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22. Supported by PHS traineeship No. ES-00098 (R.I.K.), PHS research grant No. ES-0400, and Hatch grant No. 154. The work is in partial fulfilment of the requirements for the Ph.D. degree (R.I.K.). We thank W. L. Brown, Jr., T. Eisner, J. G. Franclemont, G. L. Godfrey, R. D. O'Brien, M. J. Tauber, and R. H. Whittaker for their help.
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8 February 1971

Mycorrhizal Enhancement of Water Transport in Soybean

Abstract. *Mycorrhizae produced by* Endogone mosseae *decrease the resistance to water transport in soybean* (Glycine max *L.). The decrease was associated with an increase in the growth of shoots but not of roots.*

Vesicular-arbuscular (VA) endomycorrhizae increase the rate of growth of many plants (*1*), but the mechanism of the enhancement is only partially understood. There is evidence that increased growth could result from the increased uptake of various nutrients (*2–4*). Mycorrhizal plants are known to utilize less available forms of phosphorus more efficiently than nonmycorrhizal plants (*3*), and studies with ^{32}P show that segments of mycorrhizal roots have higher phosphorus contents after a period of uptake than comparable nonmycorrhizal segments from either mycorrhizal or nonmycorrhizal plants (*4*). On the other hand, the growth stimulation by mycorrhizae could be associated with the production of growth regulators. An ectomycorrhizal fungus has been shown to produce three growth substances identified as cytokinins (*5*).

The hyphae of VA mycorrhizae are associated with roots in such a way that they ought to increase the absorptive surface. If so, the presence of the hyphae could enhance water uptake which, in turn, might affect growth. In this report we show for the first time that mycorrhizae reduce the resistance to water uptake by plants.

Methods for growing VA mycorrhizal fungi in axenic culture are unknown, and cultures are maintained on potted plants (*6*). An Illinois isolate of *Endogone mosseae* (*7*), known to produce VA mycorrhizae on the roots of soybean and other plants (*8*), was grown on maize in an autoclaved sand-soil mixture. About 110 g of this mixture, which contained maize mycorrhizae and spores of *E. mosseae*, were used as inoculum for soybean (variety Harosoy 63) and added to 5-inch (12.7-cm) pots containing an autoclaved sand-soil mixture. A similar portion of autoclaved inoculum was added to the control pots, along with a water filtrate from unsterilized inoculum, which had been passed four times through a sieve with 44-μm openings (smaller than the spores of *E. mosseae*). This procedure ensured that contaminating microorganisms, which can affect nutrient availability and plant growth (*9*), would also be present in control pots. Soybean seeds were soaked in a soybean rhizobium culture for 3 minutes prior to planting. Plants were grown in a controlled environment [14-hour photoperiod; light intensity, 0.15 cal cm^{-2} min^{-1} (fluorescent and incandescent); day temperature, 30° ± 1°C; night temperature, 24° ±1°C)]. After planting, 50 ml of nutrient solution (*10*) was added to each pot. Plants were watered with distilled water daily.

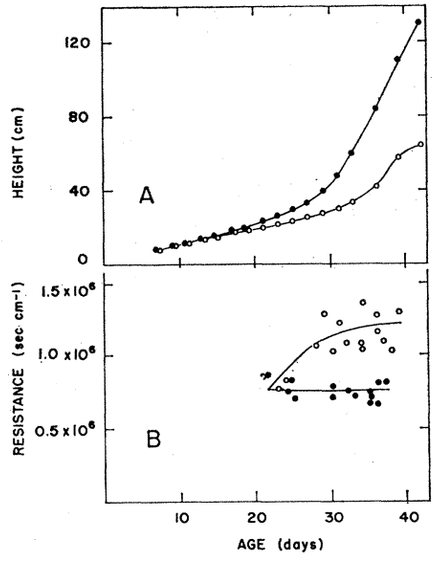

Fig. 1. (A) Height of mycorrhizal (solid circles) and nonmycorrhizal (open circles) soybean plants at various times after planting. Data represent the averages for six plants. (B) Whole plant resistances to water transport in mycorrhizal (solid circles) and nonmycorrhizal (open circles) soybeans calculated from the half-time for recovery from a moderate water deficit. Data represent resistances of single plants.

Resistances to water transport in whole plants which were intact in the soil were measured by a recently described method (*11*), in which the time required for a test leaf on the plant to recover from water deficiency was determined. The delicacy of the mycorrhizae made this method, which does not disturb the soil-plant system, particularly suitable.

Determinations were made with a thermocouple psychrometer that measures the water potentials of intact leaves (*12*). The recovery conforms to a well-known equation describing diffusive transfer in a plane sheet as a function of time (*13*). Since the resistance of the transfer system determines the time required for recovery, the resistance of the intact plant can be calculated.

The blades of intact, moderately water-deficient soybean leaves were sealed in the chamber of the thermocouple psychrometer, the soil-root or soil-mycorrhizal systems were then submerged briefly in degassed water, and the recovery in the water potential of the intact leaf was recorded. After we had determined that the data conformed to the transfer equation for a plane sheet, resistances (in seconds per centimeter) were calculated according to (*11, 13*):

$$r = \frac{t_{1/2}}{0.195\, l}$$

where $t_{1/2}$ is the half-time for recovery (in seconds) and l is half the average leaf thickness during recovery (in centimeters). Measurements of l were made with a light microscope. Since

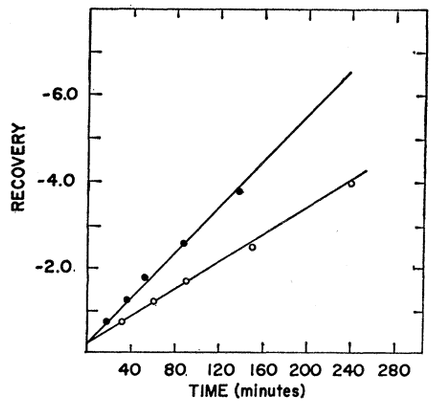

Fig. 2. Recovery from moderate water deficit in typical mycorrhizal (solid circles) and nonmycorrhizal (open circles) soybean plants. Data represent the natural logarithm of the quantity (1 — fractional recovery) at various times for single plants.

root resistances may increase when plants are subjected to severe drought (*14*), considerable care was taken not to subject plants to extreme water potentials (drought-induced changes in resistance were avoided if leaf water potentials did not decrease below — 13 bars). The leaves used to measure recovery of mycorrhizal and nonmycorrhizal plants were at similar heights above the soil and were of similar development. Experiments were carried out at 26°C.

The heights of inoculated and control soybean plants were similar until 21 days of age (Fig. 1A). After that time, inoculated plants became taller than controls. At the conclusion of the experiments, plants were examined for mycorrhizal infection. All inoculated plants had become mycorrhizal and the controls were nonmycorrhizal. No symptoms of nutrient deficiency were apparent in any of the plants.

Since mycorrhizal stimulation of growth became evident 3 weeks after planting, resistances were measured at various times to determine whether there was a relationship between growth stimulation and resistance. Mycorrhizal and nonmycorrhizal soybeans had similar resistances to water transport between 21 and 24 days of age (Fig. 1B). By the time plants were 28 days old, the resistances of nonmycorrhizal plants had increased an average of 40 percent, but the resistances of mycorrhizal plants were unchanged.

The dramatic difference in resistance of the 28-day-old plants can be seen from a logarithmic plot of leaf water potential as a function of time in representative mycorrhizal and nonmycorrhizal plants (Fig. 2). The straight line with an intercept of -0.21 at $t=0$ conforms to the equation for a plane sheet. The greater slope of the data for the mycorrhizal plant results from the lower resistance of this plant to water transport.

Resistances to transport were also calculated by a second method according to the equation

$$T = \frac{\Psi_l - \Psi_s}{R}$$

where T is the transpirational flux at the steady state (in cubic centimeters per second per square centimeter of leaf area measured from the leaf outline), Ψ_l is the water potential of the evaporating surface within the leaf (in bars), Ψ_s is the water potential of the soil immediately next to the root (in bars), and R is the resistance to water transport (in bar-seconds per centimeter). Transpiration rates were calculated from the weight loss during transpiration in a controlled environment chamber [light intensity, 0.15 cal cm^{-2} min^{-1} (fluorescent and incandescent); temperature, 26° ± 0.5°C; relative humidity, 70 ± 2 percent]. The water potential of the evaporating surface could not be evaluated directly but was estimated by measuring the average leaf water potential during transpiration by the isopiestic technique (*15*) with a thermocouple psychrometer. The soil, which was well watered, had a water potential that was essentially zero. Leaf disks for measurement of the water potential were taken from leaves midway up the stem.

Resistances estimated from the recovery curves of plants at 30 to 32 days were similar to those calculated from transpiration in the same plants (Fig. 3). The resistance to water transport in mycorrhizal soybeans was lower than that in the nonmycorrhizal plants by approximately the same amount as in the recovery experiments. However, the units of resistance were different (seconds per centimeter versus bar-seconds per centimeter), and the formal relationship between these units has not been defined.

The measurements of recovery times avoid two sources of error inherent in the transpiration experiments. First, there is a variation in water potential

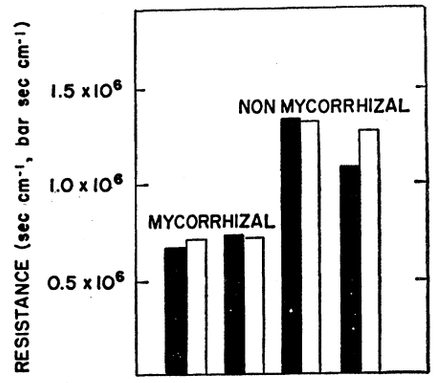

Fig. 3. Resistances (in seconds per centimeter) calculated from half-times for the recovery (solid bars) of two mycorrhizal and two nonmycorrhizal soybean plants as compared with resistances (in bar-seconds per centimeter) of the same plants calculated from transpiration (2 to 3 g hr^{-1} dm^{-2} of leaf area) and leaf water potential (open bars).

between the leaves on the same plant; and, second, there are gradients in the water potential in the leaves, which are not accounted for because the thermocouple psychrometer indicates only the average leaf water potential in excised segments. Calculations of leaf gradients and measurements of variation between leaves under our conditions indicate that the error should be of the order of ± 15 percent.

There are several possible explanations for the differences in resistance between mycorrhizal and nonmycorrhizal soybeans. First, the external hyphae might increase the total surface area of the root system much as would an increased number of root hairs. Second, the hyphae, which penetrate the root cortex to the endodermis, could provide a low-resistance pathway for water movement across the root. Third, the hyphae could enhance nutrient uptake, which, in turn, could decrease the resistance to water transport within the roots. Finally, mycorrhizal infection might increase root growth so that there is a larger root system.

There were no significant differences in root dry weights and volumes between 29- to 32-day-old mycorrhizal and nonmycorrhizal soybeans. Since most of the external hyphae are removed from the mycorrhizae during washing, these measurements provide a comparative estimate of the size of the mycorrhizae or root systems. Under our conditions, therefore, it is unlikely that the mycorrhizal effect was due to

any stimulation of growth of the root tissue.

Shoot dry weights became greater in the mycorrhizal than in the nonmycorrhizal plants after differences in plant height were evident. Thus, the results indicate that low resistances to water transport are associated with increased shoot growth in VA mycorrhizal plants. Nevertheless, the increased growth appeared slightly before differences in resistance were detectable (compare A and B, Fig. 1). Unless our experiments were incapable of measuring small initial changes in resistance, it seems probable that growth stimulation is not caused by the changes in resistance which we observed. On the other hand, if small differences in resistance were present from the onset of growth stimulation, but were unobserved, then the higher tissue water potentials which occurred in mycorrhizal plants may have contributed to the increase in growth (*12*).

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14 December 1970

Cholinergic Sensitivity: Normal Variability as a Function of Stimulus Background

Abstract. *The sensitivity of the normally innervated iris sphincter to its neurotransmitter, acetylcholine, and to related agents varies inversely with the preexisting physiological stimulus background, that is, the environmental light intensity. This normal variability suggests the existence of a negative feedback mechanism whereby sensitivity of the effector cell is modulated by a product of neuronal activity.*

Supersensitivity develops after the denervation of structures which are normally under the control of either voluntary or autonomic innervation (*1*). Development of subsensitivity to cholinomimetic drugs, as a result of topical or systemic long-term treatment with an inhibitor of cholinesterase (*2*, *3*), has also been demonstrated in a variety of tissues, and evidence has been presented that supersensitivity and subsensitivity represent opposing expressions of the same basic phenomenon (*4*). All of the methods used to induce such alterations in the sensitivity of effector organs involved drastic interference with normal physiological function.

We have recently demonstrated (*4*) that alterations in the sensitivity of the iris to pilocarpine can also be induced by alteration in the normal physiological stimulus (environmental light) for the neuronal outflow to this organ. Both eyes of the cats used in these previous experiments were, however, sympathetically decentralized to minimize the mydriatic effects of emotional state; and one eye of each cat had been parasympathetically denervated to show that the effects of environmental lighting are mediated by parasympathetic innervation.

Our experiments demonstrate that stimulus deprivation results in supersensitivity and overstimulation leads to subsensitivity of the iris to cholinomimetics in the absence of any surgical or pharmacological interference with normal innervation. Furthermore, these experiments show that sensitivity of the iris to its normal neurotransmitter, acetylcholine (ACh), is also affected by the same physiological means. These findings imply that normal cholinergic sensitivity is a dynamic rather than a static entity. Maintenance of target organ sensitivity within a normal range can best be explained, at present, on the basis of an inverse relationship between the local concentration of the neurotransmitter and the concentration of cholinergic receptors at the membrane of the effector cell (*4*).

Four cats (2.5 to 4.5 kg) were selected without regard to sex on the basis of ease of handling. The external portion of the nictitating membranes was removed under Nembutal anesthesia to eliminate interference with measurement of pupillary size and with the topical application of drugs. The cats were kept for 1 week under each of the lighting conditions shown in Fig. 1. At the end of each conditioning period a dose-response curve to pilocarpine hydrochloride (ophthalmic solution, Alcon Laboratories) was obtained. The cats were put in the dark for 30 to 45 minutes before the first measurement and remained there

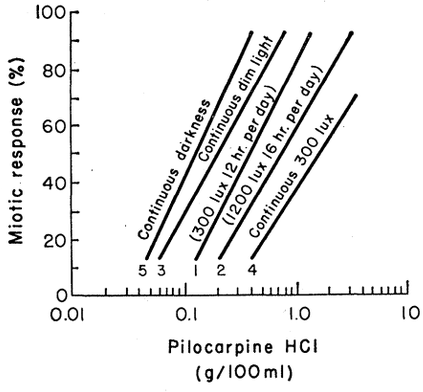

Fig. 1. The effects of preexisting stimulus background on the sensitivity of cat irises to topically applied pilocarpine. Four cats were maintained for 7 days under each of the lighting conditions indicated on the graph in the sequence shown by the number next to each line. The regression lines were obtained by the method of least-squares on all response values between 10 and 90 percent of the initial pupillary diameter. All measurements were made in complete darkness. Each regression line is based on 16 to 24 points obtained on eight eyes and covers at least a ninefold range of drug concentrations. The results show that the sensitivity of the iris to this cholinomimetic is dependent on preexisting stimulus background; 1 lux = 1 lumen/m^2.