

BLACK ROCK FOREST PAPERS

HENRY H. TRYON, DIRECTOR

THE RELATION BETWEEN MYCORRHIZAE AND THE GROWTH AND NUTRIENT ABSORPTION OF CONIFEROUS SEEDLINGS IN NURSERY BEDS

By

H. L. MITCHELL, R. F. FINN

and

R. O. ROSENDAHL



CORNWALL-ON-THE-HUDSON, NEW YORK

THE RELATION BETWEEN MYCORRHIZAE AND THE GROWTH AND NUTRIENT ABSORPTION OF CONIFEROUS SEEDLINGS IN NURSERY BEDS

By H. L. MITCHELL, R. F. FINN and R. O. ROSENDAHL¹

INTRODUCTION

IN A recent paper Hatch (1936) called the attention of foresters to various forest nursery and plantation failures which were eventually traced to the absence of a biological factor in the soil, believed to be mycorrhizae. Sixteen cases were cited. In each instance timely inoculation with soil containing mycorrhizal fungi saved from abandonment nurseries and plantations which seemed doomed to failure, since watering, cultivation and fertilization of seed beds had proved ineffective in bringing about normal seedling growth.

None of the cases cited supplies incontrovertible proof that mycorrhizal fungi were the agents responsible for recovery of the trees, since the fungi were not introduced as pure cultures. But they provide extensive circumstantial evidence which agrees with, and tends to confirm, the less extensive but more direct experimental data reported in Hatch's paper. These data indicate that pine seedlings, lacking mycorrhizae, are unable to obtain sufficient mineral nutrients to exist in certain natural soils. It is significant that a few months later Young (1936), in Australia, reported precisely similar evidence.

Both Young and Hatch introduced pure cultures of mycorrhizal fungi into half of duplicate soil samples contained in pots (Hatch) and in pots and observation boxes (Young). Pine seedlings were grown under identical conditions in both the inoculated and uninoculated soils, and various precautions were taken to prevent contamination by other organisms. All non-mycorrhizal seedlings in Young's experiment showed unmistakable signs of starvation and soon perished. The mycorrhizal seedlings were healthy and vigorous. Hatch made quantitative chemical analyses of all plants and found that the mycorrhizal seedlings absorbed, from the same substrate, 75% more potassium, 86% more nitrogen and 234% more phosphorus than the non-mycorrhizal plants. The latter contained less than the minimum amounts of nitrogen and phosphorus necessary for the health and normal growth of the species (*Pinus strobus* L.). These results, because of the technique used, prove beyond any reasonable doubt the beneficial nature of mycorrhizae under the conditions of the respective experiments.

On the basis of these and other experimental data Hatch (1935)² discarded the pathogenic and organic-nitrogen theories of ectotrophic mycorrhizae and proposed a new explanation which in many respects is identical with the mineral salt hypothesis of Stahl (1900).

¹ The writers are indebted to Professor A. B. Hatch, School of Forestry, University of Idaho, for many helpful suggestions during the course of this study, and for valuable advice regarding the presentation of the data. They also wish to express their appreciation to Professor P. R. Gast, Harvard Forest, and Dr. K. D. Doak, Division of Forest Pathology, Bureau of Plant Industry, Allegheny Forest Experiment Station, for their advice and aid in the preparation of this report.

² Published in 1937.

Hatch proposed (1) that ectotrophic mycorrhizae are more efficient organs of absorption than non-mycorrhizal roots because of enormously greater surface areas, (2) that they facilitate the absorption of any and all nutrient elements ordinarily absorbed by roots, and (3) that they are invariably produced and are essential to tree growth in all but the most fertile of natural soils or in nutrient-sand cultures and seed beds where a high level of nutrient availability is artificially maintained.

Should the explanation proposed by Hatch prove correct, the importance of mycorrhizae in all nursery and forestation programs is apparent. For this reason advantage was taken of a series of nursery experiments to assemble quantitative data on the mycorrhizal question.

EXPERIMENTAL

The data on mycorrhizae here reported were obtained from a series of experiments conducted at the Black Rock Forest in an experimental nursery. These studies were for the purpose of testing, under field conditions, data on seedling nutrition obtained from soil-sand and nutrient-sand (pot) culture experiments (Mitchell, 1934; Gast, 1937). The following brief description of the experimental details will provide the background needed for a discussion of the mycorrhizal data thus far obtained. Other phases of the experiment, which is still in progress, will be reported in subsequent papers.

SEED BEDS

The soil used in the seed beds consisted of a mixture of unwashed sand, thoroughly composted sawdust and a small amount of clay. The natural soil was excavated to a depth of 4 feet from the site of each seed bed, and a line of 3-inch tile was laid along the bottom of each of the resulting pits. These were connected to a major tile line which empties into a ravine some distance from the nursery. A 1-foot layer of gravel served for subsoil, and as a base for the seed bed frames. These are 3 feet deep, made of 2-inch plank impregnated with asphalt paint, and extend about 1 inch above the surface of the ground when in place. The frames were filled with a 1-foot layer of unwashed sand above the gravel, and then 2 feet of the sand-sawdust-clay mixture.

The arrangement just described has the following advantages: (1) there can be no interchange of water or dissolved nutrients between seed beds and the surrounding soil; (2) the soil temperature within the beds is approximately that of the surrounding soil; (3) the prepared substrate has physical properties approximating those of sandy nursery soils, but it is so infertile chemically that seedlings grown therein respond readily to fertilizer applications; (4) uniformity of soil in all beds is assured by thorough mixing; (5) adequate drainage is assured; (6) whenever desirable, the contents of the frames, which are relatively permanent, may be removed

and some other natural or prepared soil substituted; and (7) aside from precautions taken to assure uniformity, adequate drainage and aeration, and to prevent contamination, physical conditions within what might be termed the "surface horizons" of these experimental beds approximate those of the average nursery seed bed. The subsoil of the former (gravel) is of course quite different, and perhaps much better (drainage, aeration, etc.), than average.

SEEDS

White pine (*Pinus strobus* L.) seeds used in this experiment were obtained from a single tree growing on the Pack Demonstration Forest, Warrensburg, New York. They were sterilized for 1 minute in 0.1% solution of bichloride of mercury, rinsed in sterile water, stratified in moist filter paper contained in sterile flasks, and stored at a constant temperature of $+5^{\circ}$ C. for 6 weeks before planting. Previous to stratification, the fresh weight of the average seed in this lot was 17.3 milligrams. Individually weighed seeds from this sample, separated into 1-milligram fresh weight classes, were planted in certain beds scattered at random throughout the various fertilizer series.

Red spruce (*Picea rubra* Link) seeds, collected in northern New Hampshire, were obtained from a commercial seed company. They were placed in dry, cold storage until just previous to planting. At this time they were immersed in absolute alcohol for 2 minutes to remove empty seeds and to stimulate germination (Baldwin, 1932). No spruce seeds were weighed individually. The fresh weight of the average seed was 2.5 milligrams.

Three days before seeds were planted all beds were treated with acetic acid as recommended by Doran (1932)— $\frac{3}{4}$ of a quart of 0.8% acetic acid per square foot of seed bed—and no damping off was noted. White pine seeds were planted in 50 beds on April 29, 1935, and 22 beds were sown to red spruce three days later. Both species had germinated and shed seed coats by June 1. The approximate germination percentage of white pine and red spruce seeds was 85% and 70% respectively.

CARE OF SEEDLINGS

Red spruce beds were shaded during the entire initial growing season with wooden frames covered with wire screen which reduced the radiation intensity to about 60% of full light. These screens were removed at the beginning of the second season. Although it is necessary to shade white pine during, and for several weeks following germination, it has been shown (Mitchell, 1936; Gast, 1937) that, for best results, this species should receive full sunlight for the greater part of the initial growing season. For this reason all screens were removed from white pine seed beds on June 25.

Pipe lines extended to all parts of the nursery so that seedling could be watered whenever necessary during dry periods. Cello glass screens, previously described (Mitchell, 1934), were used to shelter seedlings from the heavier rains. This was done to prevent excessive leaching of fertilizer applied to the seed beds, which, it was

believed, had somewhat better drainage than the average nursery seed bed.

INOCULATION OF SEED BED

On July 1 all seed beds, except certain controls, were inoculated with samples of a soil which previous tests had shown to be rich in mycorrhizal fungi. Fifty grams of moist, screened soil were thoroughly worked into the upper 2 inches of each bed.

FERTILIZER APPLICATIONS

The experiment, which was designed primarily to study the mineral nutrient requirements of coniferous seedlings, included 8 fertilizer series, 5 devoted to white pine and 3 to red spruce. Every series was made up of 7 seed beds, each of which received a different amount of nitrogen, phosphorus or potassium. Essential nutrients other than the one varied were supplied in constant amounts to all beds in each series. These basic treatments were based upon the results of preliminary experiments. Nitrogen, phosphorous and potassium were varied in individual series as indicated in Table 1.

TABLE 1

Key to fertilizer series.

Nutrient Element Varied	Source	Species	
		White Pine	Red Spruce
Nitrogen	Dried Blood	One 7-bed series
Nitrogen	NH ₄ NO ₃	One 7-bed series	One 7-bed series
Phosphorus	Rock Phosphate	One 7-bed series	One 7-bed series
Phosphorus	Bone Meal	One 7-bed series
Potassium	KCl	One 7-bed series	One 7-bed series

The fertilizer treatments summarized in Table 1 account for 56 of the 72 seed beds planted in this experiment. Four of the remaining 16 beds were left untreated as controls (3 white pine and 1 red spruce). The other 12 beds were supplied various combinations of the three nutrient elements varied in the regular fertilizer series. The latter will be referred to as the "experimental beds," and should not be confused with the regular fertilizer series summarized in Table 1.

HARVESTING

Approximately one third of the seedlings in each bed were pulled at the end of the first growing season (Oct. 1, 1935). Smaller samples were taken on June 10 and Aug. 20 of the following year. All remaining seedlings were lifted on Oct. 20, 1936, the end of the second growing season.

After pulling, the seedlings were washed with a small stream of water to remove soil particles. Each was examined under a microscope, and a record of mycorrhizal and other features of the root system was made. Seedlings were then measured, cut into two portions, root and shoot for ratio determinations, each part placed in a labeled glassine envelope, dried at $+70^{\circ}$ C. and weighed to ± 0.1 milligram.

Representative samples from each bed were preserved for reference, photographs and morphological study.

Chemical analyses of the dried specimens were made according to methods previously described (Mitchell, 1936).

CHEMICAL ANALYSIS OF SOIL

Soil samples taken during the second growing season from various places in the unfertilized (control) beds were analyzed for "readily available" phosphorus and potassium, using the method of Truog (1930) for the former and Volk and Truog (1934) for the latter. These samples were also analyzed for ammonia, using the method cited by Mahin (1932, p. 537).

RESULTS

INITIAL GROWING SEASON

Mycorrhizal Development.—It might be well to make it clear at this point that in the present paper the two terms *mycorrhizal* and *infected* are used synonymously to designate short roots which possess the typical mycorrhizal structure, the distinctive features of which have been described by Hatch and Doak (1933); and that, as a rule, no attempt will be made to distinguish between true *uninfected* short roots (which have root hairs) and *pseudo-mycorrhizal* roots (see Hatch and Doak, *loc. cit.*, for distinguishing features), these two types, neither of which is mycorrhizal, being designated as *non-mycorrhizal* and (or) *uninfected*.

Inspection under a microscope of both pine and spruce seedlings taken from the unfertilized, uninoculated (control) beds during the first season showed that mycorrhizae were entirely absent. This was believed due to the lack of appropriate inoculum (beds uninoculated) rather than to unfavorable environmental factors, since seedlings in inoculated soils, which were otherwise identical, developed abundant mycorrhizae.

Mycorrhizae were infrequent on seedlings from beds of the fertilizer series (Table 1), even though these soils were inoculated early in the summer. Apparently fertilizer applied in the basic treatments, which included the approximate optimum quantity of all essential elements other than the one varied, resulted in mineral nutrient concentrations sufficiently high to inhibit the formation of mycorrhizae even in those beds where there was a severe deficiency of a single element. This is confirmed by the fact that mycorrhizae were frequent and well developed on seedlings in certain experimental beds which had similar single nutrient deficiencies, but differed in that they received less fertilizer in the basic treatment.

The Relation between Soil Fertility and Seedling Growth.—Seedlings in the unfertilized beds grew very slowly during the first summer. The average dry weight of white pine seedlings on Oct. 1 was only about 100 mg., and that of red spruce 20 mg. This was to be expected, for although other growth factors were very nearly optimal, the prepared soil used was, as intended, extremely infertile. But seedling growth in these beds, however poor, was much more uniform than is usual in forest nurseries. This indicated uniformity of soil within each bed.

White pine seedlings in the fertilized beds responded to treatment. Nitrogen and phosphorus applications proved particularly effective. Seedlings grown in beds which received the heaviest nitrogen application weighed approximately 200 mg., a significant increase over those grown in the unfertilized beds. But since white pine seedlings weighing 300 mg. can be grown in the same length of time if nitrogen is supplied in sufficient amounts and with proper timing (Mitchell, unpublished data), it appears that the optimum amount of nitrogen was not applied to white pine seedlings during the first growing season.

Only a few of the red spruce seedlings in the treated beds survived the first fertilizer applications, which were made, as with white pine, about 3 weeks after germination was complete. The fertilizer applied (the same as for pine) apparently resulted in external (soil) nutrient concentrations sufficiently high to produce toxic internal (seedling) concentrations (see Mitchell, 1934; Gast, 1937). This tends to prove that small-seeded, slow-growing coniferous seedlings such as spruce cannot be fertilized as heavily during the initial growing season as the larger, more rapidly growing pine seedlings.

SECOND GROWING SEASON

The Occurrence of Mycorrhizae in the Unfertilized, Uninoculated Beds.—Evidence of renewed growth was apparent on white pine seedlings in all beds by April 15 of the second season. Seedlings within each of the fertilized beds were uniform as regards needle color and development and shoot increment, but in the unfertilized, uninoculated beds certain small, scattered groups could be distinguished from the rest of the seedlings on the basis of needle color and shoot increment. During the next few weeks these rapidly growing groups spread to include many nearby seedlings, and some new groups appeared. Seedlings in immediate contact with these groups would turn from yellow-green to a deep blue-green, and become noticeably taller than adjacent seedlings within two weeks. This differential growth could not be explained on the basis of soil fertility differences, since the soil in all beds was as uniform as thorough mixing could make it. Similar rapidly growing groups appeared in the control spruce beds at about the same time. The chief difference was that, in the case of spruce, the only seedlings showing any evidence of new growth were those in small, scattered groups. All white pine seedlings were growing, although at two very different rates.

On June 10, about twenty-five days after the differential growth was first noted, seedlings were pulled from the center of each of the rapidly growing groups, which were easily distinguished even at a distance from the seed beds. At this time, these groups did not amount to more than 10 per cent of the seedlings in any single seed bed. Control seedlings were selected at random from the area outside the rapidly growing groups. Examination under a microscope showed that mycorrhizae were much more frequent on the roots of seedlings from the rapidly growing groups. These data, together with those on dry weights and chemical analyses, are presented in Table 2.

TABLE 2

Comparison of the dry weight increase and mineral nutrient absorption of mycorrhizal and non-mycorrhizal seedlings of white pine and red spruce grown in identical substrates.

	Species	Red No. ²	No. Seedlings in Sample	% Short Roots Mycorrhizal ³	Total Dry Wt. in Mg. ⁴	c_M^5	t	Odds ⁶	Root Wt. in Mg.	Shoot Wt. in Mg.	Root Shoot Ratio	Nitrogen Content		Phosphorus Content		Potassium Content	
												% Dry Wt.	Mg. N	% Dry Wt.	Mg. P	% Dry Wt.	Mg. K
Samples taken June 10, 1936 ¹	(1)	(2)	(3)	(4)			(5)		(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
	White Pine	1	36	68.6 *	207.9 ± 5.1 *	7.78	> 1000 to 1	72.3	135.6 *		0.533 *	1.485 *	3.09 *	0.129 *	0.27 *	0.496	1.03 †
			45	1.2	160.2 ± 3.4			73.0	87.2		0.836	1.233	1.98	0.078	0.13	0.549	0.88
	White Pine	2	11	64.5 *	193.0 ± 13.9 *	3.53	> 1000 to 1	67.1 *	125.9		0.533	1.476 *	2.85	0.147 *	0.28	0.587	1.13 *
			10	1.8	120.3 ± 15.2			47.3	73.0		0.647	1.272	1.53	0.103	0.12	0.584	0.70
Samples taken Aug. 20, 1936 ¹	White Pine	3	8	76.8 *	184.2 ± 18.0 *	2.66	124 to 1	61.0	123.2 *		0.494 *	1.552 †	2.86	0.126 *	0.23	0.332	0.61 *
			28	2.4	127.5 ± 11.4			51.4	76.1		0.675	1.463	1.87	0.101	0.13	0.337	0.43
	Red Spruce	4	80	62.3 *	52.7 ± 5.5 *	4.25	> 1000 to 1	12.9 *	39.8 *		0.324 *	1.665 *	0.88	0.197 *	0.11 *	0.825 *	0.44 *
			39	1.3	27.7 ± 2.1			8.3	19.4		0.427	1.164	0.32	0.077	0.02	0.627	0.17
	White Pine	1	46	70.2 *	337.2 ± 14.5 *	10.18	> 1000 to 1	127.4 *	209.8 *		0.607 *	1.599 *	5.39	0.213 *	0.72	0.630	2.12 *
Percentage increase or decrease in dry weight and N, P and K content from June 10 to Aug. 20, 1936.	Red Spruce	4	63	9.5	180.6 ± 5.1			74.6	106.0		0.703	1.204	2.17	0.070	0.13	0.450	0.81
			50	75.1 *	184.2 ± 6.8 *	22.95	> 1000 to 1	47.7	136.5 *		0.349 *	1.585 *	2.92	0.177 *	0.33	0.685 *	1.26 *
			67	1.7	25.7 ± 1.2			7.8	17.9		0.435	1.217	0.31	0.075	0.02	0.538	0.14
	Mycorrhizal Pine	1	62.1	75.9	54.7		74.4	166.6	105.8
	Non-Mycorrhizal Pine	1	12.7	2.2	21.5		1.0	none	-8.0
Percentage increase or decrease in dry weight and N, P and K content from June 10 to Aug. 20, 1936.	Mycorrhizal Spruce	4	249.5	269.7	242.9		281.8	200.0	186.3
	Non-Mycorrhizal Spruce	4	-7.2	-6.0	-7.7		-3.1	none	-17.6

¹ Data from samples taken during second growing season.

² All soils identical.

³ The number of mycorrhizal short roots is expressed as a percentage of the total number of short roots on each seedling (terminology after Hatch and Doak, 1933). Each percentage in the above table represents the mean of all individual observations for any given sample.

⁴ The yields of white pine from the different beds are not exactly comparable because of differences in the size of the seeds planted (see discussion in text).

⁵ Standard error of mean (Mills, 1931; Snedecor, 1934 and Bruce and Schumacher, 1935).

⁶ Fisher's t , which is the quotient of the actual difference between two values divided by the standard error of the difference, is the usual statistic for testing the significance of differences (Fisher, 1933 and Snedecor, 1934). Wherever possible data compared in the above table were tested for significant differences by referring to Fisher's tables, in which are given the least value of t that can be considered significant, and the least value that can be considered highly significant, for any given number of de-

grees of freedom. Significant differences, or the absence thereof, are indicated respectively in the above table by the two symbols * and †.

⁷ Odds against such a difference appearing in random samples of an homogeneous population. These values were estimated by interpolating "normal deviates," which correspond to Fisher's t , in the "probability" graph shown in Fig. 18, p. 86, Bruce and Schumacher (1935). Differences are usually considered as just significant if the odds are 20 to 1, and highly significant when the odds are 100 to 1 or greater.

Since no attempt was made to inoculate the unfertilized beds during either the first or second growing season, spores or mycelium of mycorrhizal fungi must have been carried by the wind or introduced into the beds by some other natural agency. *Hymenomyces*, many species of which are known to be mycorrhizae-formers, are plentiful in natural forest stands which adjoin the nursery on two sides. Regardless of how these beds were inoculated, the progress of mycelium, radiating out from the infected groups, was easily followed. Needles of uninfected seedlings invariably showed the yellow-green characteristic of nitrogen poverty, and the purple which, in some species, is known to be an indication of phosphorus deficiency (Deuber, 1930; Mitchell, 1934; Némee, 1935). Soon after infection, the needles of these seedlings became dark green—blue-green in the case of white pine—and the growth increased to such an extent that infected and uninfected seedlings could be easily distinguished on this basis. These observations were checked by pulling numerous seedlings at weekly intervals during the entire summer. The weekly samples were inspected for mycorrhizal development, but no record of weights was made.

By August 20 the infected groups had spread to include approximately 75 per cent of the seedlings in each of the control beds. The final harvest of infected and uninfected seedlings was made at this time. It would have been desirable to wait until the end of the growing season, but because of the rate of infection, it was necessary to sample immediately in order to obtain an adequate number of uninfected seedlings. By the end of the season practically all of the white pine seedlings were mycorrhizal, and in the spruce beds mycorrhizal seedlings were the only ones that survived. All data on seedlings harvested on August 20 are presented in Table 2.

Mycorrhizal Development in the Fertilized Beds.—The remaining seedlings in the fertilized beds were harvested on October 20. As stated previously, these beds were inoculated the first year. But unlike the first season, mycorrhizae were frequent and well developed on seedlings from every bed in which there was known to be a deficiency of one or more nutrient elements. Adsorption, leaching, absorption by seedlings or utilization by soil fungi apparently reduced the concentration of readily soluble nutrients, applied the first season in the basic treatments, to a point favorable to mycorrhizal development. Mycorrhizae were infrequent, poorly developed or entirely lacking on seedlings from seed beds in which there were no nutrient deficiencies.

Seedling Growth in the Fertilized Beds.—As in the first season, the growth of seedlings in the fertilized beds varied with nutrient environment. But it is impossible to separate the effects of the two factors, nutrition, which was purposely varied, and mycorrhizal development, which seemed to be inversely proportional to soil fertility. It is apparent, therefore, that comparative data on the dry weight increase of well-fertilized seedlings, which lacked mycorrhizae, and those grown in nutrient-deficient environments, which had abundant mycorrhizae, prove little or nothing regarding the influence of mycorrhizae

on seedling growth. Such comparisons, used by Addoms (1937), lead to assertions that mycorrhizae are not highly beneficial, a conclusion which the writers question. For this reason the fertilizer series yield data are of little importance to the present discussion, and will not be presented in detail.

THE RELATIONS BETWEEN SOIL FERTILITY AND THE INCIDENCE AND DEVELOPMENT OF MYCORRHIZAE

Observations made during the second growing season indicate that when other factors are favorable, the frequency of incidence of mycorrhizae, and the degree of their development on the roots of pine and spruce seedlings, vary inversely as the concentration of readily available nutrients in the soil. Seedlings harvested during the second growing season from infected areas of the poorest soil used—that in the unfertilized (control) beds—had the best-developed mycorrhizae and the greatest proportion of mycorrhizal short roots. Those grown in soils of intermediate fertility possessed fewer and less well developed mycorrhizae. And mycorrhizae were infrequent, poorly developed or entirely lacking on seedlings grown in all beds supplied sufficient fertilizer to preclude any possibility of nutrient deficiency.

These observations regarding the inverse relationship between soil fertility—the only apparent variable—and mycorrhizal development are believed to be highly significant. They are based upon numerous and consistent data, and it is exceedingly doubtful if, in this experiment, the differential occurrence of mycorrhizae can be explained upon any other basis. Lack of appropriate inoculum undoubtedly accounted for the absence, during the first year, of mycorrhizae on seedlings grown in the extremely infertile, but uninoculated (control) beds. Environmental factors were probably favorable since seedlings grown in inoculated soils, which were otherwise identical, developed abundant mycorrhizae. The infrequency, during the first year, of mycorrhizae on seedlings grown in nutrient-deficient beds of the fertilizer series is also in keeping with other observations regarding the occurrence of mycorrhizae. Although these beds were inoculated, as were all fertilized beds, there can be little doubt that the fertilizer applied in the basic treatment, which included the approximate optimum amount of all essential elements other than the one varied, resulted in mineral salt concentrations sufficiently high to inhibit mycorrhizae formation. That environmental factors were otherwise favorable for mycorrhizal development is evidenced by the fact that mycorrhizae were formed in abundance on seedlings grown in certain experimental beds which differed only in that they received less fertilizer in the basic treatment. It is also significant that during the second season, when no basic nutrient solution was supplied (since it was believed unnecessary), mycorrhizae were frequent and highly developed on seedlings in every one of these beds in which there was known to be a deficiency of one or more nutrient elements.

COMPARISON OF THE DRY WEIGHT INCREASE OF MYCORRHIZAL AND NON-MYCORRHIZAL SEEDLINGS GROWN IN THE SAME NUTRIENT ENVIRONMENT

The experimental evidence presented in Table 2 indicates that on June 10 of the second growing season mycorrhizal seedlings of spruce and pine, from the rapidly growing groups in the control beds, were significantly heavier than uninfected seedlings growing in the same beds. Differences in total dry weight were even greater on August 20, the second sampling date. As stated previously, growth in all of these uninoculated, unfertilized beds was very uniform during the entire initial season, indicating that soil and other growth factors were equal. It is believed, therefore, that the observed differences in growth were the result of differential inoculation by mycorrhizal fungi (see Plates I and II), introduced into the seed beds by some natural agency.

On June 10, the mean root weight of mycorrhizal seedlings of white pine was approximately the same as that of non-mycorrhizal seedlings growing in the same

beds (Table 2). It appears that the early spring superiority of the former in total dry weight was due to their shoot increment, which was significantly greater than that of non-mycorrhizal seedlings. Both roots and shoots of mycorrhizal spruce were considerably heavier than those of uninfected seedlings on both sampling dates. Root, shoot and total dry weights of infected and uninfected seedlings of both species are compared in Fig. 1.

All white pine data presented in this and other illustrations are based on samples taken from seed bed No. 1, in which, due to refinements in experimental technique, a higher degree of precision was attained. Compare the standard errors associated with mean weights of seedlings from bed No. 1 with those of samples from beds 2 and 3 (Table 2). Seedlings of the former tend to deviate less from the mean, since all were grown from individually weighed seed, none of which varied from the average of the group by more than ± 0.5 milligram. Seeds planted in the other two beds varied in weight according to the frequency distribution characteristic of the species and the sample, in this case an approximate range of from 7.0 to 27.0 mg.

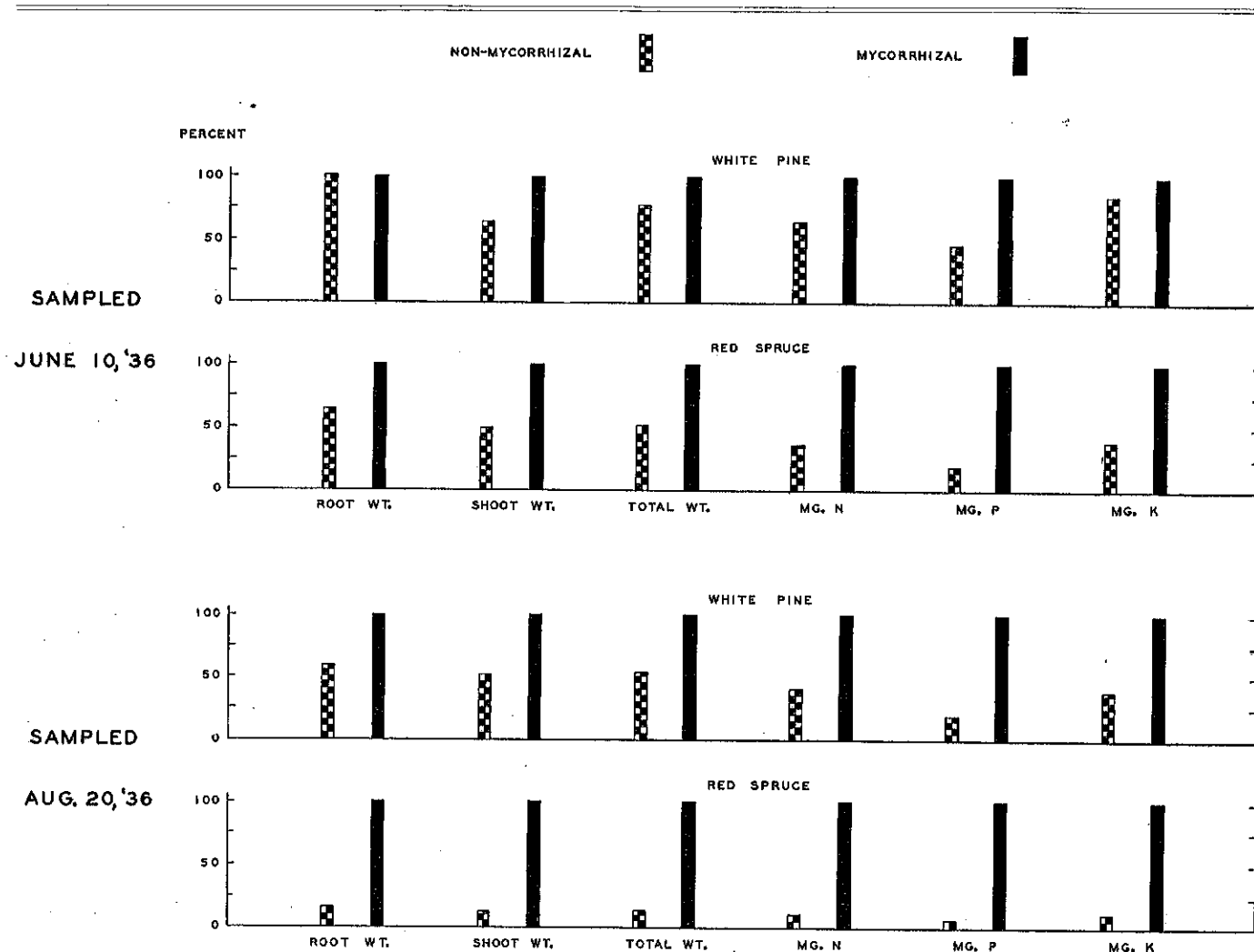


FIG. 1.—Comparison of the root, shoot and total dry weight, and the nitrogen, phosphorous and potassium content, of mycorrhizal and non-mycorrhizal seedlings of white pine and red spruce grown in otherwise identical substrates. Data from samples taken at two different times during the second growing season (see Table 2).

Seeds having a mean fresh weight of 20.5 mg. were sown in bed No. 1, whereas those used in beds 2 and 3 weighed only 17.3 mg. Since it has been shown (Aldrich-Blake, 1930; Mitchell, 1934; Gast, 1937) that seed weight influences the weight of the resulting seedling, this difference explains why seedlings (uninfected as well as infected) grown in bed No. 1 are heavier than those from beds 2 and 3. No attempt was made to correct for this factor, as it is apparent that data from beds 2 and 3, although not exactly comparable, do corroborate trends indicated by the more numerous and precise data from bed No. 1. It is interesting that the former, in spite of few observations and relatively large standard errors, show statistically significant differences between mycorrhizal and non-mycorrhizal seedlings (Table 2).

The superiority in the total yield of mycorrhizal over non-mycorrhizal seedlings of pine and spruce was greater on August 20 than at the beginning of the summer (Fig. 1). If this difference in dry weight increase was due to mycorrhizae, the only apparent variable, then it is evident that the beneficial influence of the association between fungus and tree root becomes more marked as the growing season advances. In this connection it is interesting to note that, at the end of the season, the roots as well as the shoots of mycorrhizal pine were heavier than those of uninfected seedlings.

A comparison is made in Fig. 2 of the percentage increase or decrease in the weights of mycorrhizal and non-mycorrhizal seedlings of pine and spruce between June 10 and August 20. These data indicate that non-mycorrhizal spruce made no growth whatever during this period, whereas infected seedlings increased in total dry weight by 250%. Losses in weight by non-mycorrhizal spruce, as shown in Fig. 2, were probably due to the outward movement (leaching) of mineral nutrients that usually precedes death. As stated previously, no uninfected spruce seedlings survived the entire second growing season. It was even difficult to obtain a sufficient sample on August 20. Non-mycorrhizal pine made some growth (mostly shoot increment) during this period, increasing in total dry weight by 13% as compared to 62% for infected seedlings.

THE INFLUENCE OF MYCORRHIZAE ON ROOT-SHOOT RATIO

It appears that on June 10 the relative balance between root and shoot weight, as measured by the ratios presented in Table 2, was, from the standpoint of good planting stock, less favorable for mycorrhizal than for non-mycorrhizal seedlings. It is characteristic of young coniferous seedlings to apply the majority of their total assimilate to shoot increment in the early spring. Maximum root growth takes place during the late summer and early fall. Therefore, all that the early season difference in root-shoot ratio indicates is that mycorrhizal seedlings were growing, and at a faster rate. It will be observed that later in the season, when the roots had at least partly completed their annual growth, there was less difference between the root-shoot ratios of infected and uninfected seedlings. Mycorrhizae, even though abundant and well developed, are so small and succulent

that they have but little influence on dry weights or, therefore, root-shoot ratios. As pointed out above, the root weights of mycorrhizal and non-mycorrhizal pine seedlings were almost identical on the first sampling date.

COMPARISON OF THE MINERAL NUTRIENT ABSORPTION OF MYCORRHIZAL AND NON-MYCORRHIZAL SEEDLINGS GROWN IN THE SAME SOIL

It is generally conceded that if mycorrhizae are of benefit to seedlings growing in certain environments, it is because of their influence on seedling nutrition, *i.e.*, the absorption of mineral nutrients or some other growth promoting substance. This is logical reasoning since it is well established that, other factors being favorable, dry weight increase is proportional to the internal (plant) nutrient concentration, and that, as a rule, nutrient intake is proportional to the availability of nutrients in the external (soil) solution (Aldrich-Blake, 1930; Mitchell, 1934; Gast 1937). But these relations do not hold under all conditions. As frequently demonstrated, seedlings can not take advantage of high nutrient supplies in environments where light, temperature, soil moisture or some other growth factor is limiting. Lack of proper balance between the various essential elements may also inhibit growth. Thus, difference in the mineral nutrient content of mycorrhizal and non-mycorrhizal seedlings, even though real and significant, would not, under unfavorable conditions, be reflected in dry weight increase.

In view of this fact it is difficult to understand why the majority of investigators, including many of those who believe that mycorrhizae in some way enable tree seedlings to obtain more of the essential elements, are inclined to base their conclusions regarding the mycorrhizal relationship on yield data only. When it is considered, as pointed out above, that various factors other than the one being studied may inhibit growth responses, the danger in drawing conclusions regarding the influence of mycorrhizae on seedling nutrition, without first obtaining nutrient absorption as well as yield data, becomes apparent.

The analysis data presented in Fig. 1 (see Table 2) indicate significant differences in the nitrogen and phosphorus content of mycorrhizal and non-mycorrhizal seedlings of pine and spruce on both sampling dates, the superiority of infected seedlings being more pronounced at the end than at the beginning of the season. With the exception of white pine in early spring, the same is true of potassium intake. In Fig. 2 mycorrhizal and non-mycorrhizal seedlings of both species are compared on the basis of percentage increase or decrease in N, P and K content (total milligrams) during the growing period. It is evident that non-mycorrhizal pine and spruce, from the infertile control beds, were unable to make any significant gains in N, P or K during the second growing season, whereas infected seedlings growing in the *same* substrate made very substantial increases. This difference in mineral nutrient intake undoubtedly explains the observed differences in growth previously discussed. That no other factor was limiting

is proven by the fact that well-nourished seedlings grown under otherwise identical conditions in certain beds of the fertilizer series made excellent growth—superior to that of either mycorrhizal or non-mycorrhizal seedlings grown in the less favorable nutrient environment of the unfertilized (control) beds.

Comparisons of mineral nutrient content of seedlings on a per cent dry matter basis, an expression of internal (seedling) concentration, are not always reliable in the early summer. That is why the analysis data presented in Figs. 1 and 2 are calculated as milligrams of N, P and K per seedling. Internal concentration is a function of seedling yield and the absolute amount of a given element, both of which are changing rapidly during the early part of the growing season. Compare, for example, the K content of mycorrhizal and non-mycorrhizal pine sampled on June 10 from bed No. 1 (Table 2). Although the former contain a greater *absolute amount* of K, they have a lower K *concentration* than the average

non-mycorrhizal seedling. This is due, largely, to the difference in dry weight increase, the “diluting” influence of rapid growth being reflected in the lower K concentration of the infected seedlings. However, nutrient concentration is an acceptable basis of comparison at the end of the season, since there is no shoot growth and very little mineral absorption after the seedlings become dormant.

Analysis data presented in Figs. 1 and 2 suggest that nitrogen, and especially phosphorus, were deficient in the soil used. Potassium poverty was more apparent in the case of spruce than pine. These observations are confirmed by the fact that, in beds of the fertilizer series (identical soil), white pine seedlings responded well to applications of nitrogen and phosphorus, whereas potassium additions resulted in but little stimulation in growth. It is probable that the sawdust used in the soil mixture supplied a fair amount of potash.

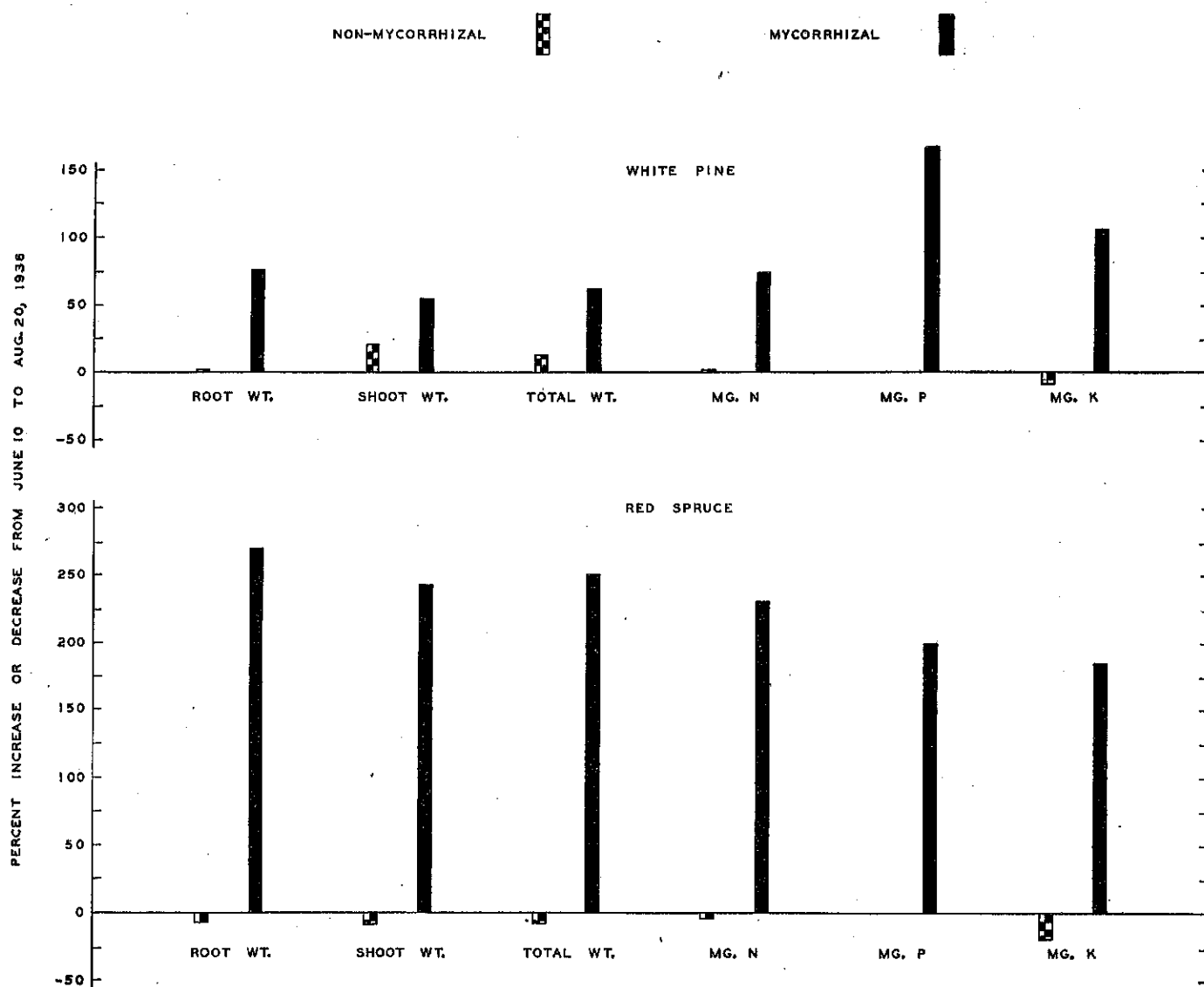


FIG. 2.—Comparison of the percentage change in the dry weight and the nitrogen, phosphorus and potassium content of mycorrhizal and non-mycorrhizal white pine and red spruce seedlings from June 10 to August 20 of the second growing season.

A QUANTITATIVE BASIS FOR EVALUATING THE INFLUENCE
OF MYCORRHIZAE ON THE NUTRIENT ABSORPTION
AND GROWTH OF TREE SEEDLINGS

The data presented in Table 2, and discussed in the preceding sections, suggest that mycorrhizal seedlings of pine and spruce are able to obtain more of the essential nutrients than non-mycorrhizal seedlings growing in the same substrate, i.e., the unfertilized sand-sawdust-clay mixture. Under conditions of the present experiment the superior absorption-capacity of the former, believed attributable to mycorrhizae, is reflected in their dry weight increase, which is significantly greater than that of seedlings lacking mycorrhizae.

But such comparisons, although they are of interest to plant physiologists and those investigating the mycorrhizal relationship, do not, in themselves, supply the nurseryman with sufficient information. From the standpoint of those concerned with the production of planting stock, it is not enough to say that seedlings equipped with mycorrhizae absorb more nutrients, grow larger or that their growth is "normal" in comparison with that of uninfected plants. "Normal growth" is too indefinite as a standard of comparison. Unfortunately many investigators consider as normal, growth which is far below that of which certain species are capable even under "average" conditions.

It would be desirable, therefore, to evaluate results obtained with experimental seedlings of a given species, infected and uninfected, in terms of the nutrient content and yield which may be expected of the species when grown for a certain length of time in a given climate under experimentally determined optimum conditions. Such comparisons require certain silvical information, much of which is available for some species of *Pinus*.

Aldrich-Blake (1930), Mitchell (1934) and Gast (1937) have presented data regarding the mineral nutrient and light requirements of Corsican, Scots and white pine seedlings. These studies show that nutrient intake and seedling yield are proportional to mineral nutrient availability, when light and other growth factors are favorable, and establish the yields and mineral nutrient contents which can be expected under approximately optimum conditions. But data from these experiments, although valuable in many respects, are not exactly comparable to those of the present study because the former are based on results obtained with the nutrient-sand (pot) culture technique. For this reason they do not constitute as reliable a standard of comparison as do data on seedlings from the various fertilizer series of the present nursery experiment—a study designed to test under nursery condition the conclusions based on data from seedlings grown in the carefully controlled but more artificial pot cultures.

In Table 3 the weights and nutrient content of mycorrhizal and non-mycorrhizal seedlings from the unfertilized (control) beds are compared with those of seedlings grown for the same length of time in unfertilized sand, in the sand-sawdust-clay beds receiving the heaviest application of N, P and K, and with the nutrient

concentration of seedlings grown for one year in nutrient-sand cultures supplied the experimentally determined optimum fertilizer ratio. As they are of some interest, data on the nutrient content of the leaves and needles of variously fertilized mature trees are also included in Table 3.

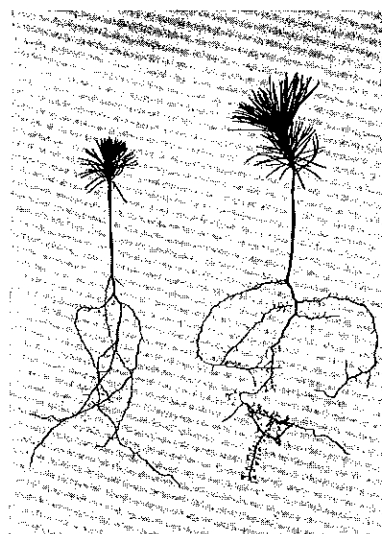
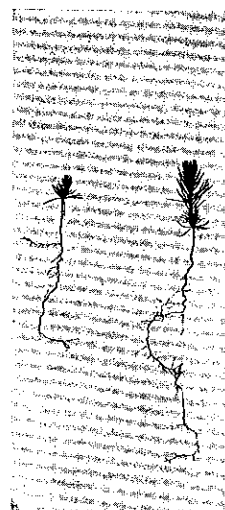
All white pine seedlings compared in this table were grown in substrates of good physical structure, assuring adequate drainage and aeration. All received full sunlight during the entire second, and for the greater part of the initial growing season. Water was supplied when needed and in sufficient amounts, and soil reaction in each instance was within the range of from pH 5.5 to 6.5. Mineral nutrition was the only factor purposely varied.

Seedlings grown for two seasons in unfertilized sand (in nursery seed beds) contained mineral elements originally present in the seeds, and small additional amounts apparently supplied by sand or water. Growth was necessarily poor. These seedlings are a product of about the poorest nutrient environment in which the species can survive for this length of time. Many succumbed during the first winter and the second growing season. Non-mycorrhizal seedlings from unfertilized beds containing the sand-sawdust-clay mixture made somewhat better growth, probably because they obtained more nitrogen from this substrate (Table 3). As previously discussed, seedlings equipped with mycorrhizae were able to extract more N, P and K from this same substrate. Their growth was also significantly better (Table 2). But, as shown in Table 3, these unfertilized seedlings, although equipped with mycorrhizae, did not absorb the quantity of mineral elements, nor did they grow as large, as the well-nourished but non-mycorrhizal seedlings grown under otherwise identical conditions in beds which received the heaviest N, P and K applications used in the fertilizer series. The latter, although larger than the 2-0 stock produced in the majority of nurseries, are not assumed to represent the very best which can be expected of white pine in this length of time. They are offered as a standard of comparison because they are the best thus far produced in this nursery in two growing seasons. It is known, however, that these seedlings did not receive as much nitrogen, especially during the initial growing season, as they are capable of utilizing.

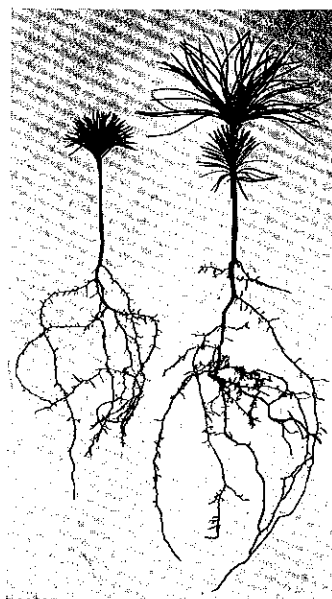
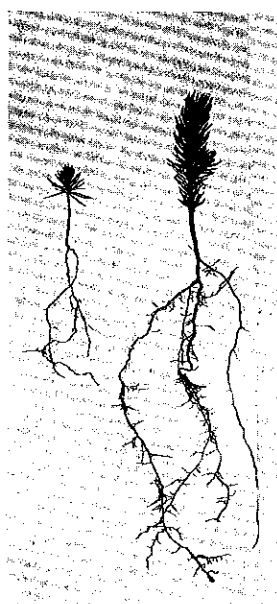
Data on seedlings from sand cultures watered with nutrient solutions of approximately the optimum fertilizer ratio for this species afford another, although probably less reliable measure of accomplishment. The yields are not comparable since the sand culture seedlings were grown for only one season. But the analysis data provide an index to the mineral nutrient concentrations of seedlings grown in substrates of high nutrient availability. It will be observed (Table 3) that these seedlings had a much higher mineral nutrient concentration than any of the nursery-grown seedlings. But in the latter environment it is probably neither possible nor desirable to attain as high a level of mineral nutrient availability, and therefore rate of absorption, as in the more artificial culture solutions. Whereas various ions are free in solution in nutrient cultures, in natural

IN.

JUNE 10



AUG. 20



RED SPRUCE

WHITE PINE

PLATE I.—Comparison of mycorrhizal (right) and non-mycorrhizal (left) seedlings of white pine and red spruce on two sampling dates.



PLATE II-A.—Mycorrhizal short roots on mother roots of white pine seedling from infected region of unfertilized bed; X8.

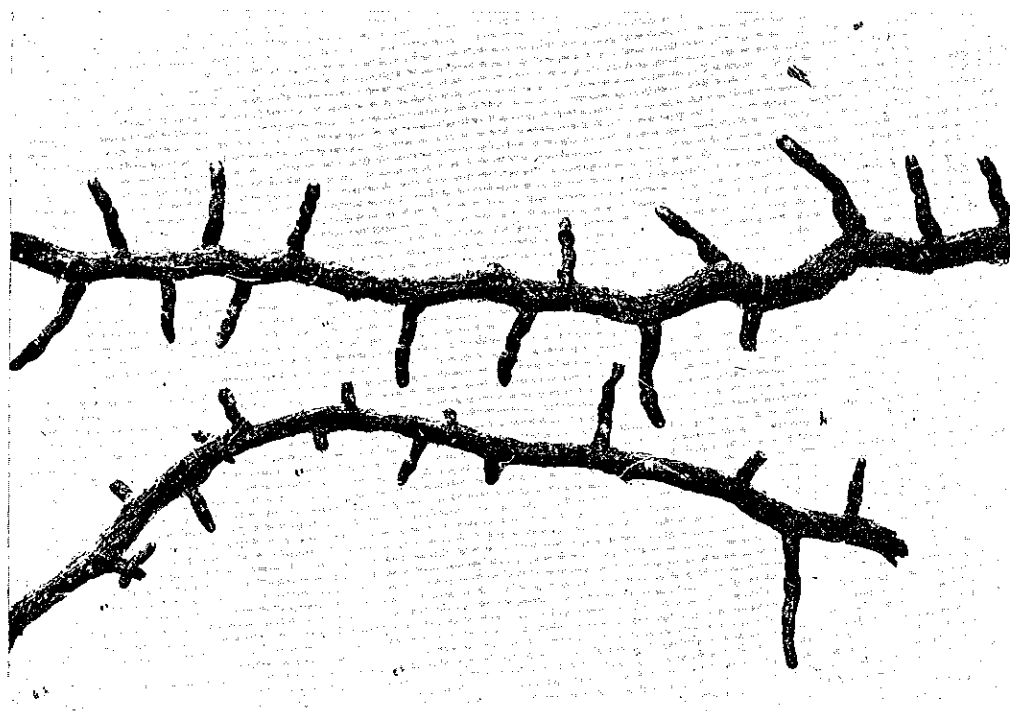


PLATE II-B.—Non-mycorrhizal short roots (that is, pseudomycorrhizae, as defined by Melin, 1917 and 1927) on mother roots of white pine seedling from region of unfertilized bed in which typical mycorrhizae were lacking; X10. The other non-mycorrhizal root type—true uninfected short roots, which possess root hairs—was infrequent in the unfertilized beds, and is not shown. (See Hatch and Doak, 1933, for distinguishing features of the various short root types).

TABLE 3

Yield and mineral nutrient content of mycorrhizal and non-mycorrhizal seedlings (from control bed) compared with similar observations on seedlings grown in different nutrient environments, and with the ash content of the leaves of variously fertilized mature trees.

Species	No. of Growing Seasons	Soil or Culture Medium	Fertilizer Treatment	Mycorrhizae	Total Dry Wt. in Mg.	Nitrogen Content		Phosphorus Content		Potassium Content		Calcium Content	
						% Dry Wt.	Mg. N	% Dry Wt.	Mg. P	% Dry Wt.	Mg. K	% Dry Wt.	Mg. Ca
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
White Pine Seedlings	2	Sand in nursery seed beds	None	Abundant	130	0.72	0.94	0.11	0.14	0.82	1.07	0.23	0.30
White Pine ¹ Seedlings	2	Sand-sawdust-clay mixture in seed beds	None	Few	181	1.20	2.17	0.07	0.13	0.45	0.81
White Pine ² Seedlings	2	Sand-sawdust-clay mixture in seed beds	None	Abundant	337	1.60	5.39	0.21	0.72	0.63	2.12
White Pine ³ Seedlings	2	Sand-sawdust-clay mixture in seed beds	Heavily fertilized	None	550	2.50	13.75	0.24	1.32	1.10	6.05
White Pine ⁴ Seedlings	1	Sand nutrient (pot) cultures	Optimum	None	...	3.26	0.60	1.80	0.36
Needles from ⁵ mature Norway Spruce	Approx. 45 yrs.	Natural soil low in P and N	None	1.67	0.17	0.88	0.72
Leaves from ⁵ mature Red Oaks	Approx. 50 yrs.	Natural soil low in P and N	None	1.66	0.22
Leaves from ⁵ mature Red Oaks	Approx. 50 yrs.	Natural soil low in P and N	Optimum N, P, K and Ca	2.70	0.42	1.01	0.81

¹ Data on non-mycorrhizal seedlings taken from the unfertilized (control) bed on August 20 (see Table 2).

² Data on mycorrhizal seedlings taken from the unfertilized (control) bed on August 20 (see Table 2).

³ Data on seedlings from the fertilizer series beds which received the experimentally determined optimum amounts of P, K, and Ca, and the highest N treatment applied in this experiment.

⁴ Data on seedlings grown for one season in sand cultures watered with a nutrient solution of approximately the optimum fertilizer ratio for the species (Mitchell, 1934 and unpublished data, and Gast, 1937). The concentration (in ppm.) of the various nutrient elements in this solution was as follows: N, 300; P, 300; K, 150; Ca, 250; S, 231; Mg, 175 and Fe, 3.4.

⁵ Leaf and needle samples were taken during that

period in which the mineral nutrient content is maximum (Mitchell, 1936).

⁶ Analyses reported here are averages of trees growing on quarter-acre plots which were supplied the optimum (for species) amounts of N, P, K and Ca (Mitchell, 1936, 1936 and unpublished data). Annual diameter increment was used as the basis for determining the most effective treatment.

soils, even those of high fertility, a large portion of the mineral elements are adsorbed in organic and inorganic base exchange compounds from which they are liberated slowly.

Analyses of the foliage of variously fertilized mature trees, shown in Table 3, are of interest in that they show the nutrient concentration of leaves from rapidly growing mature trees to be very similar to that of well-nourished white pine seedlings. The former data are based on averages of trees grown on quarter-acre plots which received different fertilizer treatments in various series similar to those of the nursery experiment. Other factors being favorable, the growth of mature trees, as with seedlings, is proportional to the nutrient content of the foliage, which is in turn regulated by the availability of nutrients in the soil solution (Mitchell, 1935, 1936 and unpublished data; Chapman, 1935; Chandler, 1936). Differences in annual diameter increment amounting to as much as 100% have been observed between mature oaks whose leaves, due to differences in soil nitrogen supply, varied in N content between 1.66% and 2.70%. Unfortunately, few data are now available regarding the influence of high mineral nutrient supplies on the incidence and development of mycorrhizae on the roots of mature trees growing in natural habitats.

SOIL ANALYSIS DATA

In order to test the hypothesis recently advanced by Burges (1936), various chemical analyses were made of soil samples taken from infected and uninfected areas—those yielding, respectively, mycorrhizal and non-mycorrhizal seedlings—of the otherwise uniform soil of the unfertilized (control) beds. Stated briefly and in general terms, Burges believes that if mycorrhizae are of any benefit to higher plants it is because of the activity of the mycelium in the substrate. In his opinion the presence of the fungus in a mycorrhizal association is an example of controlled parasitic attack. This theory is directly opposed to that of Hatch (1937), who believes that the mycorrhizal association is an example of symbiosis, and regards the intimate relationship between root and fungus as the basis of the observed mutual benefit.

In order to appreciate fully the significance of the soil analysis data presented in Table 4, it is necessary to consider first the magnitude of variance in mineral nutrient supply which is needed to produce, in seedlings, differences in nutrient content as large as those observed between mycorrhizal and non-mycorrhizal seedlings grown in the same substrate. According to the data of Hatch (1936), mycorrhizal and non-mycorrhizal seedlings differed in nitrogen, phosphorus and potassium content by approximately 2.3 mg., 0.6 mg. and 1.6 mg. respectively. Data from the present study (Table 2) indicate differences of approximately 3.2 mg. N, 0.6 mg. P and 1.3 mg. K. A measure of the influence of mycorrhizae upon the nutrition of the species (*P. strobus*) can be obtained by comparing these differences with the nutrient absorption data from carefully controlled experiments in which nutrient environment was the only variable. Mitchell (1934) and Gast (1937) have shown that, in

the "working range," nitrogen supplies differing by as much as 75 ppm. (N concentration of external solution) are necessary to produce a 3-milligram difference in the N content of resulting seedlings. It has also been found (Mitchell, unpublished data) that P supplies must differ by 125 ppm., and K supplies by 90 ppm., if the resulting seedlings are to differ by 0.6 mg. P and 1.5 mg. K. These nutrient absorption data were obtained from 1-year-old white pine seedlings. Results of the present nursery experiment indicate that similar differences in nutrient supply are necessary to produce the same order of variation in the nutrient content of seedlings during the second growing season.

According to the Burges hypothesis, any benefits attributable to mycorrhizae are due largely to the activity in the soil of the fungus which, in common with various other (non-symbiotic) soil fungi (Waksman, 1932), may influence the mineralization of complex soil compounds, thereby liberating in the soil solution nitrogen and minerals in inorganic and readily soluble forms which higher plants can absorb directly through their roots. But if differences of the magnitude observed in the present experiment (Table 2)—and that of Hatch (1936)—were chiefly the result of the activity, in localized areas, of mycorrhizal or other fungi in the non-symbiotic rôle which the Burges hypothesis suggests, then it is evident from the nutrient absorption data discussed above that there must have been highly significant differences in the available nutrient supply of infected and uninfected regions of the seed beds. The soil analysis data presented in Table 4 do not reveal any such differences.

The results reported in the first line of Table 4 are for soil samples taken from the immediate vicinity of mycorrhizal white pine seedlings when the latter were

TABLE 4

Ammonia and readily available phosphorus and potassium content of soil samples taken from around the roots of mycorrhizal and non-mycorrhizal white pine seedlings growing in the same seed bed.

Soil	Results Expressed as Milligrams NH ₃ , P and K per Kilogram of Soil (air dry basis)		
	Ammonia ¹	"Readily Available" Phosphorus ²	"Readily Available" Potassium ³
Soil surrounding roots of mycorrhizal seedlings	0.79	26.4	126.4
Soil surrounding roots of non-mycorrhizal seedlings	0.75	27.4	133.3

¹ By distillation with carbonate-free magnesium oxide (Mahin, 1932, p. 537).

² Determined according to the 0.002 N sulfuric acid extraction method of Truog, 1930 (200 parts of extractant to 1 part soil).

³ Determined according to the N ammonium acetate extraction method of Volk and Truog, 1934 (15 parts of extractant to 1 part soil).

lifted on August 20 of the second growing season. Soil from infected spots in seed bed No. 1 was rich in mycorrhizal fungi. The data presented in the second line of Table 4 are based on soil samples taken from around the roots of uninfected seedlings growing in the same seed bed—identical soil. The latter samples were inspected under a microscope and fungal hyphae were found to be almost entirely lacking.

One criticism of this method of approach is that continuous removal by seedlings growing in infected areas would tend to prevent any measurable accumulation of nutrients, even though mineral elements were liberated in the soil solution by the activity of the fungi. Because of this possibility, tests were also made of soil samples from infected and uninfected areas in which, due to removal the previous year, no seedlings were growing at the time. The results of these analyses were essentially the same as those reported in Table 4.

It is apparent that these soil analyses do not reveal sufficient differences in the ammonia and "readily available" P and K content of infected and uninfected soils to account for the observed differences in the growth and nutrient content of mycorrhizal and non-mycorrhizal seedlings harvested from the respective locations in the otherwise uniform soil of the unfertilized (control) beds. There is nothing in these results which could be interpreted as confirming in any way the hypothesis advanced by Burges.

DISCUSSION

EVIDENCE SUPPORTING THE STAHLIAN-HATCH THEORY OF THE RELATION BETWEEN SOIL FERTILITY AND THE INCIDENCE AND DEVELOPMENT OF MYCORRHIZAE

It was recently pointed out by Hatch (1937) that much of the confusion of the last 50 years regarding the function of mycorrhizae had its origin in conflicting statements about the prevalence of mycorrhizae. The majority of investigators apparently assumed that if mycorrhizae were infrequent structures they could have but little influence upon plant growth; and, conversely, if they were abundant, they might be exceedingly beneficial to the host. This reasoning led students to one or the other conclusion regarding the function of mycorrhizae, depending upon whether their observations indicated that mycorrhizae were abundant or infrequent.

The earliest conflict of this type was between Frank (1885) and Robert Hartig (1886). The former, who examined the roots of trees growing in natural forest habitats, found that practically all the short roots were mycorrhizal. Hartig, who confined his studies to trees growing in garden soils, reported that no roots were mycorrhizal. He concluded that Frank's theory of nutrition by means of mycorrhizae had no basis in fact, and that mycorrhizae, like leaf spot infections, vary according to season and soil and are rarely of any great significance.

In 1900 Stahl proposed a theory which explained these conflicting observations on the basis of soil fertility differences. It was his experience, and also that of

Schlicht (1889), that the frequency of incidence of mycorrhizae varied inversely as the fertility of the soil. This would account for the absence of mycorrhizae, as reported by Hartig (1886), in garden soils which, as a rule, are more fertile than forest soils. Stahl believed that mycorrhizae facilitate the absorption of mineral nutrients; also that they appear and are of significance only in nutrient-deficient environments. The first direct experimental proof of the correctness of Stahl's theory regarding the mycorrhizal relationship in trees was obtained by Hatch (1932, 1937) during the years 1930-35 in a series of cooperative experiments with Gast (1937) and with Mitchell (1934). These studies included soils which ranged from the extremely infertile raw humus soils of spruce stands in northern Sweden to the comparatively fertile soils of the mixed-hardwood stands of the cove type in the Black Rock Forest. The nutrient availability range of these soils was varied further by fertilizer applications so arranged that many combinations of availabilities of individual nutrient elements were obtained. Data from these studies indicate that the abundance of mycorrhizae in pine varies inversely as soil fertility and as the availability of at least four individual nutrient elements: nitrogen, phosphorus, potassium and calcium. In his summary, Hatch (1937) makes the following statement: "except as saturation, excessive dryness, or unfavorable acidities of soils affect the ability of mycorrhizal fungi to survive, the distribution of ectotrophic mycorrhizae in nature is determined by the availabilities of nutrient elements." In his opinion the susceptibility to infection by mycorrhizal fungi is controlled, either directly or indirectly, by the internal (root) concentration of nutrient elements, which in turn is regulated by the availability of nutrients in the external (soil) solution.

Observations made during the present experiment confirm conclusions drawn from the earlier studies. They show, further, that the inverse relationship between soil fertility—that is, the supply of dissolved nutrients, *i.e.*, free ions—and the incidence of mycorrhizae in pine and spruce holds under nursery conditions as well as in soil, sand-soil and nutrient-soil (pot) cultures. Although appropriate fungi were present, and environmental factors were known to be otherwise favorable for their development, mycorrhizae were few or entirely lacking on the roots of seedlings grown in every bed supplied sufficient fertilizer to preclude any possibility of nutrient deficiency. Mycorrhizae were more frequent on seedlings grown in soils of intermediate fertility. The best-developed mycorrhizae were those formed in abundance on seedlings in each control and fertilizer series bed in which there was known to be a severe deficiency of one or more nutrient elements. No results inconsistent with these observations appeared during the examination of seedlings from the 72 seed beds planted in the present experiment. These data would seem to supply the final proof in conifers of the inverse relationship between the availability of mineral salts and mycorrhizal development which was the basis of Stahl's hypothesis.

Lack of earlier experimental proof of the correctness of Stahl's theory can undoubtedly be attributed to the

fact that investigators have confined their studies of the mycorrhizal relationship almost entirely to natural forest soils of the temperate humid regions. It is well known that the majority of forest soils, particularly those supporting coniferous stands, are of poor or average fertility, the better soils being used, where possible, for agriculture. The reasons for emphasis on forest soils are both apparent and, from the more practical standpoint, justified. But it is true, although not so apparent, that in many regions it is difficult to find, among natural forest soils, a range in mineral nutrient availability sufficiently wide to thoroughly test Stahl's theory of distribution. Only the most fertile of agricultural and forest soils have high enough concentrations of readily available mineral nutrients to inhibit mycorrhizal development when the inoculum is present and other factors are favorable. The better cove soils of the Black Rock Forest, which support a near-climax type and are representative of the best forest soils in this region, approach, but have not yet attained, a level of nutrient availability sufficiently high to preclude mycorrhiza-formation (Mitchell, 1934; Hatch, 1937).

EVIDENCE SUPPORTING THE THEORY THAT MYCORRHIZAE
ARE BENEFICIAL TO TREES GROWING IN ALL
BUT THE MOST FERTILE SOILS

For two decades circumstantial evidence has been accumulating which points clearly to the mycorrhizal relationship as beneficial to tree seedlings growing in many soils, and indispensable to the life of those in very unfavorable nutrient environments (Hatch, 1936, 1937). Unthrifty, non-mycorrhizal seedlings of entire nurseries in unforested regions have recovered only after inoculation with mycorrhizal fungi contained in small quantities of soil (Kessell, 1927; Anonymous, 1931; Oliveros, 1932). Non-mycorrhizal transplants in plantations, apparently unsuccessful, recovered after interplanting with mycorrhizal stock (Roeloffs, 1930). Coniferous seedlings germinating in newly-drained peat bogs were found to survive only when infected by mycorrhizal fungi, presumably introduced as spores blown in from surrounding woodlands (Melin, 1917).

These cases supply extensive circumstantial evidence of the beneficial nature of mycorrhizae. But conclusive proof that mycorrhizae were the agents responsible for recovery of the trees is lacking in each instance, since the fungi were not introduced as pure cultures. In 1934 and 1936 Rayner reported experiments in which pure cultures of mycorrhizal fungi and (or) small amounts of humus containing specific mycorrhiza-formers were introduced into soils (also treated with various organic composts) in which, previous to treatment, conditions were such that normal mycorrhizae were infrequent (although inoculum was usually present) and seedling growth was poor. This treatment relieved the apparently "inimical soil condition" and resulted in a marked stimulus to seedling growth and, subsequently, to mycorrhiza-formation with which seedling growth and vigor seemed highly correlated. But the precise significance of these experiments is uncertain so far as the

correlation between mycorrhiza-formation and seedling growth is concerned since, as Rayner remarks, "the improvement may be masked by the relatively enormous effects produced by compost treatments."

The shelterbelt plantings in the prairie states of America led Hatch (1936, 1937) to investigate the occurrence and possible importance of mycorrhizae in prairie soils. He grew a total of 114 *Pinus strobus* seedlings, from individually weighed seed, in six pots of soil collected near Cheyenne, Wyoming, and found that mycorrhizal fungi were lacking in the soil and that seedling growth was negligible. Half the pots were then inoculated with pure cultures of mycorrhizal fungi. Within two months a remarkable increase in the growth of the inoculated seedlings was observed. Chemical analyses revealed that the mycorrhizal plants possessed 75% more potassium, 86% more nitrogen and 234% more phosphorus than uninoculated seedlings grown in the same soil. From the comparisons which Hatch made with data from experiments of the senior author (Mitchell, 1934 and unpublished data), it appeared that the non-mycorrhizal seedlings had lower concentrations of N and P than any hitherto recorded for the species, and contained absolute amounts of these elements less than the minimum necessary for health and normal growth. Subsequently Young (1936) in Australia reported similar experimental evidence. He grew pine seedlings in parallel series of a natural soil contained in pots and observation boxes. Soils of one series were inoculated with pure cultures of mycorrhizal fungi. Seedlings grown therein developed abundant mycorrhizae and were healthy and vigorous. Those in the uninoculated soil showed little growth, their needles developed the purple color which is a certain indicator of phosphorus deficiency (Mitchell, 1934), and none survived.

On the basis of these data, and in the absence of direct experimental proof to the contrary, Hatch (1937) proposed that mycorrhizae are beneficial to seedlings growing in all but the most fertile or artificially maintained soils, that they are essential for the survival of trees in very infertile soils, and that their function is to increase the absorption of mineral salts as well as organic nitrogen and carbohydrates.

The data obtained from the present study confirm this theory. In seed beds where mycorrhizal environment was the only apparent variable, infected seedlings of both pine and spruce were found to absorb significantly greater amounts of nitrogen, phosphorus and potassium, and to grow significantly larger, than non-mycorrhizal seedlings of these species grown for the same length of time in the same soil. Non-mycorrhizal seedlings of pine were able to exist—but not grow—in this infertile nursery soil, but no uninfected spruce survived the entire second growing season. The obviously superior absorption-capacity or "feeding power" of the infected seedlings, which was reflected in their dry weight increase, is believed attributable to mycorrhizae. Because of the precautions observed in the present experiment, it is exceedingly doubtful if the observed differences can be explained upon any other basis. That no other environmental factors were limiting is evidenced by the

fact that well-nourished seedlings in certain of the fertilized beds, where conditions were otherwise identical, attained a size greater than that of either infected or uninfected seedlings produced in the less fertile environment of the unfertilized (control) beds.

The data presented are based upon carefully selected samples of the numerous seedlings grown in the various nutrient environments of the present nursery experiment. Pure cultures of mycorrhizal fungi were not introduced artificially as in the experiments of Rayner (1934), Hatch (1936) and Young (1936), but in common with those of Melin (1917) were introduced by natural agencies. The results are no less significant since there is now incontrovertible proof that mycorrhizal fungi are the agents responsible for the recovery of trees growing in certain natural soils lacking in these organisms (Hatch, 1936, 1937; Young, 1936).

EVIDENCE REGARDING THE BURGESS HYPOTHESIS

The theory recently advanced by Burges (1936) can be interpreted as attributing to mycorrhizal fungi a rôle similar to, and of no greater significance than that usually ascribed to non-symbiotic soil fungi and bacteria. Burges believes that higher plants growing in certain environments frequently benefit by absorbing, directly through their roots, the nutrients made soluble as a result of the action of soil organisms—which “bear no necessary connection with mycorrhizal fungi”—in decomposing complex organic matter in the soil. Mycorrhizal fungi may in this manner stimulate the growth of higher plants. But Burges regards the presence of the fungus in a *mycorrhizal association* as an example of controlled parasitic attack and of no *mutualistic* significance. So far as tree species are concerned, Burges presents no direct experimental proof to support his theory.

Although Rayner (1934 and 1936) is apparently convinced that there is a “direct causal relation between mycorrhiza-development and thrifty growth in seedlings of various species of *Pinus*,” certain of her observations tend to support, in part, the hypothesis of Burges. In certain experiments she noted a marked improvement in the growth of pine seedlings following inoculation with specific mycorrhiza-formers, *previous to the symbiotic association* between fungus and tree root. From this she concluded that, “The *initial* stimulus cannot therefore be due to improved nutrition depending upon mycorrhizal action in the ordinary sense.” Rayner’s explanation is that the initial stimulus is due to the formation of definite growth-promoting substances (auximones) and (or) nutrients liberated by the activity of the mycorrhiza-formers in a favorable substrate. This tends to support the theory of Burges. But in all other respects Rayner’s views differ from those of Burges. In Rayner’s opinion the *initial stimulus* is directly responsible for *mycorrhiza-formation*, “which automatically follows,” and the latter—not necessarily less important—“is the end term of a series of biological activities in the rooting medium. . . .”

Hatch (1937), whose views are directly opposite to those of Burges, regards the intimate “connective” relationship between fungus and root as the basis of the

observed benefit. According to his explanation, “The mycotrophic relationship in pine, and presumably in other species possessing ectotrophic mycorrhizae, is a symbiotic mechanism which increases, chiefly by physical and therefore by relatively non-selective means, the absorption of soil nutrients.”

Hatch’s interpretation, which emphasizes the importance of the mycorrhizal structure, is strengthened by the fact that chemical tests, including those reported in Table 4 of the present paper, fail to disclose differences in the “available” nutrient content of infected and uninfected soils sufficient to account for the observed seedling responses. But these results, although they favor the explanation of Hatch, do not in themselves prove the Burges hypothesis invalid. The apparent lack of difference in the “readily available” P and K content of infected and uninfected areas of the otherwise uniform soil of the control beds may or may not be significant. It is not at all certain, for example, that the two chemical solvents which were used to extract from the soil the P and K included in the determinations reported in Table 4, imitate exactly the nutrient extractive and absorptive powers of coniferous seedlings. Thus there may have been local increases in the concentration of nutrients actually available to the seedlings—exchangeable ions liberated by the activity of mycorrhizal fungi in infected areas—which these analysis methods failed to reveal.

Although it is apparent that the phosphorus and potassium analysis data presented in Table 4 do not confirm the Burges hypothesis, it is doubtful if the validity of this hypothesis can be seriously questioned solely on the basis of these observations. But more importance is attached to the fact that there were no significant differences in the ammonia content of infected and uninfected soils. Ammonia, which is known to be available to coniferous seedlings, is one of the first decomposition products formed by the action of fungi in the decomposition of complex nitrogenous compounds (Waksman, 1932). If the Burges hypothesis is accepted as valid, the observed superiority of seedlings grown in infected soils of the present experiment can be attributed to benefits accruing from the activity of the fungi in these soils. But if, due to the activity of the fungi, ammonia and other available nitrogen compounds were liberated in the infected soils in sufficient quantities to account for the observed differences in seedling growth and nitrogen content, it is difficult to understand why differences in ammonia were not detected by the technique used. The analysis method followed, since it requires no chemical solvents which attack complex nitrogenous compounds, is such that only free and readily soluble ammonia is included in the determination. Therefore the criticism which detracts from the significance of the P and K results reported in Table 4, is not equally applicable in the case of ammonia.

On the basis of results obtained in the present experiment, particularly the data on ammonia, and in view of experimental evidence presented by Hatch (1937), the writers are inclined to favor Hatch’s explanation of mycotrophy in conifers.

SUMMARY

White pine (*Pinus strobus* L.) and red spruce (*Picea rubra* Link) seedlings were grown for two seasons in a very infertile sand-sawdust-clay mixture contained in specially constructed seed bed frames. Fifty seed beds were devoted to pine, and twenty-two to spruce. The same substrate was used throughout. Certain seed beds were unfertilized. In others nitrogen, phosphorus and potassium were varied in individual series and in several combinations. All fertilized beds were inoculated during the initial growing season with small amounts of soil containing mycorrhizal fungi. During the second season the unfertilized, uninoculated (control) beds were differentially infected (in spots) by mycorrhizal fungi, presumably introduced by some natural agency. Sample seedlings taken from different regions of each bed at various times during the first and second growing seasons were inspected for mycorrhizal development, weighed individually and analyzed for N, P and K.

Inspection of seedlings from beds in which *nutrient environment was the only variable* showed the following relationships: (1) mycorrhizae were few or entirely lacking on seedlings grown in all beds supplied sufficient fertilizer to preclude any possibility of mineral nutrient deficiency; (2) they were more frequent on seedlings in beds of intermediate fertility; and (3) the best-developed mycorrhizae were those formed in abundance on seedlings in every soil known to be deficient in one or more nutrient elements. These observations indicate that when appropriate fungi are present and other environmental factors are favorable, the frequency of incidence of ectotrophic mycorrhizae, and the degree of their development on the roots of pine and spruce seedlings, vary inversely as the concentration of readily available nutrient elements in the soil. These results agree in every respect with the Stahlian-Hatch theory of the relationship between mineral salts and mycorrhizal development.

Comparisons of infected and uninfected seedlings grown in the *same nutrient environment* revealed the following differences: mycorrhizal seedlings of pine and spruce absorbed significantly greater amounts of nitrogen, phosphorus and potassium, and their dry weight increase was significantly greater, than non-mycorrhizal seedlings grown for the same length of time, under otherwise identical conditions, in the infertile soil of the unfertilized (control) beds. The latter seedlings showed unmistakable symptoms of starvation, made little or no growth, and, in the case of spruce, did not even survive the second growing season.

It is extremely doubtful if, in this experiment, the obviously superior nutrient absorptive capacity—for P and K as well as N—of the mycorrhizal plants, as measured by chemical analyses and as reflected in dry weight increase, can be explained upon any other basis. The fungi were introduced by some natural agency rather than as pure cultures. But the results are no less significant since there is now incontrovertible proof that mycorrhizal fungi are the agents responsible for the recovery of trees growing in certain soils lacking in these organisms.

Chemical analyses of samples from infected and uninfected areas of the otherwise uniform soil of the unfertilized beds did not reveal sufficient differences in ammonia and "readily available" P and K to account for the observed seedling responses solely on the basis of the activity, in the soil, of mycorrhizal or other fungi in the non-symbiotic rôle attributed to them by Burges (1936). For this and other reasons the writers favor Hatch's (1937) explanation of mycotrophy in conifers.

These observations indicate that the benefits attributable to mycorrhizae, like their distribution in nature, vary inversely as the concentration of readily available mineral nutrients in the soil. It appears that when appropriate inoculum is present and other environmental factors are favorable, ectotrophic mycorrhizae occur on and are of benefit to coniferous seedlings growing in a wide range of nutrient environment—all but the most fertile or artificially maintained soils—and that seedlings, lacking mycorrhizae, are unable to exist in very infertile substrates.

REFERENCES

- Addoms, Ruth M. 1937. Nutritional Studies on Loblolly Pine. *Plant Physiol.* 12:199-205.
- Aldrich-Blake, R. N. 1930. The Plasticity of the Root System of Corsican Pine in Early Life. *Oxford Forest Memoir No. 12.*
- Anonymous. 1931. Establishing Pines. Preliminary Observations on the Effects of Soil Inoculation. *Rhodesian Agr. Jour.* 28:185-187.
- Baldwin, H. I. 1932. Alcohol Separation of Empty Seed, and Its Effect on the Germination of Red Spruce. *Amer. Jour. Bot.* 19:1-11.
- Bruce, D., and Schumacher, F. X. 1935. *Forest Mensuration.* McGraw-Hill, New York.
- Burges, A. 1936. On the Significance of Mycorrhiza. *New Phytologist*, 35:117-131.
- Chandler, R. F. 1936. Nitrogen Deficiency as a Problem in Certain Forest Soils of New York State. (Paper read before joint session of the Society of American Foresters and the Ecological Society of America at the A. A. A. S. Meetings, Atlantic City, Dec. 29, 1936).
- Chapman, A. G. 1935. The Effects of Black Locust on Associated Species with Special Reference to Forest Trees. *Ecological Monographs*, 5: (1):37-60.
- Deuber, C. G. 1930. Fertilization of Hardwoods. *National Shade Tree Conference Proceedings.*
- Doran, W. L. 1932. Acetic Acid and Pyroligneous Acid in Comparison with Formaldehyde as Soil Disinfectants. *Jour. Agr. Res.* 44:571-578.
- Fisher, R. A. 1933. *Statistical Methods for Research Workers.* Fourth revised edition. Oliver and Boyd, Edinburgh.
- Frank, A. B. 1885a. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Ber. d. deut. Bot. Ges.* 3:128-145.
- 1885b. Neue Mittheilungen über die Mycorrhiza der Bäume u. der Monotropa Hypopitys. *Ber. d. deut. Bot. Ges.* 3:27-33.
- Gast, P. R. 1937. Studies on the Development of Conifers in Raw Humus III. The Growth of Scots Pine (*Pinus Silvestris* L.) Seedlings in Pot Cultures of Different Soils under Varied Radiation Intensities. *Meddel. fr. Statens Skogs-försöksanstalt* 29:587-682.
- Hartig, Robert. 1886. Ueber symbiotische Erscheinungen im Pflanzenleben. *Bot. Centralbl.* 25:350-352.
- Hatch, A. B. 1932. Correlation between the Development of Pine Mycorrhizae and the Nutrient Content of Soils. (Paper read before joint session of the American Society of Plant Physiologists and the Ecological Society of America at the A. A. A. S. Meetings, Atlantic City, Dec. 28, 1932).
- , and Doak, K. D. 1933. Mycorrhizal and Other Features of the Root System of Pinus. *Jour. Arnold Arb.* 14:85-99.
- 1936. The Role of Mycorrhizae in Afforestation. *Jour. For.* 34:22-29.

- Hatch, A. B. 1937. The Physical Basis of Mycotrophy in Pinus. Black Rock Forest Bull. No. 6.
- Kessell, S. L. 1927. Soil Organisms. The Dependence of Certain Pine Species on a Biological Soil Factor. Empire For. Jour. 6:70-74.
- Mahin, E. G. 1932. Quantitative Analysis. McGraw-Hill, New York.
- Melin, Elias. 1917. Studier över de norrländska myrmarkernas vegetation. Almqvist & Wiksells, Uppsala. Norrländskt Handbibliotek VII Sonderbdr. A. Akad. Avhandl. p. 1-426.
- Mills, F. C. 1931. Statistical Methods. Henry Holt and Co., New York.
- Mitchell, H. L. 1934. Pot Culture Tests of Forest Soil Fertility. Black Rock Forest Bull. No. 5.
- 1935. A Method for Determining the Nutrient Needs of Shade Trees with Special Reference to Phosphorus. Black Rock Forest Papers, 1:1-4.
- , and Finn, R. F. 1935. The Relative Feeding Power of Oaks and Maples for Soil Phosphorus. Black Rock Forest Papers, 1:5-9.
- 1936. The Effect of Varied Solar Radiation Upon the Growth, Development and Nutrient Content of White Pine Seedlings Grown Under Nursery Conditions. Black Rock Forest Papers, 1:15-22.
- 1936. Trends in the Nitrogen, Phosphorus, Potassium and Calcium Content of the Leaves of Some Forest Trees During the Growing Season. Black Rock Forest Papers, 1:29-44.
- Nömc, A. 1935. Příspěvek k seznání chemismu t. zv. kareňních zvěvů u borovice. Lesnická Práce, 14:442-451. (Biol. Ab. 10:11676. 1936).
- Oliveros, S. 1932. Effect of Soil Inoculation on the Growth of Benguet Pine. The Makiling Echo. 11:205-214.
- Rayner, M. C. 1934. Mycorrhiza in Relation to Forestry. I. Researches on the Genus *Pinus*, with an Account of Experimental Work in a Selected Area. Forestry, 8:96-125.
- 1936. The Mycorrhizal Habit in Relation to Forestry. II. Organic Composts and the Growth of Young Trees. Forestry, 10:1-22.
- Rocloffs, J. W. 1930. Over Kunstmatige Verjonging van Pinus Merkusi Jungh. et de Vr. en Pinus Khasya Royle. Tectona, 23:874-907.
- Schlicht, A. E. C. 1889. Arbeiten aus dem pflanzenphysiologischen Institute der Königlich landwirtschaftlichen Hochschule in Berlin, XIII. Beitrag zur Kenntniss der Verbreitung und der Bedeutung der Mycorrhizen. Landwirtsch. Jahrb. 18:478-506.
- Snedecor, G. W. 1934. Calculation and Interpretation of Analysis of Variance and Covariance. Collegiate Press, Ames, Iowa.
- Stahl, E. 1900. Der Sinn der Mycorrhizenbildung. Jahrb. f. wiss. Bot. 34:534-668.
- Truog, E. 1930. The Determination of the Readily Available Phosphorus of Soils. Jour. Amer. Soc. Agronomy, 22:874-882.
- Volk, N. J., and Truog, E. 1934. A Rapid Chemical Method for Determining the Readily Available Potash of Soils. Jour. Amer. Soc. Agronomy, 26:537-546.
- Waksman, S. A. 1932. Principles of Soil Microbiology. Second revised edition. Williams and Wilkins, Baltimore.
- Young, H. E. 1936. A Mycorrhiza-Forming Fungus of Pinus. Australian Inst. of Agr. Sci. 2:32-34.