} \times \frac{14.4 \text{ cm}^2 \text{ (b)}}{251.44 \times 10^4 \text{ cm}^2 \text{ (c)}} = 3.05 \text{ ml}$$

(a) - 117 imp. gal per pass
(b) - area of each cavity
(c) - area under the spray system

11. If using 2 passes per fertilization treatment:

Volume per cavity = 3.05 ml X 2 = 6.1 ml

Total volume needed = 2 x 117 gal x 4.546 = 1,063.76 L

12. Concentration of fertilizer solution:

$$\frac{0.681 \text{ mg (P)}}{6.1 \text{ ml}} \times 1{,}000 = 111.63 \text{ ppm (P)}$$

13. If using concentrate injection system:

$$1{,}063.76 \text{ L} \times \frac{1}{190 \text{ (a)}} = 5.598 \text{ L}$$

(a) - actual distribution factor of the injector

14. Concentration of fertilizer concentrate:

$$\frac{1{,}362{,}99 \text{ g}}{5.598 \text{ L}} = 243.478 \text{ g/L (20-20-20)}$$

Note: To avoid solubility problems, the concentrate should contain less than 150 g/L. Total volume of fertilizer solution or distribution factor of the injector may be modified to achieve this.

15. Concentrate to prepare:

6 L X 243.478 g/L = 1,461 g (20-20-20)