THE MYCORRHIZAL ASSOCIATION BETWEEN *LILIUM* TAXA AND THE PHYCOMYCETE *ENDOGONE FASCICULATA*

by

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A special thanks to my wife, Dee, who assisted me in laboratory work and in the preparation of this thesis.

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ABSTRACT

This study was initiated to determine the effect mycorrhiza have on the growth of Lilium. It was conducted in the Plant Science greenhouses at Newark, Delaware during 1970 and 1971. The experimental design was a split plot replicated forty times.

This experiment analyzed the effect that two soil types, two Lilium taxa and two innoculations had on growth.

The effect that the innoculations had on the growth of Lilium was highly significant. The tissue analysis yielded results indicating an increased ability of mycorrhizal plants to obtain nutrients.

This study showed that visual discrimination of mycorrhizal plants is possible.

INTRODUCTION

This investigation was begun to establish the possibility of an endotrophic mycorrhizal association in the genus Lilium and to determine whether or not it is beneficial to the plant.

Researchers and gardeners alike have long underestimated the role the rhizosphere plays in the quality of plants they grow. Most growers think only of supplying water, nutrients, the correct pH, aeration, and other commonly appreciated requirements. We have only recently come to realize that the microflora of the root zone is also a very important consideration.

We now recognize that many of the practices associated with the growing of horticultural crops may have harmful effects. The rhizosphere, the area surrounding the root where interactions among soil microflora and fauna occur, is in a state of sensitive balance that can be unknowingly disrupted by seemingly unrelated activities. We can not ignore the potentially harmful effects of soil sterilization and other soil amending processes.

As early as 1820, the presence of the mycorrhizal-forming fungus was observed. However, it was not until recently that any of the functions were understood. At that time, some believed the mycorrhizal association to be a disease while others believed that plants were growing parasitically on the fungus.

The mycorrhizal association is mutually beneficial to the plant and the fungus. The plant benefits by the increased nutrient uptake and the fungus benefits by recieving carbohydrates from the higher plant's reserves.

Researchers were slow to realize that two types of mycorrhiza exist. Much of the early work was done by foresters who found the ectotrophic mycorrhiza growing on the roots of pine. Ectotrophic mycorrhiza have a large mass of mycelium visible outside of the root. Even though the fungus was clearly visible, they did have misconceptions about its function. Much work was done with ectotrophic mycorrhiza before researchers realized that endotrophic mycorrhiza existed. Endotrophic mycorrhiza have only a small mass of mycelium outside the root while a much greater percentage is found internally. The endotrophic mycorrhiza were more common but since they did not alter the gross morphology of the root they were overlooked.

The fungus Endogone fasciculata (Thaxter), found in almost any soil, is very active in the rhizosphere. It readily attacks roots that supply carbohydrates on which the fungus could feed. These organisms are destroyed by soil sterilization. The result is that the mycorrhizal association that Endogone is capable of forming with plants is not established. This fungus, in a mycorrhizal association, provides the plant with nutrients that they are not otherwise able to get.

It is important to understand the exact role mycorrhiza play in plant nutrition. Mycorrhizal plants are found almost everywhere

in the world and the benefits they offer could yield significant economic benefits.

LITERATURE REVIEW

The presence of mycorrhiza in the rhizosphere was established in the early 1800's. The discoverers knew that mycorrhiza existed because they could see its mycelium on the surface of the roots. The reaearch since then has yielded information on the habits, functions, types and visual expressions of mycorrhiza.

Gerdemann (2) came to the conclusion that "vesicular-arbuscular mycorrhiza occur on most cultivated crops and on many herbaceous and woody non-cultivated plants." The difficulty in transferring innoculum has held back research with mycorrhiza. Researchers began to notice that there were other indications of infection that were not as obvious as the mycelium present on the roots. Many of these indications had been noted before but they had never been associated with mycorrhiza.

Daft and Nicolson (1) in 1966 listed specific outward expressions of the presence of a mycorrhizal association. They noted that the endotrophic mycorrhiza did not cause the root structure to change as did the ectotrophic. Endotrophic mycorrhiza caused a color change in the roots. Maize roots that were uninfected remained white while infected roots turned a pale yellow. This color disappeared quite rapidly upon exposure to light so its value as a criterion of infection is questionable. Other observational

criteria indicating mycorrhizal presence include such things as a reduction in the number of root hairs, and thicker, more brittle and contracted roots. None of these criteria are definitive enough to be positive indicators.

Daft and Nicolson (1) also observed the most obvious and probably the most important criterion, which is the size and vigor of the plants affected by the mycorrhiza-forming fungi. Gerdemann (2) observed similar results in maize when his mycorrhizal test plants grew four times larger than the controls in both fresh and dried weight. This, according to Gerdemann (2), serves as an excellent indicator of mycorrhizal presence if results can be compared to a control planting.

Neill (6) stated that the degree of infection was influenced be the vigor of the roots. The more vigorous roots tended to be less mycorrhizal. This seems to indicate that plants can be infected more rapidly when they are in a stress condition. The plants do not seem to benefit from the mycorrhizal association when they have an adequate supply of nutrients. This adequacy of nutrients resulted in vigorous growth which, according to Neill, resulted in a decreased percentage of infection. Mycorrhiza, then, serve to provide plants under nutrient stress with the less available forms of nutrients.

The beneficial affects of mycorrhiza seem to relate to the ability of the fungus to obtain unavailable nutrients. Gerdemann(4) found that in both maize and tuliptree there was a significant in-

crease in growth of mycorrhizal plants over non-mycorrhizal plants attributable to enhanced phosphorus uptake. Soil samples drawn from these plantings indicated a greater depletion of phosphorus and potassium by the mycorrhizal plants. The ability of mycorrhizal plants to more effectively assimilate nutrients is still not understood completely but it is believed to be related to the fungus' superior ability to utilize the less available forms of nutrients. Gerdemann (4) also stated that the fungus benefits from the association. He found that the fungus is initially attracted by carbohydrate exudates from the plant roots and, once attracted, they would penetrate the root cells and begin to utilize the carbohydrate reserves found in the cells. This "feeding" caused the soil microflora to multiply to an extent far greater than the normal population in the soil. Gray and Gerdemann (5) in their work with radioactive phosphorus found that mycorrhizal plants accumulate a greater percentage of radioactive phosphorus than did the non-mycorrhizal plants. Under low phosphorus conditions the non-mycorrhizal plants showed phosphorus deficiencies while those with the symbiotic relationship grew more vigorously.

Daft and Nicolson (1) collected evidence which indicates that mycorrhizal plants are better able to utilize the less available forms of phosphorus. They added rock phosphate and monocalcium phosphate to the soil in which test plants were to be grown. Plants innoculated with a mycorrhiza-forming fungus were better able to utilize these relatively unavailable forms of phosphorus than the non-mycorrhizal

control.

Gerdemann (3) observed that an extremely low level of available phosphorus hampers mycorrhiza formation and also inhibits plant growth. Small amounts of phosphorus are enough to encourage mycorrhiza formation. He stated that a high level of available nutrients tends to reduce the degree of infection and, to an even greater degree, to reduce the effect that mycorrhiza have on plant nutrition and growth. He also noticed that high levels of carbohydrates seem to increase the degree of infection. To further substatiate his findings, he conducted tests under greenhouse conditions during the winter months to show that when the light intensity is reduced, mycorrhizal infections were also reduced. In the winter months, with less light, reduced photosynthesis would result in a decreased amount of carbohydrates stored in the roots.

Woodcock and Stearn (7) found that lilies respond favorably to phosphorus which seems to promote root growth.

METHODS

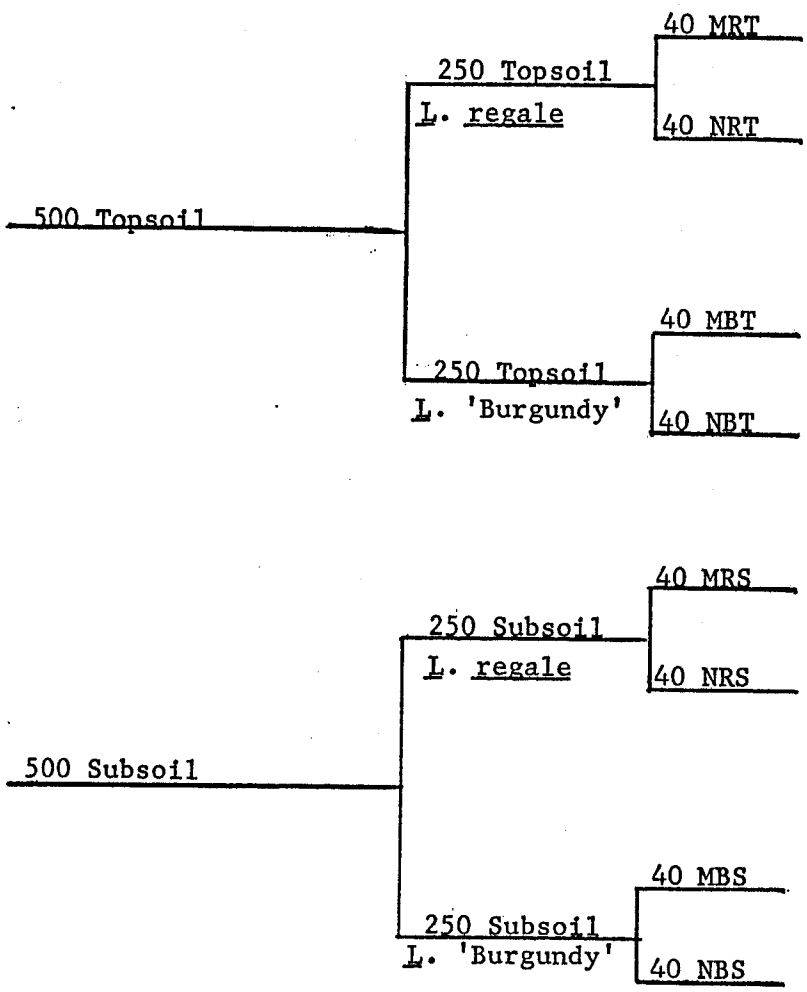
This experiment was conducted in the Plant Science greenhouses in Newark, Delaware. Seeds of Lilium regale (Wils) and Lilium 'Burgundy' were purchased from Rex Bulb Farm in Oregon. Seeds were chosen for this experiment because they would not be infected by mycorrhizal fungi and any infestation could be removed chemically. The seeds were soaked for twenty seconds in a three percent solution of sodium hypo-chlorite to destroy any fungal infestation. The seeds were then washed in three changes of distilled water and sown in vermiculite in ten inch seed pans on December 1, 1970. Vermiculite was chosen as a sterile medium to insure an uninfected seedling lot. The seedlings were grown in isolated seed pans until they reached the first true leaf stage.

The soil used was a Manor Silt Loam collected from two different horizons. A pit was dug five feet deep to reach subsoil levels. Samples were taken from both the lower topsoil of the A horizon and the subsoil from the B horizon. The topsoil had a pH of 4.8 and the subsoil a pH of 5.3. Samples of each were exposed to leaching on the greenhouse bench along with the experimental plants. The topsoil was mixed with equal parts, by volume, of medium textured sand and perlite. The subsoil was amended in the same manner to yield a suitable medium for lily

growth.

Five hundred one and one fourth inch diameter plastic pots were each filled with two hundred grams of each of the two soil mixes. Table 1 illustrates the experimental treatments.

TABLE 1. DIVISIONS OF THE EXPERIMENTAL TREATMENTS



These are the divisions that were made to yield the treatments.

M Innoculated
N Non-Innoculated
B Lilium 'Burgundy'
R Lilium regale
T Topsoil
S Subsoil

These seedling transplants were grown in flats on the greenhouse benches until they were well established and had reached a slight root bound condition.

From each group of two hundred fifty plants, eighty plants of uniform size and vigor were selected. Each group was further divided into two groups of forty. One group of forty was left uninnoculated while the other group of forty was innoculated with soil collected from beneath a vigorously growing clump of garden lilies. The soil and root segments were tested to determine mycorrhizal presence. The roots were found to be heavily infected. It is assumed that the soil probably contained spores that served as reproductive structures. A ten gram mass of soil was applied to the bottom of each of the pots. Each treatment was replicated forty times. The experiment was set up as a partial split plot where innoculations were kept separate while soil groups and lily taxa were handled factorially. Innoculations were split or kept separated to reduce the chance of mycorrhizal contamination. The plants were kept seaprated by eight feet of bench space. The extra plants not used in the experiment were placed between the two groups to give an added buffer. The plants within each innoculation were randomly arranged in flats and placed on the greenhouse bench. They were periodically repositioned to give an even sunlight distribution.

To determine the effects of mycorrhiza on phosphorus and

potassium uptake, no phosphorus or potassium fertilizer was applied to the soil mix at any time. Two applications of nitrogen fertilizer (33½-0-0) were applied during the experiment to keep the plants in a vigorous condition.

Mycorrhizal effects on the plants were visually evaluated three weeks before the conclusion of the experiment. This was done by ranking all the plants by size. They were arranged from largest to smallest by leaf area.

At the conclusion of this experiment all the plants were harvested to determine the fresh weights of the tops, roots, and bulbs. The tops were cut at ground level on all plants. All top growth was saved for a tissue analysis to determine relative nutrient uptake in the different treatments. Because of the small quantity of top growth produced by the plants, it became necessary to combine all replications into eight treatment samples to get enough tissue to run the analysis. Tissue was analyzed using the perchloric acid digest. A .333 gram mass was digested and brought to volume with distilled water. These samples were run on the flame photometer to determine levels of phosphorus, potassium, calcium and magnesium.

Root samples were collected to determine which plants were mycorrhizal. The roots were processed and stained to determine infection. The staining procedure is described in Appendix I.

MATERIALS

The materials and quantities used were as follows:

Items	Quantity
1) 1½" plastic pots	1000
2) *Lilium regale* seed	2 ounces
3) *Lilium* 'Burgundy'	2 ounces
4) Ammonium Nitrate	50 grams
5) Perlite	5 pounds
6) Sand (medium texture)	50 pounds
7) Topsoil	100 pounds
8) Subsoil	100 pounds
9) Innoculating soil	5 pounds

RESULTS AND DISCUSSION

The addition of Endogone to the test plants resulted in a four-fold increase in plant growth. This increase was evident in both visual ranking of the plants and in the fresh weight of the plant parts.

The relative uptake of phosphorus, potassium, calcium, and magnesium, which seem to be the nutrients that mycorrhiza make more available, was determined by tissue analysis. A complete listing of significance levels is in Table 3.

The ability to visually recognize the presence of mycorrhizal and non-mycorrhizal conditions in the plants, by their increased vigor and size, is an indication of the magnitude of the effect of mycorrhiza on plant growth. Table 2 graphically illustrates the results of a visual ranking of the test plants from largest to smallest by leaf area only. If mycorrhizal plants are more vigorous than non-mycorrhizal plants, the first half, or one hundred sixty plants, ranked by size should all be mycorrhizal and the second half should all be non-mycorrhizal. In the table, non-mycorrhizal plants judged mycorrhizal are denoted by a small white dot in the center of a shaded block while mycorrhizal plants judged non-mycorrhizal are denoted by a small black dot in the center of a white block. All plants ranking as expected are represented by a solid white block.

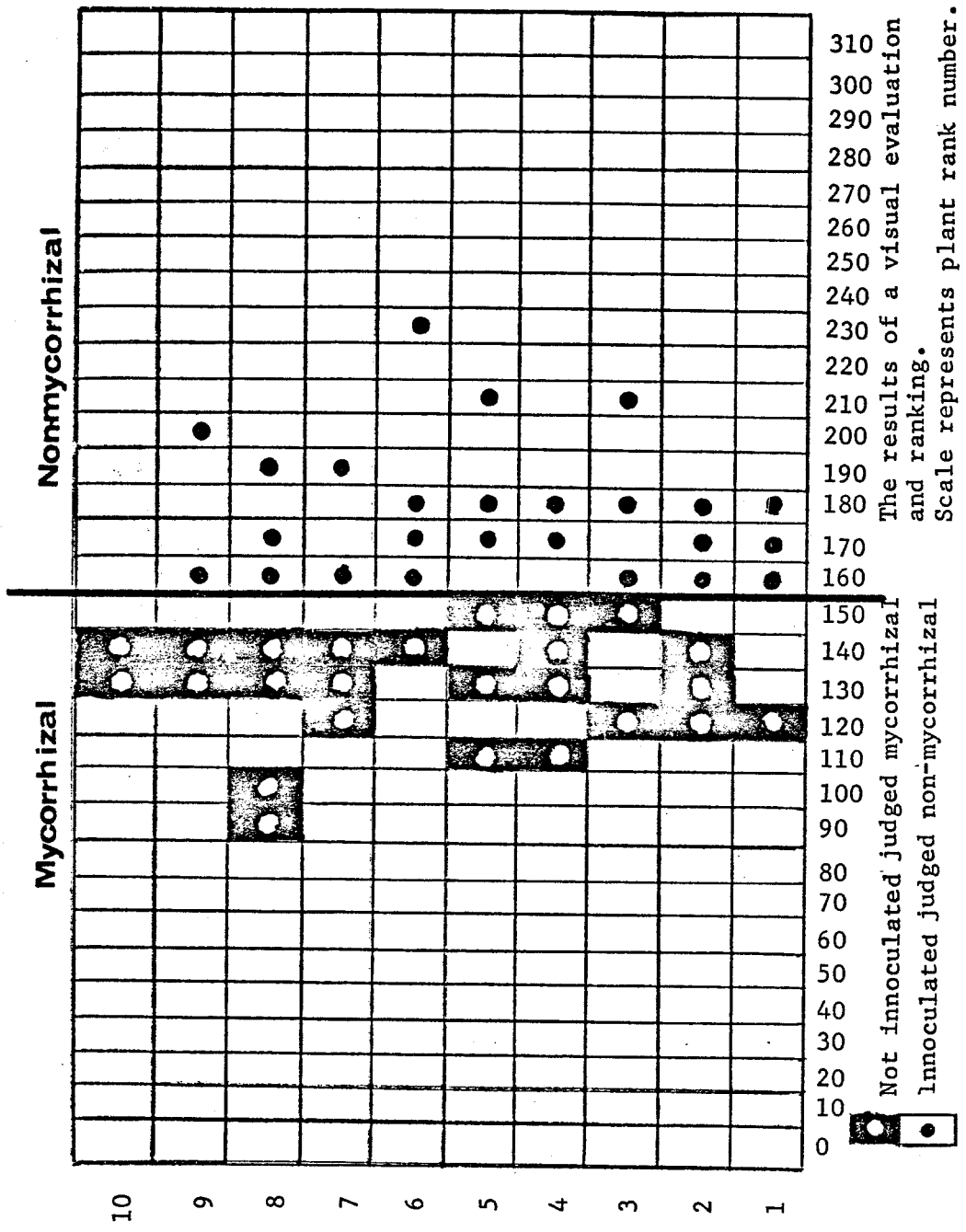
As the table shows, nearly eighty-five percent of the plants fell into their expected places. Twenty-five of the plants intended to be non-mycorrhizal fell into the area of the mycorrhizal plants. It is possible that these plants accidentally became mycorrhizal and, as a result, they fell into the range which, by size, would indicate a non-mycorrhizal condition.

A record of those plants that differed from their expected ranking was kept. These plants were checked later by root sampling to determine if the plants that failed to rank as expected had taken on the condition of the range into which they fell. Almost with-

out exception, those plants that were intended to be non-mycorrhizal, but fell into the mycorrhizal side of the range dividing line, had been accidentally infected. Those plants that were intended to be mycorrhizal, but ranked non-mycorrhizal, actually failed to establish an association. It is interesting to note that most of the plants that were incorrectly classified were clustered around the center line.

There was a gradation of infection approaching the center line. Those closest to the center line had only small amounts of stained mycelium visible.

TABLE 2. VISUAL RANKING OF PLANTS BY LEAF AREA



An evaluation was conducted to determine the extent to which mycorrhiza increased the amount of dry weight produced by the plant parts. An F.test was used to determine the significance of the differences between the means.

TOP GROWTH

Innoculations

The innoculations played a large part in the increase in plant growth. The differences that occurred between the mycorrhizal and non-mycorrhizal means were highly significant. The presence of Endogone fasciculata caused a greater increase in the growth than all other factors.

Taxa

The differences between the means for the top growth of the lily taxa were highly significant although it seems that these results are not consistent with the other data collected. It is possible that there was a difference where one taxon produced more top growth than the other. This would result in significantly different means. When the visual observation was made, all the plants were removed from their flats. Many plants lost roots that had begun to grow out of the pots. This caused a corresponding reduction in the leaf area. The ability of Lilium regale to recover more rapidly than the other member of the taxa was not measured but could have caused the observed differences.

Soils

In comparing the means for the soils effect on the top growth, the result was found to be highly significant. The topsoil, because of its higher percentage of organic matter, probably had more readily available nutrients. The plants ability to respond to increased fertility was the reason a difference of this magnitude occurred.

BULBS

Innoculations

Results similar to those from the tops were obtained when the means of the bulbs from test plants were compared. The highly significant differences in the means for innoculations bears out the supposition that mycorrhiza have a beneficial effect on bulb growth.

Taxa

The effect of taxa bulb size was found not to be significant. The bulb weights of the taxa had closely similar means.

Soils

The soils had no detectable effect on the bulb size. The difference between bulb means were no greater than would be expected if only chance were operating. The benefits that tops and roots received from the soil was not found in the bulb. However, the bulbs' stage of growth could have affected the results obtained. The bulbs, because of the loss of tops, as noted above, could have been in a

depleted condition.

ROOTS

Innoculations

Innoculation caused a difference between the means of the roots that was highly significant. This result was expected because the innoculation, if an association was established, provided the roots with additional phosphorus, which tends to promote root growth.

Taxa

The root growth differences between taxa again were not significant. The root growth of the taxa were similar.

Soil

The soil, with its difference in total and available nutrients, caused a difference in the means that was highly significant. Differences in soil nutrient levels are found in Table 4. The root growth could have been promoted by the nutrients made available in the soil.

The interactions were not evaluated because of the dominating effect of a large F value. There is a tendency for a large F value to carry over into the interactions, making them less valid.

The figures 1 through 8 show some of the differences that occurred among the treatments.

TABLE 3. F VALUES FOR THE COMPARISON OF MEANS

	TOPS OBSERVED F	BULBS OBSERVED F	ROOTS OBSERVED F	REQUIRED F .05	REQUIRED F .01
INNOCULATIONS (I)	207.002**	373.81**	366.98**	4.08	7.31
REPLICATIONS	1.29n.s.	1.10n.s.	1.220n.s.	1.680	2.11
TAXON (V)	8.654**	0.561n.s.	0.068n.s.	1.83	2.34
IxV	30.038**	0.979n.s.	0.779n.s.	1.83	2.34
SOILS (S)	14.436**	1.490n.s.	8.830**	1.83	2.34
SxV	7.321**	1.410n.s.	4.90**	1.83	2.34
IxSxV	32.718**	4.80**	8.170**	1.83	2.34

* (.05)
** (.01)
n.s. (not significant)

The results of the comparisons of the plant part means.

F values and significance level

TABLE 4. SOIL SAMPLE RESULTS

	MAGNESIUM	PHOSPHORUS	POTASSIUM
Topsoil	108.0	73.0	206.0
Subsoil	63.0	53.0	114.0

The values represent pounds of available nutrients per acre. Samples were taken before this experiment was started.

COMPARISONS OF TREATMENTS

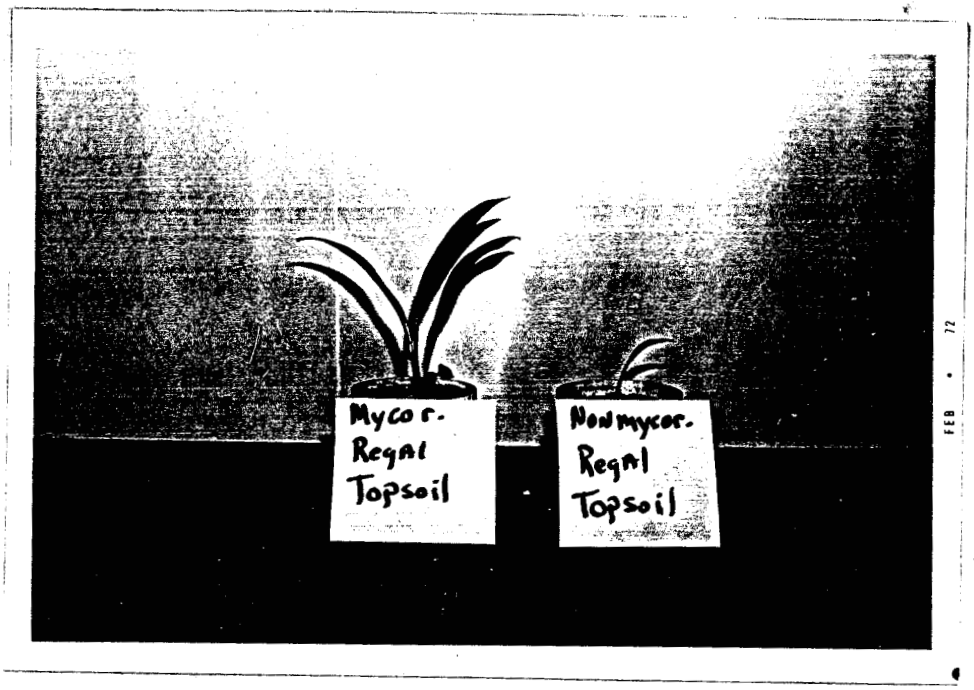


FIGURE 1. Lilium regale tops, mycorrhizal and non-mycorrhizal, topsoil.

FIGURE 2. Lilium regale roots, mycorrhizal and non-mycorrhizal, topsoil.

COMPARISONS OF TREATMENTS (CONTINUED)

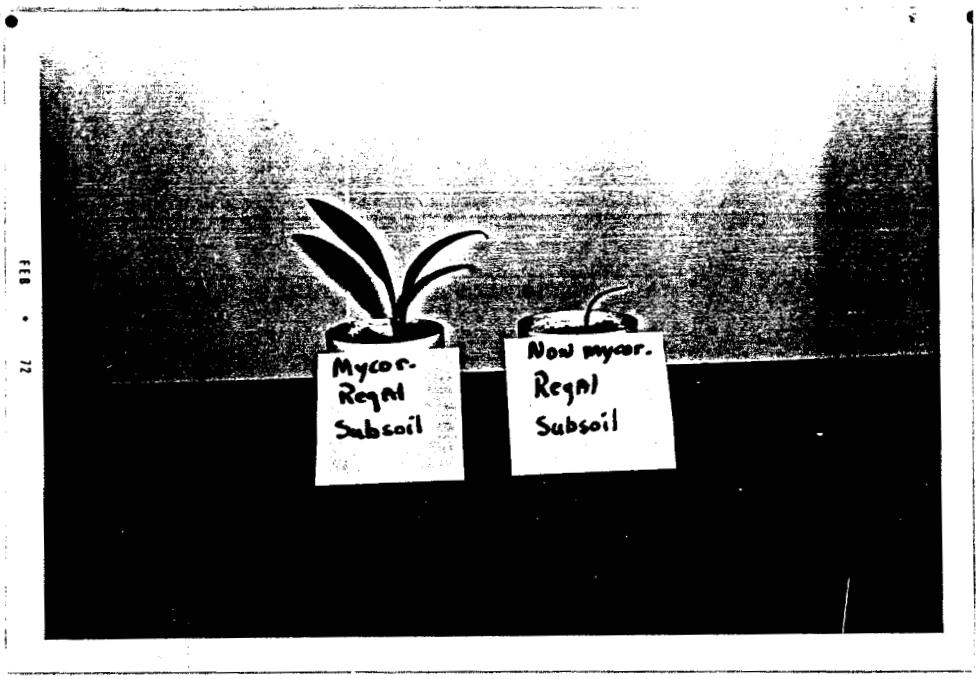


FIGURE 3. Lilium regale tops, mycorrhizal and non-mycorrhizal, subsoil.

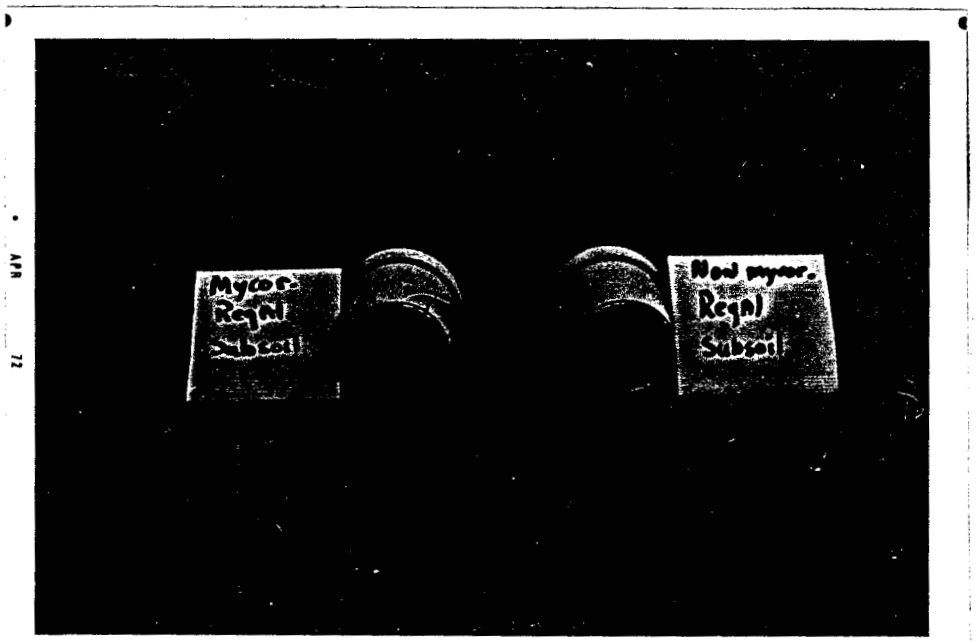


FIGURE 4. Lilium regale roots, mycorrhizal and non-mycorrhizal, subsoil.

COMPARISONS OF TREATMENTS (CONTINUED)

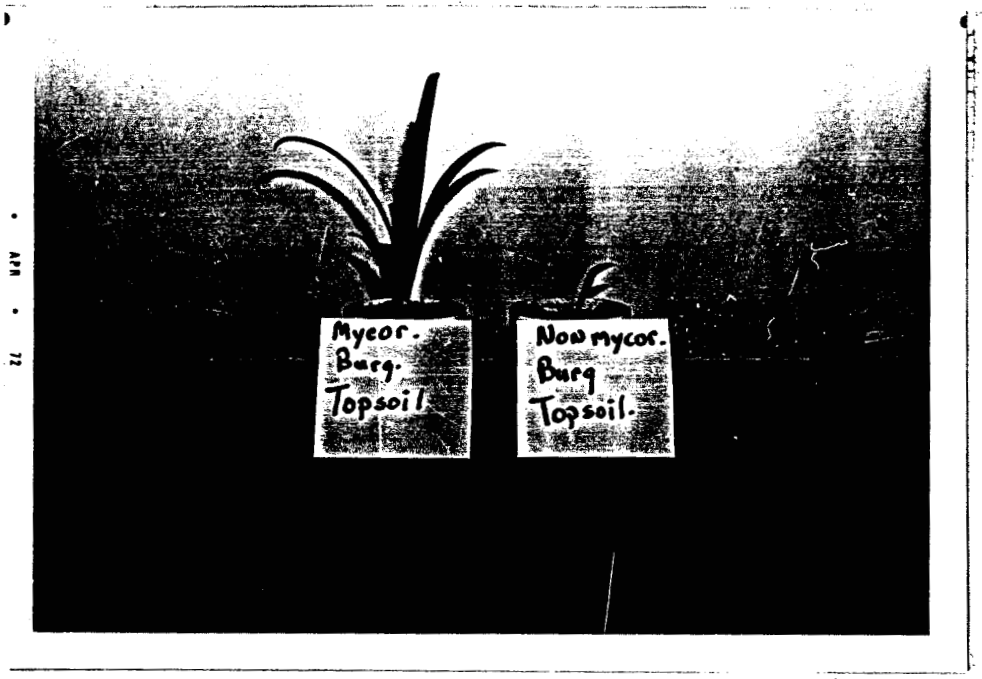


FIGURE 5. Lilium 'Burgundy' tops, mycorrhizal and non-mycorrhizal, topsoil.

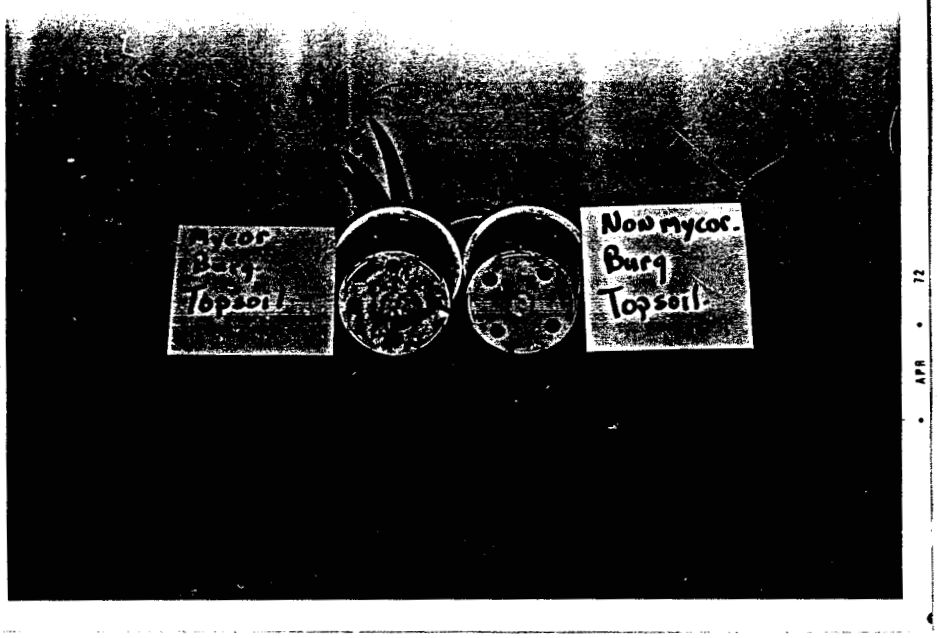


FIGURE 6. Lilium 'Burgundy' roots, mycorrhizal and non-mycorrhizal, topsoil.

COMPARISONS OF TREATMENTS (CONTINUED)

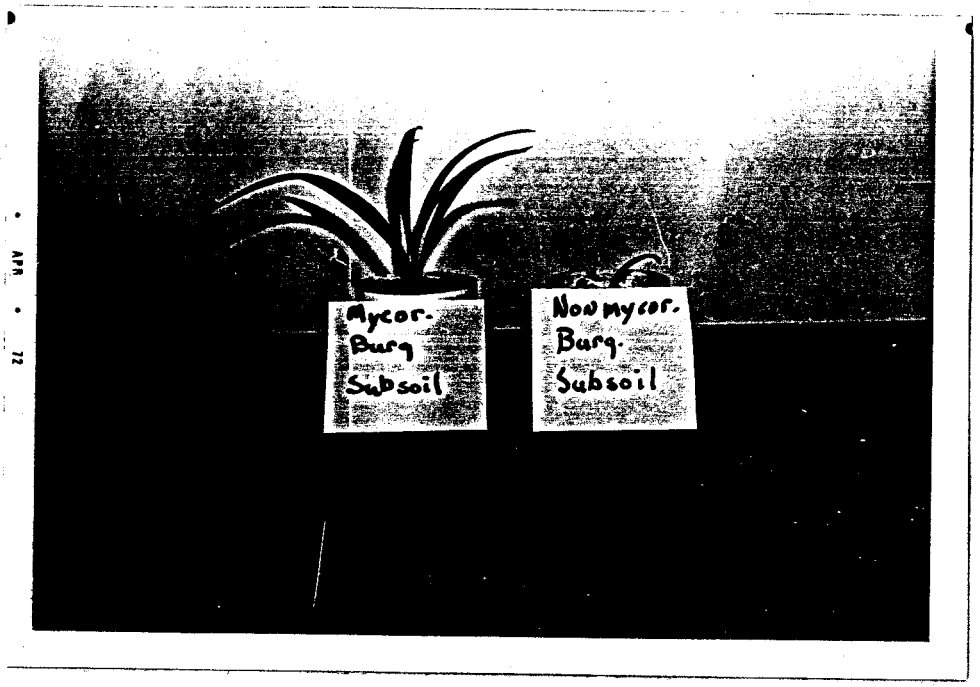


FIGURE 7. Lilium 'Burgundy' tops, mycorrhizal and non-mycorrhizal, subsoil.

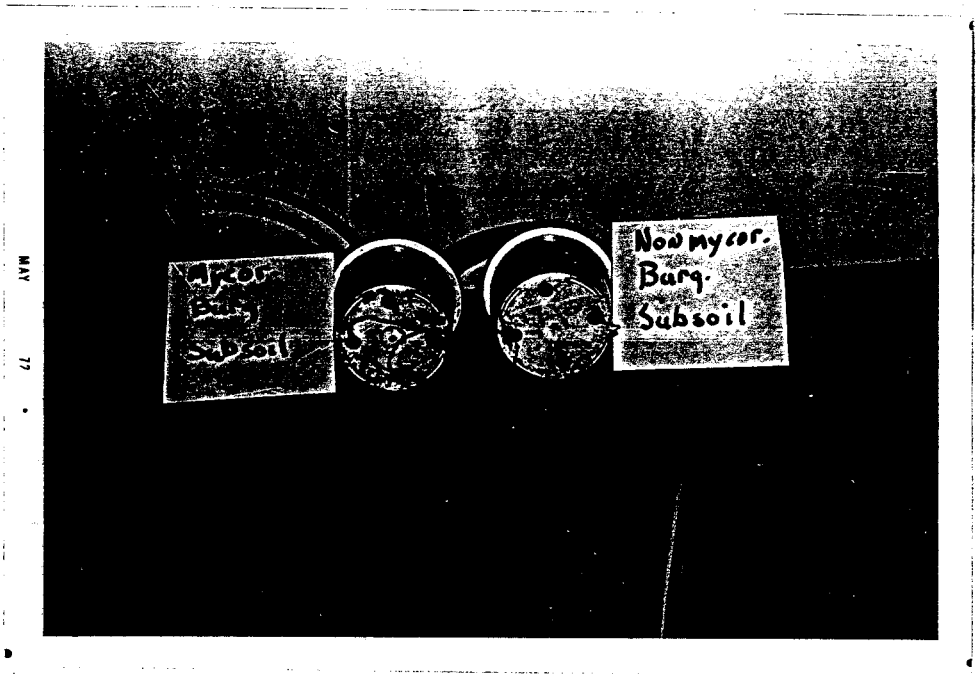


FIGURE 8. Lilium 'Burgundy' roots, mycorrhizal and non-mycorrhizal, subsoil.

TISSUE ANALYSIS

The plant tops were dried and analyzed for magnesium, phosphorus, potassium, and calcium (Table 5). There is an obvious difference between the nutrient content of the mycorrhizal and non-mycorrhizal groups. The mycorrhizal plants accumulated nutrients to an extent far greater than the non-mycorrhizal plants. The infection played a major role in the nutrition of these test plants. The mycorrhizal plants' ability to extract less available forms of nutrients from the soil seems to parallel the results obtained from the fresh weights.

The tissue analysis indicates that the taxon of lily does not influence the nutrient uptake and accumulation.

The effect that soil has on the nutrients accumulated was significant. Comparing the results of the different soils shows that in every case, except potassium, there is a significantly higher amount of nutrients made available and subsequently picked up by the plant when grown in topsoil. This is true of the mycorrhizal plants only. When the values of non-mycorrhizal plants are compared, the soil fails to make any difference. If a fungal innoculum were added to the uninnoculated plants, they would probably divide into distinct groups as a result of the mycorrhiza-soils interaction just as the original innoculations did.

The infection in lily roots and its effects was the primary concern of this experiment. The three types of roots in

lilies were investigated. There seems to be a possibility that the basal, contractile, and adventitious stem roots might be differentially susceptible to mycorrhizal association. The presence of a infection in the basal roots was established and all the previous work reported in this paper was primarily on the basal roots. The structurally and functionally different adventitious stem roots and contractile roots could possibly effect the mycorrhizal association. Observation showed that an association occurred in the stem roots and contractile roots although these seemed to have a reduced intensity of infection.

TABLE 5. TISSUE ANALYSIS

SAMPLE	% Magnesium	% Phosphorus	% Potassium	% Calcium
MBT	0.60	0.75	2.65	1.51
MRT	0.46	0.58	1.73	2.15
MBS	0.02	0.21	2.75	0.35
MRS	0.02	0.22	2.73	0.43
NRT	0.02	0.11	0.50	0.52
NBT	0.03	0.05	0.25	0.20
NBS	0.02	0.06	0.02	0.03
NRS	0.01	0.05	0.02	0.03

Results of the tissue analysis of the tops showing percentages of magnesium, phosphorus, potassium, and calcium.

M Mycorrhizal
N Non-mycorrhizal
B Burgundy

R regale
T Topsoil
S Subsoil

CONCLUSIONS

The effect of a mycorrhizal association on the growth of selected taxa in the genus Lilium is very pronounced. The effect of various taxa and soils contributes only slightly when compared to the effect of the mycorrhizal association. Significant differences in the means of the fresh weights of the plant parts and the results of the nutrients accumulated all indicate the beneficial effects of the association. Mycorrhiza seem advantageous to plants growing in a nutrient stress situation.

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APPENDIX I

Procedure for Staining Endogone

Use fresh rootlets.
Autoclave for 10 minutes at 12#/in.^{2} in 1 N. potassium hydroxide or boil in two changes of KOH.
Rinse twice in distilled water.
Transfer to 3% sodium hypo-chlorite (Clorox).
Accidify with a few drops of 5 N hydrochloric acid.
Soak 3-10 minutes to a pale straw color. DO NOT BLEACH COLORLESS.
The roots are then washed in distilled water and stained with alcoholic lacto-phenol cotton blue.
Transfer this to the autoclave for 10 minutes at 12#/in.^{2}.

APPENDIX II

MEAN WEIGHTS OF THE PLANT PARTS IN GRAMS

		TOPS	BULBS	ROOTS
Innoculation(I)	Innoculated	0.592	2.608	3.490
	Not Innoculated	0.087	0.696	0.700
	LSD .05	0.071	0.202	0.295
	LSD .01	0.095	0.271	0.395
Taxon(V)	*L. regale*	0.385	1.606	2.075
	'Burgundy'	0.294	1.698	2.117
	LSD .05	0.061	0.242	0.316
	LSD .01	0.081	0.319	0.419
IxV	Mycorrhizal *L. regale*	0.670	2.502	3.401
	Non-Mycorrhizal 'Burgundy'	0.074	0.681	0.650
	Mycorrhizal 'Burgundy'	0.513	2.715	3.584
	Non-Mycorrhizal *L. regale*	0.100	0.710	0.749
	LSD .05	0.087	0.341	0.447
	LSD .01	0.115	0.450	0.589
Soil(S)	Topsoil	0.399	1.727	2.334
	Subsoil	0.280	1.577	1.858
	LSD .05	0.061	0.242	0.316
	LSD .01	0.081	0.319	0.419

APPENDIX II (CONTINUED)

		TOPS	BULBS	ROOTS
SxV	L. regale/Topsoil	0.487	1.75	2.36
	L. regale/Subsoil	0.284	1.46	1.78
	'Burgundy'/Topsoil	0.310	1.70	2.28
	'Burgundy'/Subsoil	0.276	1.70	1.93
	LSD .05	0.087	0.341	0.447
	LSD .01	0.115	0.450	0.589
IxSxV	Mycorrhizal/L. regale/Topsoil	0.872	2.778	3.973
	Mycorrhizal/'Burgundy'/Topsoil	0.546	2.756	3.927
	Mycorrhizal/'Burgundy'/Subsoil	0.481	2.673	3.240
	Mycorrhizal/L. regale/Subsoil	0.469	2.226	2.829
	Non-Mycorrhizal/L. regale/Topsoil	0.102	0.728	0.759
	Non-Mycorrhizal/'Burgundy'/Topsoil	0.076	0.645	0.676
	Non-Mycorrhizal/'Burgundy'/Subsoil	0.072	0.718	0.624
	Non-Mycorrhizal/L. regale/Subsoil	0.099	0.692	0.740
	LSD .05	0.122	0.543	0.632
	LSD .01	0.162	0.752	0.835