INFLUENCE OF ROOT TEMPERATURE ON THE ABSORPTION OF FOLIAR APPLIED RADIOPHOSPHORUS AND RADIOCALCIUM

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ABSTRACT

INFLUENCE OF ROOT TEMPERATURE ON THE ABSORPTION OF FOLIAR APPLIED RADIOPHOSPHORUS AND RADIOCALCIUM

by Richard L. Phillips

Radioisotopes were used to assess the influence of root zone temperature on absorption and distribution of phosphorus and calcium applied to leaves of bean and pea plants. Absorption and distribution of P^{32} applied to primary leaves of bean seedlings growing in solution culture at root temperatures of 7°, 13°, 18° and 24°C. increased with increasing temperature. Similar results were obtained with the pea. Calcium was also absorbed at higher rates with increasing root temperatures but translocation from the treated leaf was negligible.

Simultaneous use of different air and root temperature combinations resulted in a comparable influence of air and root temperatures on foliar absorption of P^{32} by the bean. Translocation to the root, however, was greater at the higher root temperature regardless of air temperature. Pre-absorption root temperature affected subsequent absorption of P^{32} while pre-absorption air temperature did not. However, both air and root temperatures had an effect during absorption.

Effects of translocation on absorption were eliminated by the use of excised bean leaves floated on plastic with their petioles extending into water. Absorption of P^{32} and Ca^{45} in a uniform environment continued to be greater by primary leaves of bean plants previously grown at a root temperature of 24°C. than at 13°C. after

removal from the plant. Similarly, primary leaves excised from bean plants growing at root temperatures of 7°, 13°, 18° and 24°C. and immersed into a solution containing a known concentration of phosphate continued to absorb at rates which were a function of the temperature to which the roots were previously exposed.

Growth and anatomical studies were conducted in an attempt to explain these differences. Bean plants grown at a root temperature of 24°C. increased in dry weight and moisture content more than those grown at 13°C. over a twenty day period. Expansion of the primary leaves as determined by the area of leaf tracings was approximately twice as great at 24°C. as at 13°C. after 10 days at these temperatures. Measurements of transverse sections of primary bean leaves revealed that the dimensions of the epidermal, palisade and spongy mesophyll cells were significantly greater in leaves grown at the higher root temperature.

Electron microscopy was employed for a study of the fine structure of cuticle from leaves of plants grown at different root temperatures. Samples from the center of the leaves were embedded in an epoxy resin and transverse sections were cut with an ultramicrotome. Good sections of the fragile cuticle of the bean and pea were secured with difficulty but it appeared that low root temperatures result in slightly thicker cuticles which impregnate the outer epidermal walls to a greater extent.

Replicas of leaf surfaces were prepared for examination with an electron microscope with cellulose acetate as the negative and shadowed aluminum as the final positive. Surface wax appeared as rod-like deposits on bean leaves while pea leaves had ribbon-like deposits.

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Accumulation of the wax on leaf surfaces decreased with increasing root temperatures in both plants.

The contact area of 10 µl droplets of water containing India ink and placed on the primary leaves of bean plants grown at root temperatures of 7°, 13°, 18° and 24°C. increased with increasing temperatures. The addition of a surfactant increased the area of all drops and completely eliminated differences associated with root temperatures.

Transpiration by primary leaves of bean plants previously grown at a root temperature of 24°C. was 80 percent greater than that of plants grown at 13°C. as revealed by potometer experiments in a comparable environment. Closure of stomata with phenylmercuric acetate reduced transpiration of both groups by 70 percent but transpiration by plants grown at the higher root temperature continued at a greater rate.

Root temperature appears to influence both physiological and anatomical modifications of leaves and leaf surfaces that, in turn, alter foliar absorption and subsequent transport.

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by

Richard L. Phillips

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INTRODUCTION

The temperature of the soil environment has long been considered an important factor in the production of horticultural crops. For development of agriculture in northern areas it is important to know the effect of low soil temperatures on the uptake and assimilation of nutritive elements by plants. In the spring, when the air temperature is conducive to plant growth, the root temperature in northern soils is often inhibitive to the uptake of adequate nutrients.

A logical solution to this problem would be to determine the practicality of supplying the necessary nutrient elements through the leaves. Although numerous experiments have shown the effect of various factors on the absorption of foliar-applied nutrients, relatively few have demonstrated the influence of root temperature. Since there is an inseparable interdependence between the activity in the roots and in aerial plant parts, it would not be surprising to find a resultant relationship between root temperature and foliar absorption. The research presented in this thesis was undertaken to determine the extent of this influence and to develop a better understanding of this relationship.

In addition, the temperature of the root environment will be related to physiological and anatomical modifications of leaves and leaf surfaces that, in turn may alter foliar absorption and subsequent transport.

REVIEW OF LITERATURE

Influence of Root Temperature on Plant Behavior

Effect on Plant Growth - - Marked differences in development (36, 40, 64, 80, 85, 90, 125) and in growth rates (87) have been attributed to soil temperature. The optimum soil temperature for plant growth is probably determined genetically by endogenous mechanisms.

Locascio and Warren (80) found that the dry weight of tomato plants grown at a root temperature of 13°C. was less than at 21° or 29°C.; however, there were no differences between the two higher root temperatures. Wilcox et al. (125) reported a striking difference in the growth of tomato plants between root temperatures of 13.3° and 14.4°C. but the difference between 14.4° and 15.5°C. showed no significant temperature effect. Apparently there is a critical range of root temperatures through which wide differences in growth can occur.

The growth of corn is also directly influenced by root zone temperature conditions (64, 65, 85, 91). Nielsen et al. (91) found that both top and root growth of corn increased with increasing root temperatures through a range of 5° to 27°C. A substantial increase in growth of corn was observed (64) when the root temperature was elevated from 15° to 20°C. and a further increase occurred at 25°C. Apparently corn is adapted over a wider root temperature than is the tomato.

In working with soybeans, Early and Cartter (40) found that the temperature of the root environment influenced the dry weight of the root less than the top. Root growth increased with root temperature from 2° to 27°C., while top growth increased with increasing root

temperature from 2° to 17°C., remained uniform from 17° to 27°C. and decreased sharply at 37°C.

When strawberry plants were grown at root temperatures of 7°, 13°, 18°, and 24°C., maximum dry matter accumulation in the top during the vegetative phase was obtained at the two highest temperatures, while no significant difference was found in the dry weights of the roots among the given temperatures (103).

Davis and Lingle (36) have suggested that differences in soil temperature may result in a differential production of root-produced substances having shoot regulatory activity, and retardation of movement in the phloem resulting in the congestion of substances in the shoot.

Decreased root growth at low root zone temperatures may result from a decreased movement of leaf-produced thiamine to the roots or the requirement for more thiamine at lower temperatures. Bonner (17) demonstrated that low temperature effects could be partially eliminated by the addition of thiamine to the nutrient medium. With some plants the temperature of the root zone is more important for maximum growth than is the air temperature. Nelson (90) found this to be the case with hemp when different combinations of air and root temperatures were used.

The optimum soil temperature for plant growth at different stages of plant development declined with an increase in physiological age (102).

Water Relations - - A direct relationship between transpiration

and water absorption by the root system is proposed by the transpiration-cohesion-tension theory, favored by most plant physiologists (18). The evaporation of water from mesophyll cells sets up a diffusion pressure gradient that is communicated from cell to cell until the xylem is reached. The pull exerted by the movement of water from the uppermost xylem cells of leaves is transmitted through the water columns in the xylem of stems down into the roots. Thus water is pulled up through stems by the evaporation-pull of transpiration and the water column is kept intact by the cohesive force of water molecules.

A reduction in absorption and transpiration of water by plants growing in cold soils has been reported by various authors (3,4, 10, 26, 31, 42, 67, 72, 73, 101, 121). Arkley (3) found that the production of dry matter and the amount of water transpired during active growth are proportional under constant conditions of climate and soil fertility. This is not surprising since water is directly involved in photosynthesis and transpiration influences nutrient uptake from the soil.

Some suggested causes for decreased absorption at low root temperatures as listed by Kramer (72) are:

1. Low temperatures retard elongation of roots. This is a limiting factor in soils where roots must come into contact with moist soil but is not a problem in solution cultures.

2. The permeability of cells decreases as the temperature is lowered.

3. The viscosity of protoplasm and of the colloidal gels in the cell walls is much higher at low temperatures. The increased viscosity probably retards the movement of water across the mass of living cells lying between the

soil and the xylem of the roots.

4. The viscosity of water increases as the temperature decreases slowing down its movement through the soil and its entrance into and through the roots.

5. The physiological activity of the root cells, especially the rate of respiration, is decreased by low temperature. This would be particularly important if the absorption of water is dependent upon energy by the roots themselves.

Kramer (72) proposed that the principal cause of decreased water absorption by plants at low soil temperatures appears to be the combined effects of decreased permeability of the root membranes and the increased viscosity of water, resulting in increased resistance to water movement across the living cells of the roots. Although other factors may contribute to this effect, he feels that they are of secondary importance. These conclusions agree closely with those of Collander (32) who stated that the diffusion process in highly viscous media often shows high temperature coefficients. Therefore the influence of temperature on permeation rates might be an indication of a high viscosity of the plasma membranes.

The reduction in water absorption at low soil temperatures may vary considerably with different plants and is greater for plants normally grown in warm soils than those adapted to lower soil temperatures. For example, watermelons and cotton absorbed only 20 percent as much water at 10°C. as at 25°C. while collards absorbed 75 percent as much at 10°C. as at 25°C. (73). It may be that the protoplasm of cotton and watermelon undergoes much greater changes in viscosity and permeability than the protoplasm of collards. This difference may even occur in plants of the same genus. Water absorption of a species

of southern pine was depressed much more than that of anorthern species when root temperatures were varied between 5° and 15°C. (71).

Ehrler (42) demonstrated a depressing effect of low root temperature on water absorption by alfalfa. Loss of turgor during wilting depended on the relative rates of water absorption and transpiration. A 5°C. root temperature caused wilting and reduced water absorption to approximately 70 percent of that by plants grown at 26°C. in the first 24 hours.

Cotton plants wilted on clear days when the root temperature was lowered to between 10° and 18°C. The amount varied, depending on air temperature, relative humidity and light intensity (4). Wilting was probably due to a lowered capacity of roots to absorb water and transmit it to the conducting channels.

Other authors have reported similar observations. Clements and Martin (31) found that transpiration in Helianthus annuus varied little between 13° and 38°C., but dropped rapidly below 13°C., approaching zero at 0°C. Muskmelons in culture solution used 84 percent less water at a root temperature of 10°C. than at 26.7°C. over a 1½ day period (101). With the aerial parts of rooted lemon cuttings subjected to constant environmental conditions, Bialoglowski (10) observed that transpiration remained stable in the temperature range of 25° to 30°C., but marked reductions occurred as the soil temperature went above or below 25°C. Cameron (26) reported a marked reduction in soil water consumption with orange trees as the soil temperature was reduced from 32° to 0°C.

Low soil temperatures decreased transpiration rates during early

stages of plant development but did not exert any appreciable effect during later periods. The amount of free water decreased and bound water increased in thermophilic plants with a lowering of the soil temperature, while no definite relation between the amount of bound and free water and soil temperature existed in cold resistant plants (67).

At low root temperatures, the environmental conditions surrounding sunflower leaves had little influence on transpiration, thus water intake by roots was probably the rate-limiting factor (119). However, soil temperature had a marked influence on stem and leaf temperatures. At a soil temperature of 10°C. stem, leaf and air temperatures were essentially the same (24°C.). At a soil temperature of 25°C. leaf and stem temperatures were both lower than the air temperature and at a soil temperature of 40°C. leaf and stem temperatures were both higher than the air temperature. Optimum root temperatures probably result in lower leaf temperatures due to greater transpiration.

Jensen and Taylor (57) applied the Arrhenius theory to obtain activation energies for water flow from its temperature dependence. They found that the activation energy for water flow through the plant stem was in good agreement with those calculated for self-diffusion and viscous flow of water. The apparent activation energies were higher for water moving through roots than through stems or leaves, and higher for leaves than for stems. This indicated that mechanisms of water movement through leaves and roots is more complex than either simple diffusion or viscous flow.

Nutrient Uptake - - The uptake of nutrients by plants is similarly affected by low root temperatures and may, in turn, contribute to reported growth depressions (27).

Because energy is required for the intake of nutrients, the process is governed in part by metabolic activities within the plant and not merely by the permeability of root cell membranes. In this respect, Korovin and Barskaya (68) found that lowering the root temperature decreased root respiration in thermophilic plants more than in cold resistant plants.

Korovin et al. (69) reported that a decrease in root temperature resulted in a decrease in phosphorus absorption and incorporation into organic compounds, primarily into nucleoprotein fractions. A decrease in the amount of organic phosphorus in the leaves was also detected. Thus, they concluded that the decrease in uptake of phosphorus and its primary assimilation in the roots of plants in cold soil leads to a decrease of the reformation of high energy phosphorus bonds and to a reduction of the processes of activation of hexoses, glycolysis and respiration which are basic to cellular metabolism.

Lingle and Davis (79) reported that the response of tomato seedlings to phosphorus fertilization increased with an increase in soil temperature. Similarly, an increase in dry matter accumulation and phosphorus absorption has been noted for corn (65, 91) and alfalfa (78) with an increase in soil temperature.

Korovin et al. (69) contended that an increase in phosphorus content in the soil or nutrient solution favored an intensification of its assimilation into plants and favorably affected yield at lowered

root temperatures. Increasing the available phosphorus in the soil has been found to increase the optimum temperature range for the growth of barley (99). However, Wilcox et al. (125) reported that, although phosphorus uptake by tomato plants grown at 14.4° and 15.5°C. root temperatures was much greater than at 13.3°C., the stunting effect of low root temperatures could not be corrected by increasing the phosphorus content in the plant whereas decreased root activity due to low root temperatures was partially overcome by phosphorus fertilization (63).

With tomatoes growing at soil temperatures of 13°, 21°, and 27°C., Locascio and Warren (80) found that the relative response to phosphorus was greatest at the lowest temperature. Apple and Butts (2) similarly reported that growth increases due to phosphorus application were greater at low soil temperatures than at high temperatures.

A reduction in soil temperature reduced the assimilation of phosphorus to half, whereas a reduction in air temperature had little effect (136). In contrast, the assimilation of calcium was highest when both soil and air temperatures were favorable, whereas a reduction in temperature of either soil or air reduced assimilation approximately 40 percent.

Foliar Absorption

General Considerations - - Foliar application of fertilizers has long played an important role in the nutrition of horticultural crops and many benefits to agriculture have resulted from research done in this field (20, 21, 74, 84, 117, 121, 122, 127, 129, 130). Few ex-

periments, however, have been reported which concern the influence of root temperature on foliar absorption (112).

Studies have shown that all essential nutrients which are taken up by the roots can also be taken in by the above ground parts. One interesting experiment with bean plants (121) showed that they could be grown from seed to seed with all the mineral nutrients being supplied through the foliage. In some cases foliar application is the only practical way to correct nutritional disorders (20, 74, 84, 112, 127, 136). Greater control of fruiting and vegetative response may also be obtained.

Radioisotopes have proven to be very useful in tracing the absorption and translocation of foliar applied nutrients (7, 15, 25, 60, 118, 129, 131). Before their use only visible effects such as leaf symptoms, plant growth and changes in mineral concentration of plant tissues could be measured. The use of radioisotopes makes it possible to distinguish foliar absorbed nutrients from those already in the plant and those simultaneously being taken up by the roots. Furthermore they permit tracing the path of nutrients as they pass through the plant.

Wittwer (127, 130) and Bukovac and Wittwer (25) have reviewed the various techniques used when radioisotopes have been used for studies of foliar absorption and translocation. Methods of application have included leaf injection (11), vacuum infiltration (33), momentary dipping of leaves (113), spraying of leaves (66), the application of droplets onto leaf surfaces (23), the "Sticking Method" (93) and the leaf immersion technique (60). The first four methods are suitable for studies of transport but have limitations for determining absorption.

The last three methods also lend themselves to experiments concerning foliar absorption since the non-absorbed residue may be more easily removed.

Methods for removing the residue include the removal of a disc containing the site of treatment and the "washing technique" developed by Jyung (60) which involves washing the site of treatment with a designated amount of water or some other solvent. A limitation of the disc removal method is that some of the absorbed nutrient is removed with the disc resulting in an underestimation of absorption. Sources of error in the leaf washing technique might be due to the strong adsorption of some nutrients to the leaves which would result in an overestimation of absorption or the easy leachability of some other nutrients resulting in underestimation.

There has been much work done on the mechanism and factors which affect foliar absorption and translocation (6, 19, 24, 35, 29, 30, 52, 62, 96, 112, 118, 120, 126). For the treating solution such factors as pH, carrier ion, surfactants, addition of sucrose and concentration have been studied. Work has been done on the effects of temperature, light, humidity, time of application and nutritional status of the plants. Also number of stomata, site of application, age of leaf, presence of surface moisture and stage of plant development have been considered.

Jyung and Wittwer (61, 62), reported that foliar uptake of rubidium and phosphate ions is an active process. They found that foliar uptake of ions was enhanced by light, reduced by dinitrophenol, accumulated against a concentration gradient and had a temperature

coefficient greater than that of simple diffusion.

Variable results have been obtained with the use of surfactants, depending on their chemical nature and concentration (66, 118). According to Koontz and Biddulph (66), surfactants were ineffective in increasing absorption. However, Boroughs and Labarca (19) showed that a much greater uptake of P^{32} labeled KH_2PO_4 or $NH_4H_2PO_4$ occurred in the presence of either anionic, cationic or non-ionic surfactants. The anionic and cationic surfactants were superior to the non-ionic two days after application.

According to Barinov and Ratner (7) there is a time lag between the application of various nutrients to the leaves and their appearance in the tissues. Among the factors affecting this phase are: thickness of the cuticle, hygroscopicity and solubility of the salts applied and the selective properties of the protoplasm.

Since the earliest extensive studies dealing with time course of salt uptake by roots, it has been recognized that a relatively brief interval of rapid uptake is normally followed by a slower but more prolonged period of absorption (76). The initial phase appears to be non-metabolic, may occur anaerobically, has a temperature coefficient typical of a physical process and is predominantly concerned in cation absorption. Laties (76) suggested that the first phase is due to the exchangeable binding of ions to negatively charged biocolloids or to non-diffusible anions in the cell wall and that the second phase is an active uptake into protoplasmic constituents of the cell by processes which are metabolically controlled.

The apparent free space was described (75, 76) as that part of the

tissue which is in free diffusion communication with the environment without permeability barriers. It was suggested (55) that phase 1 might be the uptake of ions into this apparent free space while phase 2 occurs inside the permeability barrier. These concepts have been applied to the familiar pattern of foliar absorption curves (130). The Donnan free space is that part of the apparent free space which contains a high concentration of negative charges (76). The entry of ions into this space depends on Donnan equilibrium considerations. Therefore positive ions should enter more readily than negative ions.

Jyung (60) proposed that the shoulder of the typical foliar absorption curve coincides with drying, concentration and crystallization of the applied solution. This agreed with results obtained by Koontz and Biddulph (66) who found that a marked reduction of uptake and translocation occurred at the time of crystallization of the applied salts on the surface of the leaf.

The nutrition of plants through the leaves is closely interrelated with the entire complex of the major physiological processes, including photosynthesis, respiration, enzyme activity and the root nutrition of plants. Substantial evidence that foliar applied nutrients have an effect on photosynthesis has been shown (37, 56). A sharp increase in photosynthetic activity was obtained following foliar applications containing nitrogen, phosphorus and potassium (7).

It has also been observed that foliar feeding may accelerate the absorption of nutrients by plant roots (56). Since roots utilize carbohydrates and respire at the expense of photosynthetic products; growth, absorbing surfaces and absorption of mineral nutrients will all

increase proportionately with the intensity of photosynthesis.

Surface characteristics - - It is generally assumed that plants originated in the water as simple unicellular organisms, and that by specializations of many sorts they have attained their present complexity of form and organization. It was not until plants were able to acquire a cutinized surface that they could grow permanently in a terrestial environment with foliar organs exposed to the air. The aerial exposure was undoubtedly of great advantage for the provision of adequate CO_2 for rapid growth but this very exposure provided for rapid water loss. The cuticle, a layer laid down of products of metabolism of all cells exposed to the air, was the answer to this problem. Whereas, in water these compounds were largely washed away, in the air they oxidized and polymerized and formed a coating that greatly inhibited water loss (100). Since the coating also prevented rapid uptake of CO_2, the terrestial plant developed another new feature, the stoma.

The cuticle was first described and named by Brongniart (22) who showed that it was noncellular in structure. Lee and Priestly (77) stated that cuticle formation is associated with synthesis of fatty compounds of dividing protoplasts. According to Priestly (100) long chain fatty acids and alcohols formed by condensations of shorter chained acids migrate to the outer epidermal walls where they are deposited with their polar groups in the water phase and their hydrocarbon chains in the air. In the presence of oxygen these oxidize and gradually condense to form a more or less continuous film over the outer surface of the plant. As water is lost and oxidation proceeds,

a "varnish-like" protective coating is formed.

Chibnall and Piper (28) obtained mixtures of long-chain paraffins, alcohols and ketones from the waxes of leaves. More recently Matic (83) isolated from the cuticle of Agave a mixture of hydroxylated octa- and hexa-decanoic acids. Although the major components are fatty acid poly-esters (cutin) and fatty acids (wax), smaller amounts of pectin, cellulose, protein and amino acids have been found to exist in cuticular membranes (131).

The cuticular layers of different plants vary in thickness and in degree to which the vertical walls of the epidermal cells are cutinized. In the vertical wall, cutinization may occur as pegs of teeth where walls of two adjacent cells meet or as flanges or skirts when the entire wall is cutinized (94, 115). Since in its early stages of deposition cutin may be liquid, it tends to follow the laws of capillarity making thick deposits in crevices and being thinner over convex surfaces. Without exception, more cutin has been found in the upper surface than in the lower (107).

Environment also has a great influence on cuticle development, high insolation as in desert areas or at high altitudes being conducive to heavy cuticle whereas shade and moisture are conducive to thin cuticle (115). Water stress has also been shown to increase cuticle deposition (115).

Walls of palisade and spongy parenchyma cells, particularly those bordering the sub-stomatal chambers, are cutinized (110). Therefore, even though nutrients may enter the stomatal cavities, they must still pass through a cuticle. These films are first detectable in expanding

tissues when intercellular spaces begin to appear.

In many plants the cuticle is covered with a deposit of wax in a variety of patterns. This phenomenon has been investigated by electron microscopic studies of plastic and carbon replicas of the leaf surfaces of many plants (51, 58, 88, 107, 108). These wax projections from the surface of the cuticle take different forms which may or may not be characteristic of the species, the genus or the family. The wax appears to be extruded through the intact cuticle and accumulates in a semi-crystalline or irregularly crystalline form (58).

The wax formation shows variation with environment while the leaves are expanding and just mature. A reduction in light results in a reduction of wax deposits. Juniper (58) found no wax projections on pea leaves when the plants were grown in the dark but they soon developed upon exposure to light. No wax was present on leaves of intact maize coleoptiles but appeared as the leaves broke through (107). The wax was solid in light and firmer at higher temperatures.

Surface wax appears to be deposited only on young leaves and essentially only during or shortly after the period of leaf expansion (53, 58, 107, 108). The margins of growing epidermal cells are commonly covered with a loose layer of cuticular substance (107, 108). These border areas are not present on fully grown leaves which suggests that these may be the regions of rapid growth of epidermal walls. When young but non-expanding leaves are dewaxed, the wax is not replaced but, when the wax is removed from growing regions, it is replaced at the cell margins but not in the center (58, 108). This accounts for the unchanging wax distribution on growing leaves and the unimpeded growth of

epidermal cells.

Much controversy has occurred concerning the deposition of this waxy material upon the leaf surface. Wax rodlets on some plants suggest a concentrated extrusion of wax through channels in the outer epidermal walls. However, wax canals have not been found in the walls either before or after wax deposition (58, 107). Mueller et al. (88) observed shallow pits in the cuticle of Musa but saw no relationship to wax extrusion since they were tightly closed. Electron micrographs show generally random extrusion through otherwise intact cuticle (108). Therefore, since wax is extruded only in young leaves, one might conclude that wax is extruded through the young, fragile cuticle and that normal thickening of this layer stops further wax extrusion. Wax patterns appear to remain uniform as leaves grow (58, 88).

The cutinized part of the epidermal wall beneath the cuticle has a complicated structure. It contains a cellulose framework and pectic compounds, cutin, waxes and other compounds as encrusting substances. By dissolving the cutin in hot alcoholic alkali, Roelofsen (106) isolated the cellulose skeleton from the cuticular layer of Clivia nobilis leaves and determined fibril orientation. The double refraction of the outer layer was negative which indicated a radial orientation of molecules. He presumed that the wax molecules crystallized in tangentially oriented platelets. The inner parts of the cuticular layers contained, in addition, cellulose and pectin.

Sitte and Rennier (114) reported that the cuticular walls appear to be laid down as layers and that the thickening of these layers is due in part to the interposition of cutin and wax between layers deposited

earlier. During development, cutin with only little wax appeared first, with the main quantity of wax appearing afterwards.

The cuticle has been likened to a rubber sponge in which the holes are filled with wax. van Overbeek (124) suggested that, when the cuticle is in a hydrated condition due to wetting or turgidity of leaf tissues, the swelling of the cutin will spread the wax components further apart thus facilitating penetration.

Roberts et al. (104) demonstrated the presence of pectinaceous materials in the cuticle of apple leaves which are continuous with similar layers in the walls of the epidermal cells. Microchemical tests (97) indicate the solutes can move along these pathways.

One of the more obvious functions of the cuticle is its restriction of water loss by transpiration. Thicker cuticles are generally found under dry conditions (115) and often contain a higher percentage of wax which undoubtedly further restricts the movement of aqueous solutions both inward and outward. The position of wax deposits may also influence the ability of plants to resist excessive water loss. Schieferstein and Loomis (108) found that the wax of Nicotiana glauca (a mesophyte) was largely on the surface and highly volatile while that of Agave americana (a xerophyte) was mostly subsurface and non-volatile.

A more critical study of the cuticle and its function is possible through its isolation by enzymatic (94, 95, 115, 131, 132) or chemical methods (51, 121). Orgell (95) described initially rapid penetration of the isolated cuticle, influenced by concentration, pH, polarity of solvent and solute, and charge on the penetrating particles. He (94) suggested that cracks and imperfections in the cuticle or an imbricated

cuticle of small platelets cemented together by pectic substances may result in ready penetration of foliar applied polar substances. Parallel changes in the wax content and wettability of leaves grown under different conditions was observed by Skoss (115) who concluded that the amount of wax in the cuticle largely controls the movement of water into leaves.

Permeability of the cuticular membrane for many chemicals and ions in solution is much greater from the outer to inner surface than from the inner to outer surface (51, 108, 131, 132). For inorganic ions the rate of penetration is positively related to the extent of ion binding on the surface opposite the site of entry (132). Since cuticles are fat-like in nature they are more permeable to non-polar compounds (34, 82). Also, cations penetrate more readily than anions. The fatty acids, alcohols and unsaturated esters of the plant cuticle are dissociable and non mobile, hence they impart to the cuticle a negative charge in the presence of water; this charge attracts cations and repels anions (34). The cuticle appears to function as a semi-lopoidal cation exchange membrane (95).

In order for penetration of the cuticle or stomates by an aqueous solution to occur, the leaf surfaces must first be wetted. The wettability of plant surfaces may vary considerably according to their physical and chemical characteristics. Boynton (20) emphasized the importance of contact angle and surface wetting in foliar absorption. Ebeling (41) demonstrated that high contact angles are associated with poor wettability. The addition of wetting agents reduced the contact angle on the leaf surface.

The contact angle has been shown to decrease with a decrease in surface wax (53, 58). Roughening a surface increases the contact angle

(1) which may explain the increase due to wax projections. It appears that water relations of the leaf are also of considerable importance in determining the magnitude of the angle. As leaves wilt, the contact angle tends to increase.

Preferential pathways for the absorption of aqueous solutions includes epidermal cells above veins, leaf hairs, anticlinal walls of epidermal cells and stomatal guard cell walls (39). Stomatal entry has been suggested but it has been largely discounted (39, 47, 118) unless surfactants are used (39).

There is much evidence for the existence of structures called ectodesmata in the outer epidermal cell walls which extend from the protoplasm of the cells up to the cuticle (45, 46, 47), and often are concentrated where penetration is the greatest (39, 45, 47). At first it was thought that ectodesmata were plasmic structures but electron microscope studies showed fine strings and not a tube of plasmalemma (46). Franke (45) proposed that they exist in the walls at all times but conditions affect their activity. Turgid leaves contained more ectodesmata than wilted and the number was greatest during night and early morning.

Through microautoradiography Franke (47) has confirmed that guard cells and anticlinal walls are the favored sites of absorption of radioactive solutions applied to the cuticle. These areas also corresponded to the areas of greatest ectodesmata density. These studies conclusively showed that, although the guard cells participate in foliar absorption, penetration does not take place through the stomata.

Ectodesmata also appear to be connected with cuticular transpiration

since excretion of water droplets appeared to be at sites of high ectodesmata concentration (46). Therefore perhaps the same pathways serve for absorption and excretion. Franke (46) proposed that ectodesmata may be formed by mechanical stresses which result in a separation of fibrils in the epidermal cell wall since these structures commonly occur where stresses are greatest.

Absorption and Translocation of Phosphorus and Calcium - - Inorganic phosphorus is absorbed by plants as $H_2PO_4^-$ or $HPO_4^=$ ions depending on pH and then esterified into the various classes of organic compounds found in plants (48). Among these are phytin, phospholipids, phosphorylated sugars, nucleoproteins and nucleic acids, and various coenzymes (89). It is critically involved in all energy-transfer steps in the cell (49). Participation of phosphorus in phosphate carriers, phosphorylation and the energy of phosphate bonds are some of its primary metabolic roles in plant cells. In growing plants, phosphorus is most abundant in meristematic tissues (86, 92).

Foliar absorbed radio-phosphorus is rapidly incorporated into sugar phosphate esters (14). Analysis of the phosphate fraction in the bean stem two hours after treatment showed that most of the foliar absorbed P^{32} was present as inorganic phosphate (118). It was therefore suggested that initially large quantities of hexose phosphates are synthesized in the leaf following foliar absorption but phosphorus is transported primarily as inorganic phosphate (118).

Foliar applied phosphorus is rapidly absorbed by the leaves of numerous plants and translocated to other parts of the plants (5, 8, 23,

109, 113, 128). Phosphorus has been classified as a mobile element in plants following foliar absorption (23). Downward movement of phosphorus occurs principally in the phloem (12, 13) with some movement upward through the phloem to developing buds and some lateral movement at high levels to the xylem (12). Phosphorus may be continually circulated throughout the plant (15).

The absorption of foliar applied phosphorus may result in an increase in dry weight of both the top and the roots (5) particularly when phosphorus is not supplied to the root medium (109). Silberstein and Wittwer (113) found that tomato, bean and corn plants grown at low phosphorus levels gave definite growth responses to foliar applied phosphorus as indicated by height and fresh weight measurements. Although foliar applied phosphorus was utilized more efficiently than when broadcast on the soil, the latter gave a higher total yield. The amount of uptake of phosphorus by leaves depended largely on the amount supplied to the roots (9).

Teubner et al. (117) reported as much as 12 to 14 percent of the total phosphorus in the parts of various fruit and vegetable crops harvested for food may be supplied through foliar sprays. The accumulation of foliar applied radiophosphorus in these organs was not reflected by a change in total phosphorus nor was the yield altered. They concluded that the phosphate absorbed by the foliage of plants grown in soils adequately supplied with phosphorus was replacing, or was utilized in preference to, phosphate that would otherwise have been absorbed by roots from the soil.

Absorption and translocation of foliar applied P^{32} is largely de-

pendent upon the physiological state of the plant and upon external factors. Absorption of phosphorus is greater in younger leaves (120) but more is translocated from older leaves (65). Teubner et al. (118) found greater absorption of P^{32} through the upper surface of bean leaves but others (35, 51) found greater absorption when it was applied to the lower surface.

The absorption of foliar applied phosphorus is apparently stimulated by light and its movement in the plant is greater during the day than at night (11, 118). It is likely that its movement in the phloem is associated with the movement of sugars in a mass flow of nutrients (18). Barrier and Loomis (8) reported that absorption of P^{32} was not reduced by the depletion of leaf carbohydrates but translocation from the leaves may be slowed or stopped. Phloem transport was greatly reduced in leaves depleted of carbohydrate reserves (30). Shading was shown to decrease uptake and translocation of foliar-applied P^{32} (50).

The absorption and translocation of foliar applied phosphorus in the bean plant increased directly with temperature from 10 to 21°C. (117). The greatest increases occurred at the lower temperature increments. The effect is primarily on transport (118). Localized low temperatures on the petiole or stem were found to markedly retard translocation of foliar applied phosphorus to portions below the temperature zone (116). However, low stem temperatures did not retard movement of P^{32} through stems when it was applied to the roots.

Shtrausberg (112) noted that air temperature had greater influence on assimilation and translocation of foliar applied nutrients than did root temperature. However, this may depend on the plant used since

warm weather crops such as tomato are affected more by root temperature than are cool weather crops.

Soil moisture stress was shown to greatly retard absorption and translocation of phosphorus applied to the leaves of sunflower (126) and red kidney bean (96). An increase in foliar uptake of phosphorus may be induced by high humidity (120). This agrees with the proposal that hydration of the cuticle favorably influences absorption.

Maximum uptake of phosphate by bean plants has been reported to be at pH 2 or 3 (118). The effect of pH appeared to be on the rate of absorption through the cuticle (116).

Foliar applied phosphorus may be lost from the plant either by foliar leaching (123) or excretion by the roots (9, 43). It may subsequently be assimilated by the root system of the same or adjacent plants (9). Phosphorus was leached with difficulty from leaves and especially from very young leaves (123). Loss by the roots decreased as the concentration of phosphorus in the root medium increased (43).

Calcium is one of the few essential elements entering into the structural skeleton of the plant, namely as calcium pectate, a constituent of the middle lamella (89). It forms salts with organic acids, other ions and enters into chemical combination in protein molecules. Calcium is necessary for the continued growth of apical meristems and serves as an activator of several enzymes in plants (86). A large part of the calcium in most plants is located in the leaves and more calcium is found in older than in younger leaves (86).

Calcium is readily absorbed by leaves (7, 23) but, in contrast to phosphate, is immobile in plants following foliar application (15,23,

98). Biddulph et al. (16) stated that calcium is not entirely immobile but the amount exported from the treated leaf is very small, the amount being related to the amount applied. Anesthetization by diethyl ether greatly increased translocation of foliar applied calcium from the site of application to all other parts of the plant (24). This movement was shown to occur via the xylem (16).

METHODS AND MATERIALS

Plant Material and Culture

Phaseolus vulgaris L., cv. Black-Seeded Blue Lake (Rogers Bros., Twin Falls, Idaho) and *Pisum sativum* L., cv. Little Marvel (Ferry-Morse Seed Co., Mountain View, Calif.) were selected as test material because of uniform growth characteristics and differential temperature requirements for optimum growth and development.

All seeds were germinated in coarse quartz sand in the greenhouse. Bean seedlings were transferred to solution cultures maintained at desired temperatures when the primary leaves were approximately 10 percent expanded (three cm in length). Pea seedlings were transferred when the first leaves were about 25 percent expanded. Great care was used to select plants of uniform height and leaf size and appearance.

Environmental Conditions - - The majority of the experiments were conducted with four thermostatically controlled temperature tanks, each containing ten quart-sized mason jars immersed in a water bath. Water temperature was maintained at selected temperatures by refrigerator compressor units working against a heating element. Two plants were grown in each jar which contained one-half the supply of nutrients recommended by Hoagland and Arnon (54). The solution culture was aerated from a central compressed air supply which entered each container through an aerating stone.

Long term growth studies were conducted in two-gallon glazed

crocks positioned in large temperature tanks of similar design to the units described above. Each tank contained twelve crocks in each of which six plants could be grown. Generally the temperature of the root environment was maintained at 7°, 13°, 18° and 24°C. When fewer temperatures were used for comparative purposes, 13° and 24°C. were selected. For simplicity the temperature of the root environment will hereafter be designated root temperature.

For maximum control of environmental conditions the smaller tanks were placed within growth chambers (108" x 72" x 90"). The plants were grown in a daily cycle of 14 hour light and 10 hour dark periods unless otherwise specified. The temperature during the light period was maintained at 24°C. and that of the dark period at 17°C. Light intensity in most cases was 1200 foot candles at the leaf surface.

Maintenance of uniform environmental conditions was more difficult in the greenhouse. Air temperature and light intensity varied with the outside weather. The temperature during the day ranged from approximately 21° to 38°C. and the night temperature was maintained at about 17°C.

Absorption Studies

Treating Solutions - - For studies of phosphate absorption using the droplet method (23), a solution containing 0.2 percent ortho-phosphoric acid was labeled by adding $H_3P^{32}O_4$ to give a final activity of approximately 50 μc per milliliter and the pH was adjusted to 2.5 or 3.0. Similarly, treating solutions containing calcium chloride were prepared with a specific activity of 50 μc per milliliter but in this

case a pH of 4 was used. Tween 20 (Polyoxyethylene (20) sorbitan monolaurate) was used as a surfactant at the rate of 0.1 percent in all experiments with peas. The solutions thus prepared were used for subsequent experiments until the activity decreased to approximately 10 μc per milliliter.

Absorption of phosphorus was also followed from a 10^{-3}M orthophosphoric acid solution prepared with an activity of approximately 100 μc per m mole. The pH was adjusted to 3.5 with a sodium acetate buffer.

Application of Radioisotopes - - Two general methods were employed for applying the labeled nutrients to the leaves. For most experiments the droplet method was used (23). A 10 μl droplet of the treating solution was applied to the upper surface of one of the primary bean leaves on the midvein and midway between the apex and the base. A leaflet of the first leaf was the site of application for the pea. A tuberculin syringe with a no. 27 guage stainless steel needle was used and treatment was performed between 8 and 8:30 A.M.

The "leaf immersion technique" developed by Jyung (60) was employed for "specific absorption" studies. Excised bean leaves were immersed in Petri dishes containing forty milliliters of the treating solution. A mylar cover was placed over each Petri dish to reduce evaporation and contamination. The leaves were removed after 12 hours and the non-absorbed phosphate was removed by washing.

Harvest and Removal of Non-absorbed Nutrients - - The plants were harvested at designated intervals, usually 12, 24, 48 and 96 hours after

treatment. The disc removal technique was used in all experiments involving whole bean plants. A disc one centimeter in diameter containing the site of treatment was removed with a cork borer. The plant was further separated into the remainder of the treated leaf, the stem including the opposite primary leaf, and the root. Each plant part was placed in one-ounce paper cups. Pea plants were similarly harvested but the treated leaflet was taken as a sample instead of a disc.

The "washing technique" (59) was used in two experiments with excised leaves using the droplet method. The non-absorbed residue was removed with 20 ml of distilled water.

Removal of the non-absorbed nutrient on the immersed leaves was accomplished as described by Jyung (60). Then a disc two cm in diameter was removed for radioassay.

Sample Preparation and Assay for Radioactivity - - The samples were dried in a forced draft oven at 21°C. for 24 to 48 hours. Before assay the plant material was crushed against the bottom of the paper cups with a rubber stopper to insure uniform geometric placement. The samples were counted directly using an end-window Geiger-Müller tube and standard scaler circuit.

Estimates of Absorption and Transport - - Most measurements were expressed as a percentage of the total labeled element applied to the leaf surface that was recovered in various parts of the plant. The amount of the applied nutrient not recovered in the disc or leaf washings was considered absorbed. "Specific absorption" was expressed as mμ-moles H_3PO_4 per cm^2 of leaf surface in 12 hours.

Morphological Studies

Growth Studies - - Fresh and dry weights were determined for bean plants grown at root temperatures of 13° and 24°C. Plants were harvested at intervals of 0, 2, 4, 6, 8, 10 and 20 days, divided into leaves, stems and roots and weighed immediately to determine fresh weight. Following drying for two days at 21°C. in a forced draft oven, dry weights were determined. These data were used to ascertain the percent moisture in each plant part.

Leaf tracings were made at daily intervals by inserting the primary bean leaf between cardboard and a clear plastic covered with tracing paper. By careful manipulation no apparent damage to any of the leaves was observed. The surface area of the leaf was obtained by use of a planimeter. This technique facilitated the determination of expansion rates of individual leaves.

Measurements of fresh transverse sections of the primary leaves of bean plants were made with a light microscope containing a micrometer. Hand sections were prepared by splitting a cylinder of pith, inserting a piece from the center of the leaf and cutting very thin transverse sections with a stainless steel razor blade. Measurements were obtained of the lamina, upper cuticle, upper epidermis, palisade cells, spongy mesophyll and lower epidermis for primary leaves from plants grown at 13° and 24°C.

Cellulose acetate replicas of surfaces of primary leaves of bean plants grown at 13° and 24°C. root temperatures were prepared for examination under a microscope.

Cuticle Studies - - An electron microscope (Philips 100 B) was used to study transverse sections of cuticles of bean and pea leaves in detail not possible with a light microscope. Small sections of leaves were fixed in Zetterquist at pH 7.0. These were then embedded in Epon according to Luft's method (81) and sections approximately 100 mμ thick were obtained with a glass knife on a Leitz ultramicrotome.

Surface characteristics of the cuticle of primary leaves of bean plants grown at different temperatures were studied by preparing replicas and observing them with an RCA type EMU electron microscope. Since replicas of surfaces were photographed, all details observed were due to surface characteristics and not to underlying structures. By using a two stage technique any contamination on the leaf surface remained with the first stage and was not evident in the final stage.

A negative replica was prepared by coating the leaf surface with acetyl cellulose and carefully stripping after drying. This negative replica was placed on a glass slide and shadow cast at an angle of 30° with vaporized aluminum. A vertical cast was then made with vaporized aluminum resulting in a shadowed replica of aluminum. Melted paraffin was brushed over the composite replica and it was cut into squares approximately two millimeters on a side which were then placed in methyl acetate. The cellulose acetate dissolved below 40°C. and the paraffin melted above 40°C., freeing the aluminum replica. The aluminum replicas were washed in fresh methyl acetate and picked up on specimen grids. After selecting representative samples under a light microscope, they were observed in an electron microscope and photographed.

For comparative purposes the double-stage plastic method used by

Mueller, et al. (87) was also employed. The leaves were dipped in graded concentrations of Tween 20 until thoroughly wetted. The wet leaves were then coated with a 15 percent aqueous solution of polyvinyl alcohol. After drying, the negative replica was carefully removed and a positive replica was made with Formvar (polyvinyl formaldehyde). The Formvar replica was backed with a layer of collodion (nitrocellulose) and the composite replicas were cut into small squares approximately two millimeters on a side. The polyvinyl alcohol was dissolved in water and the remaining film was picked up on specimen grids. The collodion was dissolved with amyl acetate. The final Formvar replicas were observed and photographed with an electron microscope. The appearance of the wax deposits was somewhat different with this method than with aluminum replicas (Fig. 1). Perhaps some surface wax was dissolved or otherwise altered by the surfactant. In view of this possibility most replicas were prepared with aluminum since that method may depict the natural surface more accurately.

Surface Wettability

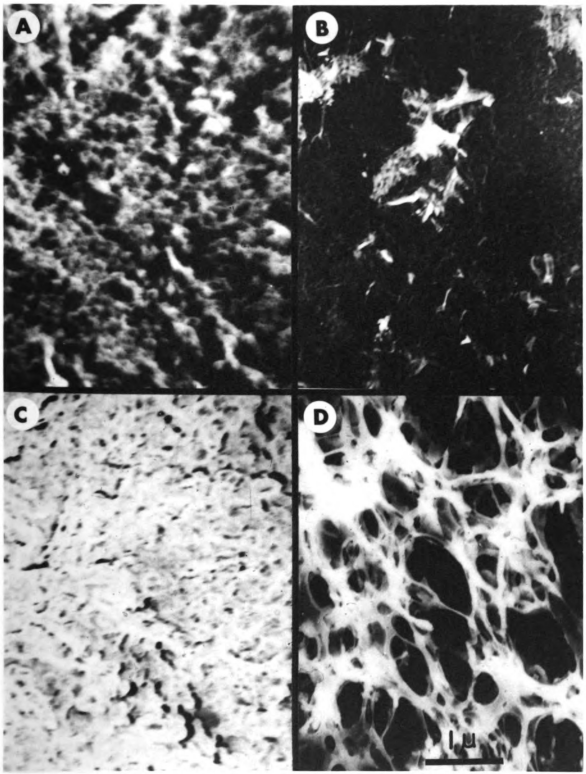
The effect of root temperature on the wettability of aqueous solutions applied to the primary leaves was next determined. Six 10 µl droplets of water containing India ink were applied to the upper surface of leaves from plants grown for six days at root temperatures of 7°, 13°, 18° and 24°C. Ten leaves were used for each temperature. The diameter of each droplet was measured after drying and the calculated area was designated as the contact area of the droplet. The effect of a surfactant was determined by the addition of Tween 20 (0.1 percent)

Figure 1

Electron micrographs of aluminum and Formvar replicas of bean and pea leaf surfaces showing a comparison between the two methods. All at 18,000 X.

Top - Aluminum (A) and Formvar (B) replicas of leaf surfaces of bean plants grown for 7 days at a root temperature of 7°C.

Bottom - Aluminum (C) and Formvar (D) replicas of leaf surfaces of pea plants grown for 24 days at a root temperature of 7°C.


A
B
C
D
1 μ

to a solution used for one-half of the droplets.

Transpiration Studies

Studies of transpiration in a uniform environment were made with plants previously grown at root temperatures of 13° and 24°C. Transpiration rates were calculated by measuring the volume of water absorbed per unit time. Bean plants pre-treated at a given root temperature were decapitated above the primary leaves and the cut surface sealed with lanolin. All data presented were based on primary leaves. Plants with and without roots were used.

Cuticular transpiration was assessed by dipping the leaves for 10 seconds in a 10^{-4}M solution of phenylmercuric acetate containing 0.1 percent Tween 20. The leaves of control plants were dipped in a 0.1 percent Tween 20 solution. All plants were then placed in the dark for one hour. This treatment effectively closed most stomates and prevented opening in the light (133, 134, 135).

The plants were then placed in potometers under a bank of fluorescent lights with an intensity of 1200 foot candles. The air temperature was 29°C. After thirty minutes readings were commenced for one hour to determine the amount of transpiration by each plant. At the end of the experiment negative impressions of the leaf surfaces were made with silicone rubber which solidified within two minutes. Positive replicas were then made with cellulose acetate and observed to confirm the degree of stomatal closure. Leaf tracings were made to document area. Transpiration was expressed as microliters per hour per square centimeter of leaf surface.

In connection with the transpiration studies, replicas were made

at various times of the day of leaves of plants growing in the greenhouse at root temperatures of 13° and 24°C. The replicas were observed with a microscope and measurements were made of the stomatal openings. Also, the degree of wilting by plants grown at different root temperatures was estimated.

Statistical Design and Estimates of Variability

All experiments were performed with plants grown in temperature controlled tanks. Since only four tanks were available and a limited number of plants could be grown in each, it was not practical to replicate temperature in each experiment. However, frequent checks were made of the equipment and, in none of the experiments reported, was there a deviation of more than one or two degrees from the temperatures given. The greatest difference might be expected within each tank since differences in aeration and in plant material is possible. However, these too were minimized by careful control and plant selection. Furthermore, all treatments within a given temperature were replicated at least five times. Temperature effects were confirmed by repeating certain phases of experiments and relationships were similar. With these facts in mind, the author is confident that the various experiments showed true differences between temperatures without their replication.

The data were subjected to analysis of variance and regression analysis where desired. When root temperature served as a pre-treatment to provide plant material, all subsequent treatments were replicated and placed in a split-plot, randomized block or completely ran-

domized design. Arc sine transformation of all percentage data was done previous to analysis of variance. The significance of differences between treatment means was determined by using the t-test for a comparison of two means and Duncan's multiple range test (38) for a comparison of more than two means.

RESULTS

Influence of Root Temperature on Foliar Absorption of P^{32} and Ca^{45} by Intact Bean and Pea Plants

Effect of Root Temperature - - The influence of different root temperatures on the absorption of foliar-applied phosphorus by bean plants grown at the same air temperature was studied in growth chambers. Root temperatures were maintained at 7° 13° 18° and 24°C. A fifteen hour light period with a light intensity of 800 foot candles was maintained throughout the experiment. The air temperature was maintained at 21°C. during the light period and 15°C. in the dark. After three days at the designated root temperatures, a 10 µl droplet of a P^{32} labeled solution was applied to one of the primary leaves and the plants were harvested at intervals of 12, 24, 48 and 96 hours after treatment.

Absorption and translocation of the applied P^{32} increased with an increase in temperature and time (Fig. 2). At all times of assay the greatest amount of P^{32} was absorbed by plants grown at the 24°C. root temperature and the least by those grown at 7°C. The absorption of P^{32} by the plants grown at root temperatures of 13° and 18°C. fell between these extremes. A good linear relationship ($r=+0.85$) in absorption between the various temperatures is indicated in Figure 3, A.

Little difference in the amount of P^{32} recovered in the treated leaf and stem was found, but transport to the roots followed a pattern similar to absorption. The transport of P^{32} to the roots readily oc-

Figure 2

Total absorption and distribution at various intervals after treatment of P^{32} applied to the primary leaves of bean plants grown at root temperatures of 7^{o}, 13^{o}, 18^{o} and $24^{o}C$.

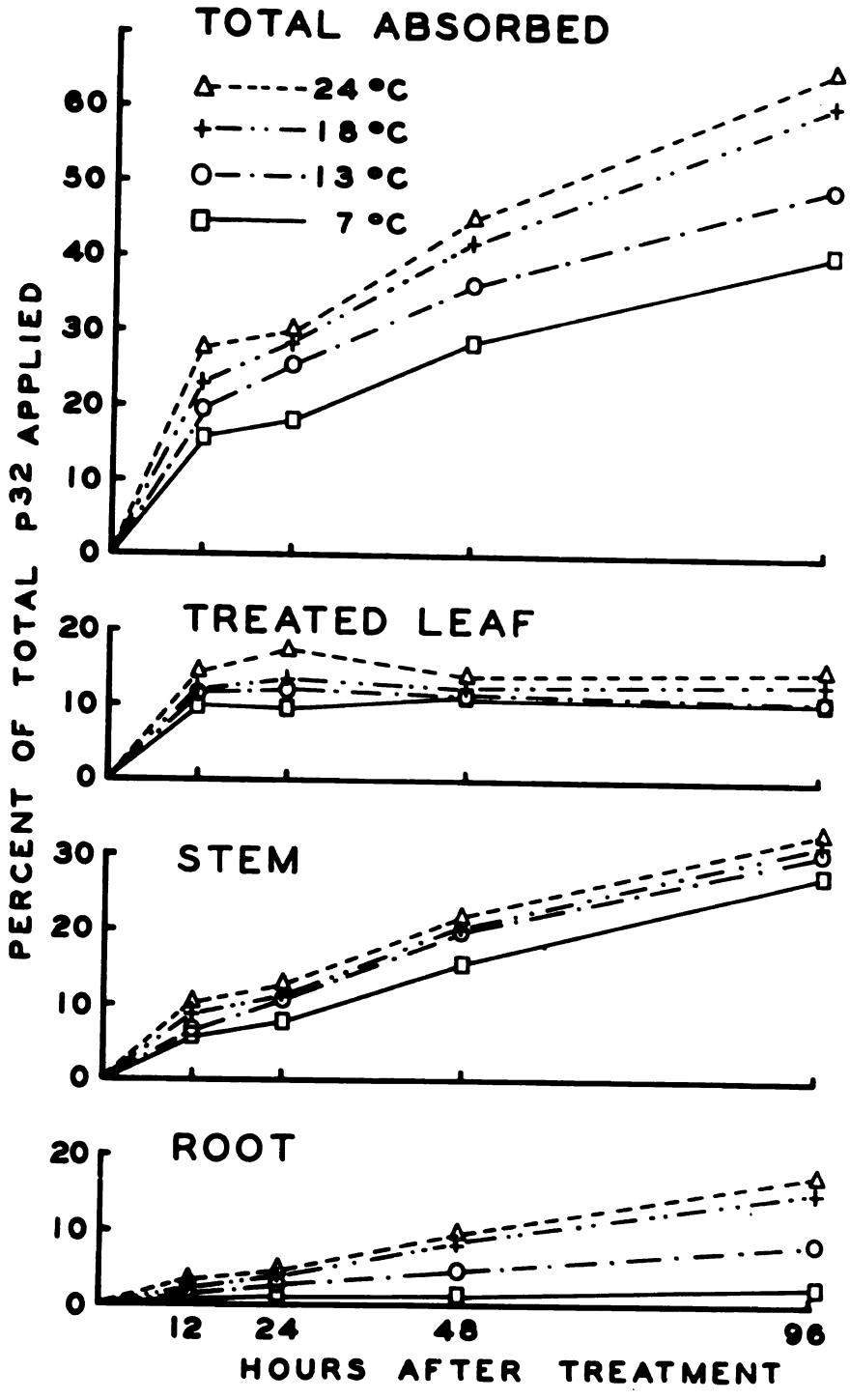

TOTAL ABSORBED
24 °C
18 °C
13 °C
7 °C
60
50
40
30
20
10
0
TREATED LEAF
20
10
0
STEM
30
20
10
0
ROOT
20
10
0
12
24
48
96
HOURS AFTER TREATMENT
PERCENT OF TOTAL P32 APPLIED

Figure 3

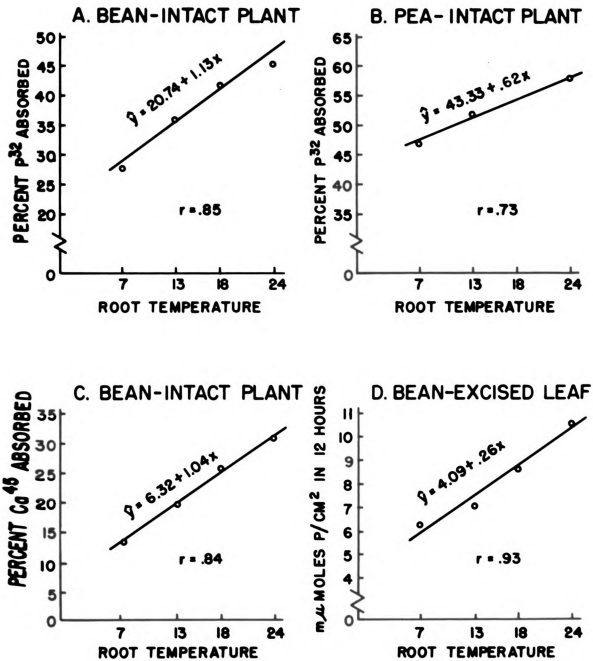
Effect of root temperatures (7°, 13°, 18° and 24°C.) on the absorption of P^{32} and Ca^{45} applied to the leaves of bean and pea plants.

A. Absorption of P^{32} by intact bean plants 48 hours after application.

B. Absorption of P^{32} by intact pea plants 96 hours after application.

C. Absorption of Ca^{45} by intact bean plants 96 hours after application.

D. Absorption of phosphate by excised primary leaves of bean plants 96 hours after application.


A. BEAN-INTACT PLANT
PERCENT P32 ABSORBED
y = 20.74 + 1.13x
r = .85
ROOT TEMPERATURE
B. PEA- INTACT PLANT
PERCENT P32 ABSORBED
y = 43.33 + .62x
r = .73
ROOT TEMPERATURE
C. BEAN-INTACT PLANT
PERCENT Co 46 ABSORBED
y = 6.32 + 1.04x
r = .84
ROOT TEMPERATURE
D. BEAN-EXCISED LEAF
mμ MOLES P/CM2 IN 12 HOURS
y = 4.09 + .26x
r = .93
ROOT TEMPERATURE

curred at the higher root temperatures but was much reduced at the lower root temperatures, particularly at 7°C. (Fig. 2).

Absorption of P^{32} by leaves of the pea followed a pattern similar to that of the bean (Fig. 4), but the temperature effect was less as evidenced by the slope of the regression line (Fig. 3, B). The transport of the absorbed P^{32} to the root was also affected less by root temperature (Fig. 4) than in the bean.

The absorption of Ca^{45} by bean plants also increased with increasing root temperatures although the total amounts absorbed were less than with P^{32} (Fig. 5, Top). At all times of assay the greatest amount of Ca^{45} was absorbed by plants at the 24°C. root temperature and the least amount by plants grown at 7°C. This response fitted a linear ($r=+0.84$) relationship between 7° and 24°C. (Fig. 3, C). Transport of the applied Ca^{45} from the treated leaf was negligible.

Absorption of Ca^{45} by the pea plant as illustrated in Fig. 5 (bottom) was negligible for the first 48 hours after application. This is probably due to the use of the treated leaflet in place of a disc and designating that amount recovered in the rest of the plant as absorbed. A substantial amount of the applied Ca^{45} had moved out of the treated leaflet at the highest root temperature after 96 hours giving some indication of greater absorption.

Relative Effects of Air and Root Temperatures - - The relative influence of air and root temperatures was assessed by following foliar absorption of P^{32} by bean and pea plants grown at 13° and 24°C. air temperatures with root temperatures of 13° and 24°C. at each air

Figure 4

Total absorption and distribution at various intervals after treatment of P^{32} applied to the leaves of pea plants grown at root temperatures of 7°, 13° and 24°C.

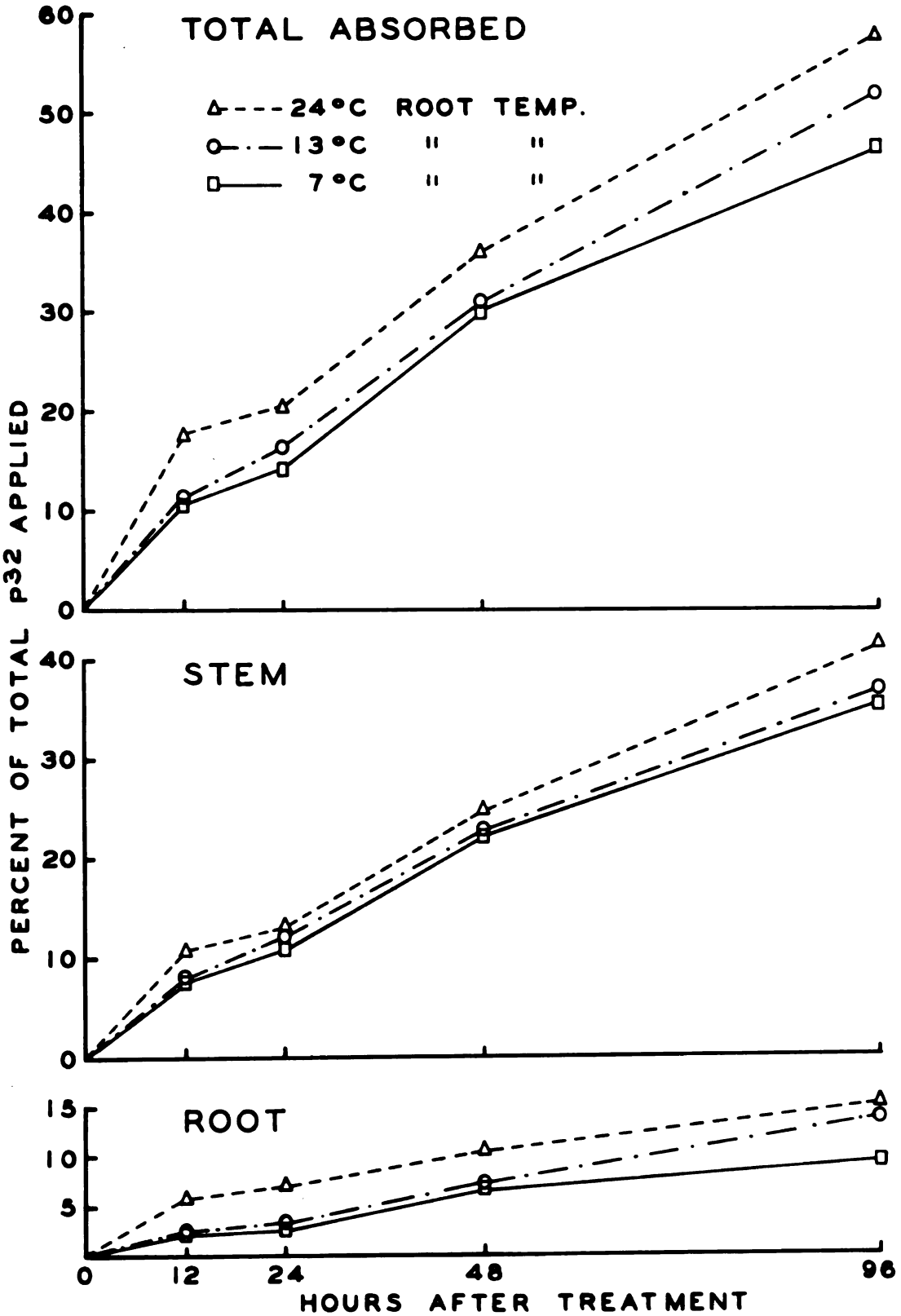
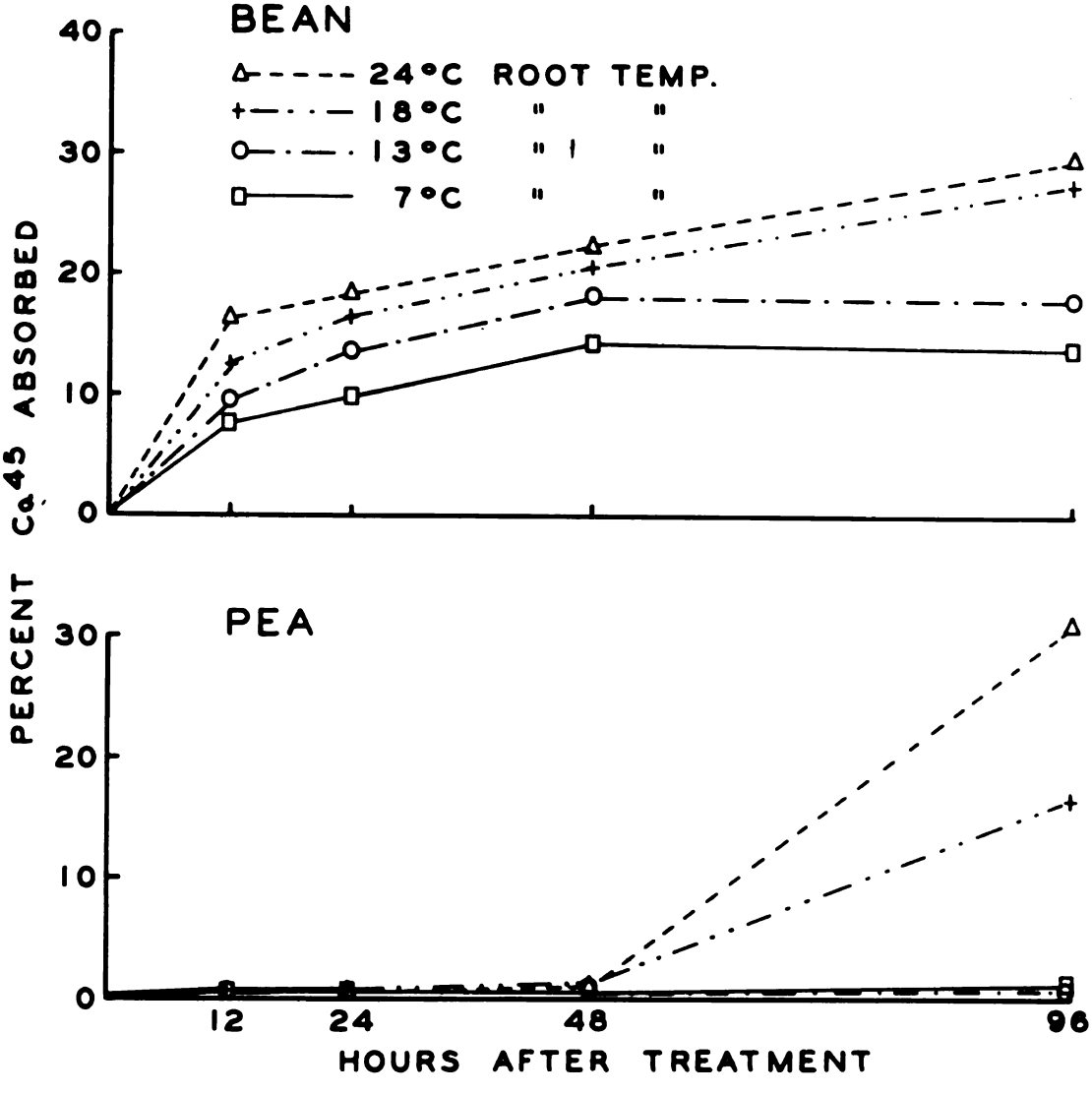

TOTAL ABSORBED
24°C ROOT TEMP.
13°C " "
7°C " "
STEM
ROOT
PERCENT OF TOTAL P32 APPLIED
HOURS AFTER TREATMENT
0
10
20
30
40
50
60
5
12
24
48
96

Figure 5

Absorption of Ca^{45} applied to the leaves of bean (top) and pea (bottom) plants grown at root temperatures of 7°, 13°, 18° and 24°C.


BEAN
24°C ROOT TEMP.
18°C " "
13°C " " "
7°C " "
PERCENT Ca45 ABSORBED
40
30
20
10
0
PEA
30
20
10
0
12
24
48
96
HOURS AFTER TREATMENT

temperature.

Air and root temperatures apparently have a comparable influence on foliar absorption by young bean seedlings (Fig. 6). The absorption of P^{32} was greatest at all harvests when both air and root temperatures were 24°C. and the least when both temperatures were 13°C. Intermediate results were obtained with plants grown at the 13°C. air - 24°C. root and 24°C. air - 13°C. root temperature combinations.

Translocation of the absorbed phosphate to the root was greatest at the higher (24°C.) root temperature regardless of air temperature (Fig. 6). Although little difference was found in the treated leaf, a greater amount of P^{32} was recovered in the stems of plants grown at the 24°C. air - 13°C. root temperature combination than in plants grown at the 13°C. air - 24°C. root temperature combination while the reverse was true in the root.

Similar results were obtained with pea plants grown at different air and root temperature combinations (Fig. 7). The greatest absorption of foliar applied P^{32} occurred when both air and root temperatures were 24°C. while the least amount was absorbed when both temperatures were 7°C. The other temperature combinations were intermediate but there is an indication that root temperature has a greater initial influence while air temperature has a greater influence at 96 hours after treatment.

Influence of Root and Air Temperatures before Treatment - - Because of observed differences in plant growth at various root temperatures, a comparison between the influence of temperature before and

Figure 6

Total absorption and distribution of P^{32} applied to the primary leaves of bean plants grown at various air-root temperature combinations.

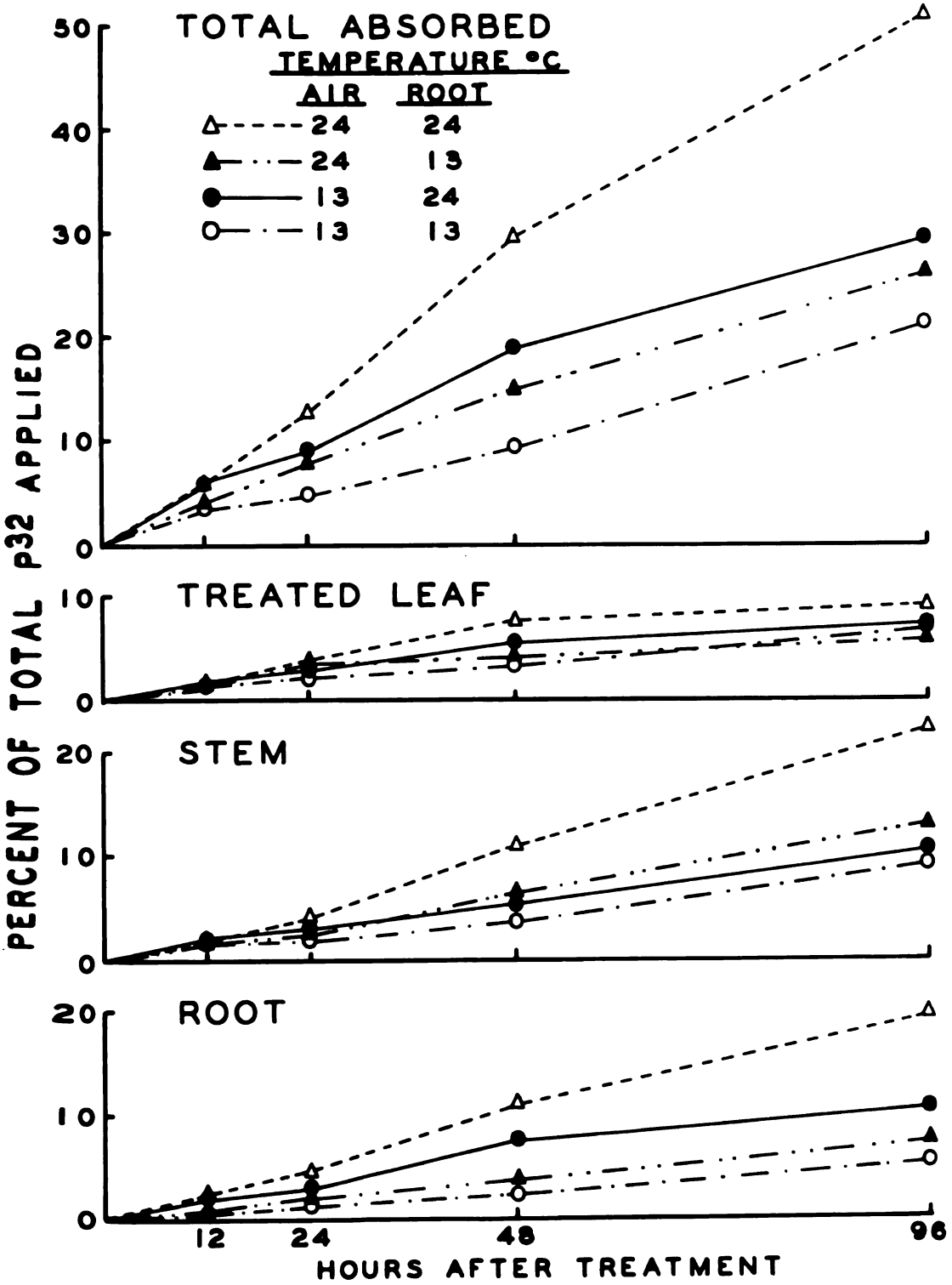
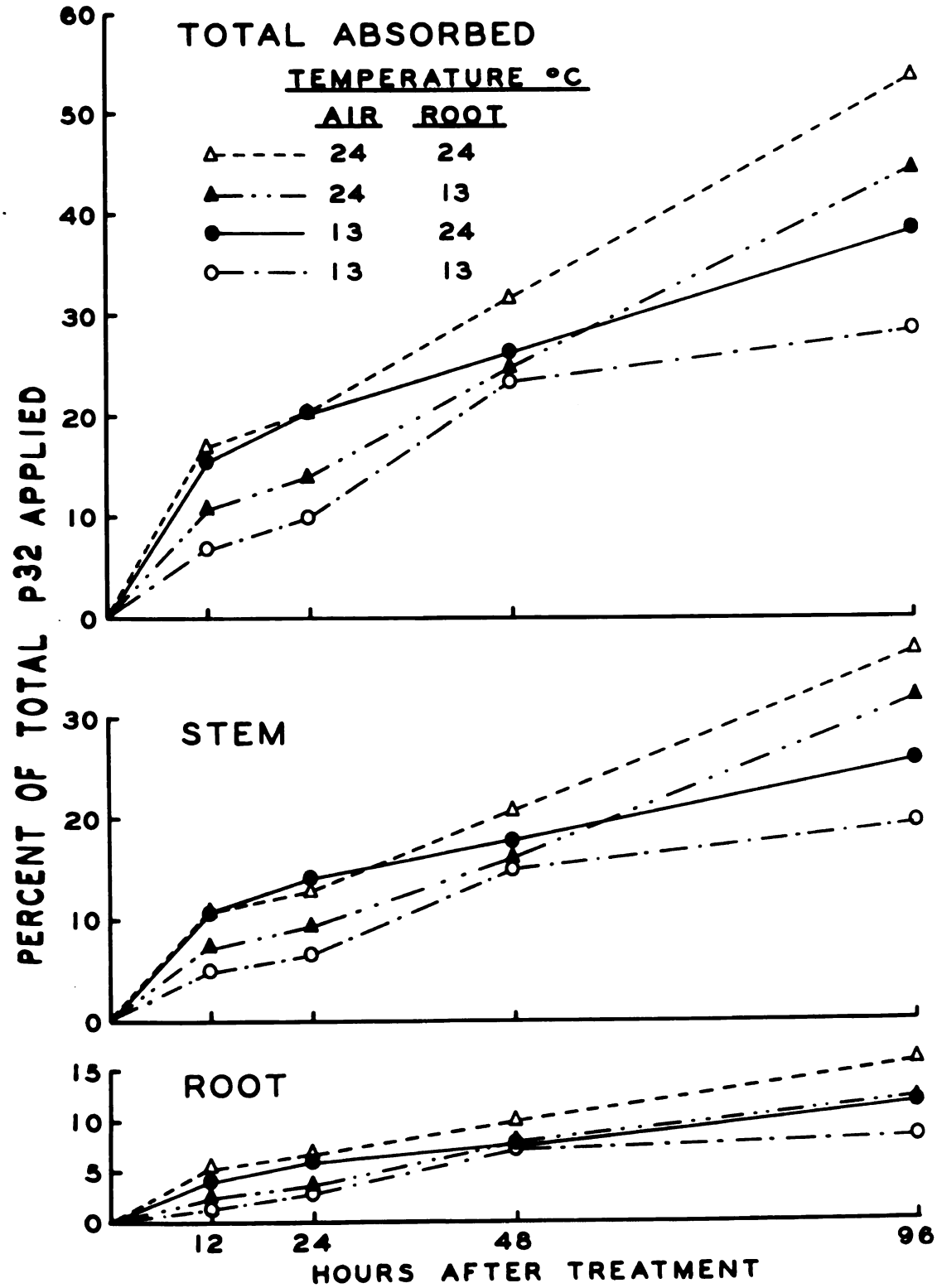

TOTAL ABSORBED
TEMPERATURE °C
AIR ROOT
24 24
24 13
13 24
13 13
TREATED LEAF
STEM
ROOT
PERCENT OF TOTAL P32 APPLIED
HOURS AFTER TREATMENT
0
10
20
30
40
50
12
24
48
96

Figure 7

Total absorption and distribution of P^{32} applied to the leaves of pea plants grown at various air-root temperature combinations.


TOTAL ABSORBED
TEMPERATURE °C
AIR ROOT
24 24
24 13
13 24
13 13
STEM
ROOT
PERCENT OF TOTAL P32 APPLIED
HOURS AFTER TREATMENT
0
10
20
30
40
50
60
5
15
12
24
48
96

during absorption was desired. The effect of pre-absorption root temperature was determined by growing one half of the bean plants for four days at 13°C. and the remainder at 24°C. After four days one half of the plants at each temperature were transferred to the other temperature just before P^{32} treatment. The air temperature was maintained at 24°C. throughout the experiment.

The air temperature pretreatment was similarly performed with bean plants at 13° and 24°C. After four days, one half of the plants at each air temperature were interchanged. The temperature of the root environment was kept continuously at 24°C.

Pre-absorption root temperature and root temperature during absorption similarly influenced absorption of foliar-applied P^{32} (Table 1). At both times of assay the greatest amount of P^{32} was absorbed by those plants grown at a continuous root temperature of 24°C. while the least amount was absorbed by those grown at a continuous temperature of 13°C. Intermediate effects were obtained with plants which had the root temperature interchanged at the time of treatment.

Root temperatures before and during absorption also influenced the distribution of the absorbed P^{32} within the plant. Pre-absorption temperatures had little effect on the amount of P^{32} found in the treated leaf but this amount was increased by a higher temperature during absorption (Table 2). The temperature before and during absorption influenced the amount of P^{32} recovered in the stem and the root. The greatest amount was recovered in roots of plants grown at a continuous root temperature of 24°C. while the least amount was found in roots of plants grown at a continuous 13°C. Transport to the roots was sig-

Table 1. Influence of root temperature on the absorption of foliar applied P^{32} by the bean.

Pre-absorption Temperature	Absorption Period	Temperature During Absorption		Means[1/]
		13°C	24°C	
(°C)	(hrs)	(percent absorbed)		
13	24	25.3	26.2	39.1
	96	49.5	55.4	
24	24	26.6	29.9	41.2
	96	52.4	55.8	
Means[2/]		38.5	41.8	

[1/] Means for influence of pre-absorption temperature significantly different at P=.01.

[2/] Means for influence of temperature during absorption significantly different at P=.01.

Table 2. Influence of root temperature on the distribution of foliar applied P^{32} in the bean.

Root Temperature		Distribution[1/]		
Pre-Absorption	During Absorption	Treated Leaf	Stem	Root
(°C)	(°C)	(% of applied P^{32})		
13	13	12.6[a]	13.1[ab]	11.8[a]
	24	15.6[b]	12.3[a]	12.9[a]
24	13	12.2[a]	15.5[b]	12.0[a]
	24	13.8[ab]	13.3[ab]	15.8[b]

[1/] Based on average of 24 and 96 hours absorption periods. Means within each group not followed by the same letter are significantly different at P=.05.

nificantly (P=.05) retarded when the root temperature was 13°C. either before or during absorption.

The air temperature before absorption had no significant effect on subsequent absorption of foliar applied P^{32} by bean plants (Table 3). However, absorption by plants grown at 24°C. during the absorption period was significantly higher than by plants grown at 13°C. The greatest amount of P^{32} was absorbed by plants grown at a continuous root temperature of 24°C. and the least amount by plants grown at a continuous 13°C. temperature. Absorption by plants initially grown at the lower temperature and changed to the 24°C. air temperature during absorption was higher than by plants initially grown at the higher air temperature and transferred to the 13°C. temperature.

The amount of P^{32} found in the stem was influenced more by the temperature during absorption than before absorption (Table 4). The greatest amount of the foliar applied P^{32} was transported to the root in plants maintained at a continuous air temperature of 24°C. while the least occurred in plants grown continuously at 13°C.

Influence of Root Temperature on Foliar Absorption by Excised Leaves

Leaves excised from plants previously grown at root temperatures of 13° and 24°C. were employed in an attempt to separate the confounding effects of absorption and translocation in intact plants.

Leaf Exposed to Air with Petiole Submerged - - Leaves from plants previously grown for four days at root temperatures of 13° and 24°C. were placed on a plastic material floated in pans of water. The petioles extended through the plastic and into the water thus maintaining

Table 3. Influence of air temperature on the absorption of foliar applied P^{32} by the bean.

Pre-Absorption Temperature	Absorption Period	Temperature During Absorption 13°C	24°C	Means[1/]
(°C)	(hrs)	(percent absorbed)		
13	24	20.9	26.7	28.4
	96	29.1	36.9	
24	24	22.4	28.7	30.4
	96	30.4	40.2	
Means[2/]		25.7	33.1	

[1/] Means for influence of pre-absorption temperature not significantly different.

[2/] Means for influence of temperature during absorption significantly different at P=.01.

Table 4. Influence of air temperature on the distribution of foliar applied P^{32} in the bean.

Air Temperature		Distribution[1/]		
Pre-Absorption	During Absorption	Treated Leaf	Stem	Root
(°C)	(°C)			
13	13	14.8^{ab}	4.1^{a}	6.2^{a}
	24	15.3^{b}	7.6^{c}	8.9^{b}
24	13	24.4^{bc}	6.2^{b}	7.9^{ab}
	24	11.1^{c}	7.6^{c}	15.8^{c}

[1/] Based on average of 24 and 96 hour absorption periods. Means within each group not followed by the same letter are significantly different at P=.05.

the leaves in a turgid condition for the duration of the experiment. A droplet of the P^{32} labeled solution was applied to each of the leaves and they were harvested at intervals of 1, 3, 6, 12, 24, 48, 96 and 144 hours after treatment.

Excised leaves of bean plants previously grown at the 24°C. root temperature continued to absorb P^{32} at a greater rate than leaves from plants previously grown at the 13°C. root temperature (Fig. 8, Top). The greatest difference occurred during the first hour of absorption but this effect continued throughout the experiment.

Initial absorption of Ca^{45} by leaves from plants previously grown at the 24°C. root temperature was also greater than by those from plants grown at the 13°C. root temperature (Fig. 8, Bottom). However, little difference was found after the first 12 hours of absorption.

The apparent difference between absorption curves for intact plants and excised leaves may be explained by the method used for removal of the non-absorbed residue. The disc removal technique, used with intact plants, removed some of the absorbed nutrient while the washing technique, used with excised leaves, did not.

Leaf Immersion Method - - When leaves from plants previously grown at root temperatures of 7°, 13°, 18° and 24°C. were immersed in a P^{32} labeled solution containing a known quantity of phosphate, it was found that absorption increased with increasing pre-absorption temperature treatment (Table 5). This difference increased with time. A linear relationship between temperature and absorption was found (Fig. 3, D).

Figure 8

Absorption of P^{32} (top) and Ca^{45} (bottom) under similar conditions by excised primary leaves from bean plants previously grown for four days at root temperatures of 13° and 24°C.

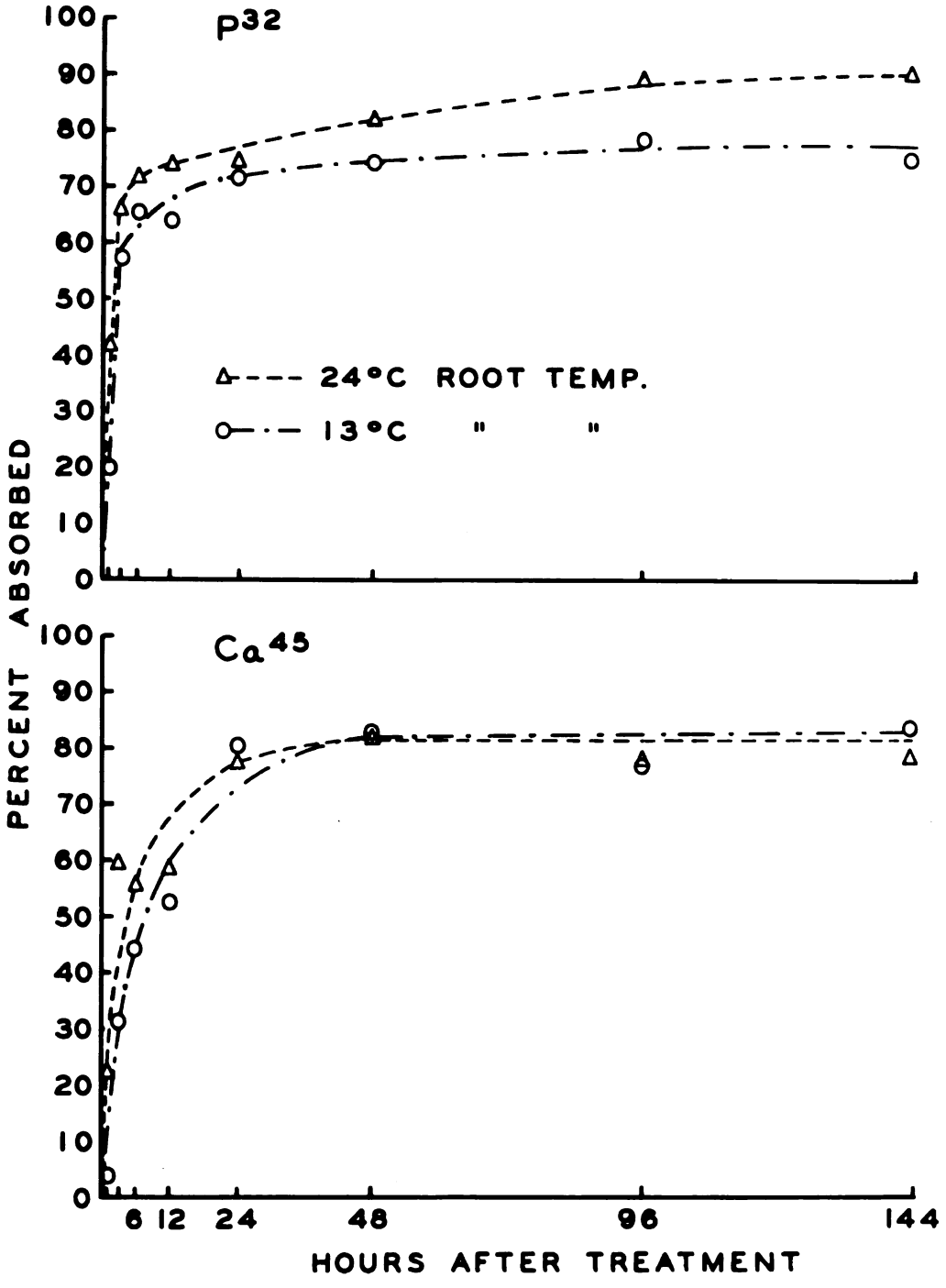

P32
Ca45
24°C ROOT TEMP.
13°C " "
PERCENT ABSORBED
HOURS AFTER TREATMENT
0
10
20
30
40
50
60
70
80
90
100
6
12
24
48
96
144

Table 5. Absorption of phosphate by excised leaves of bean plants grown at different root temperatures.

Root Temperature	Days Parent Plant Grown at Given Temperatures [1]					Means
	0	1	2	4	3	
(°C)	(mu moles/cm^2 leaf x 12 hours)					
7	6.69	7.23	7.01	6.29^{a}	4.79	6.40
13	6.69	6.67	7.88	6.96^{ab}	5.31	6.70
18	6.69	9.71	8.60	8.71^{bc}	6.88	8.12
24	6.69	9.94	12.21	10.60^{c}	11.45	10.12

[1] Treatments followed by different letters significantly different at P=.05. The mean effect of temperature, time and interaction of temperature and time were significant at P=.01.

Growth Responses

Dry weight increases were greater in plants grown at the 24°C. root temperature than in plants grown at 13°C. (Fig. 9). A gradual increase in dry weight occurred throughout the twenty day interval with plants grown at 13°C. root temperature, but the growth rate of plants grown at 24°C. root temperature increased sharply after 8 days. This coincided with a marked increase in leaf growth.

An interesting relationship was revealed when top: root ratios were determined (Table 6). This ratio increased slightly in plants grown at 13°C. root temperature in the first four days and then decreased to a more less steady ratio. This was due to a greater retardation of root growth than top growth at the lower temperature during the first few days after the plants were transferred. The opposite effect occurred with plants transferred to the 24°C. root temperature. The top: root ratio decreased greatly during the first four days and then gradually increased until, at the end of twenty days, the ratio was much higher than that of plants grown at 13°C. This resulted from an initial acceleration of root growth followed by an increased growth rate of the top.

The percent moisture was calculated for the various plant parts at the different harvest dates (Table 7). With the exception of the twenty day harvest, all differences between temperatures were found to be significant at P=.01 for the total, leaf and stem but not for the root. There was greater hydration of the aerial parts at the higher root temperature (Table 7).

Leaf tracings made at daily intervals and reproduced in Figure 10

Figure 9

Influence of root temperature (13° and 24°C.) on the dry weight of bean plants grown for 20 days in the greenhouse.

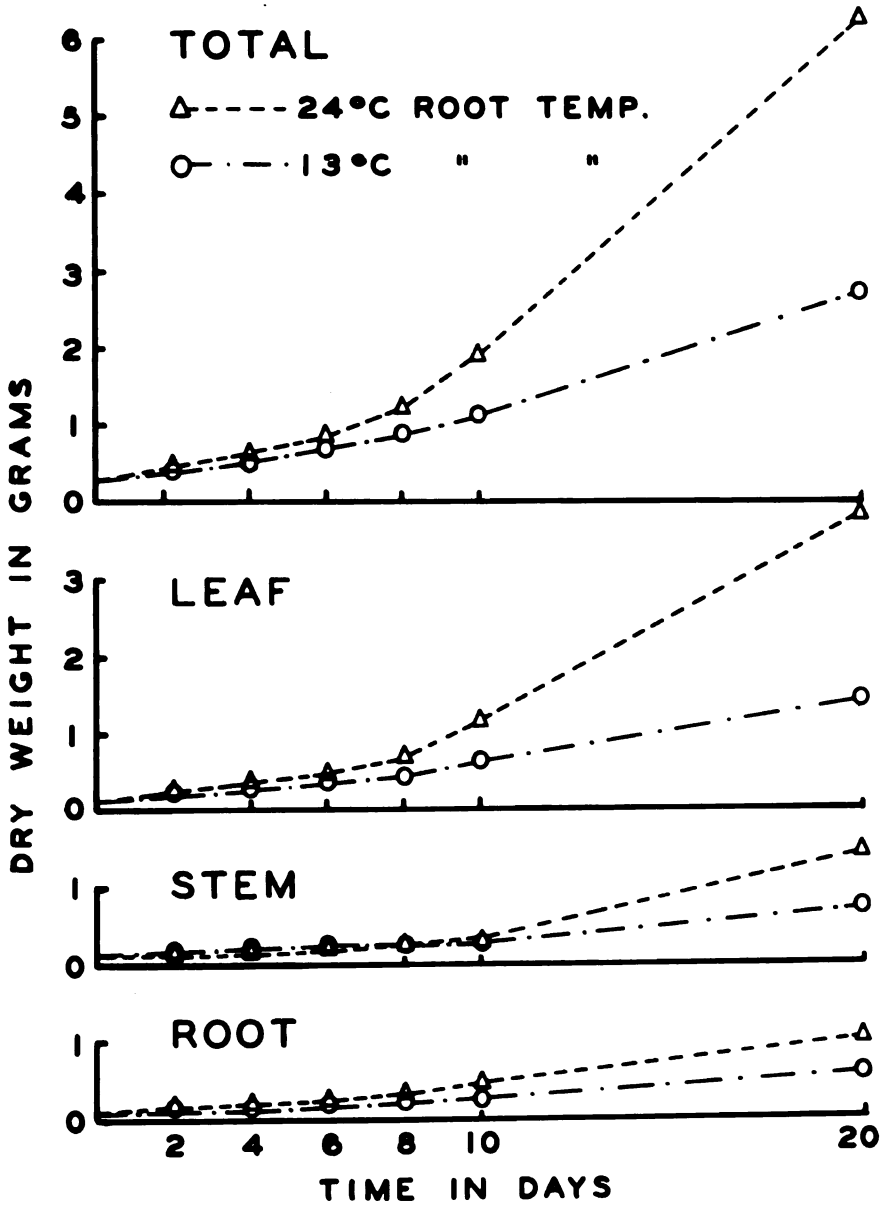

TOTAL
△----- 24°C ROOT TEMP.
○—·—· 13°C " "
LEAF
STEM
ROOT
DRY WEIGHT IN GRAMS
TIME IN DAYS
0
1
2
3
4
5
6
2
4
6
8
10
20

Table 6. Top: Root ratios for bean plants grown for twenty days at different root temperatures.

Root Temperature	Days at Given Temperatures						
	0	2	4	6	8	10	20
(°C)							
13	4.06	4.21	4.76	4.10	3.51	3.59	3.64
24	4.06	2.79	2.70	3.05	3.08	3.61	5.20

Table 7. The effect of root temperatures on moisture content of bean plants.[1]

Plant Part	Root Temp.	Days at Given Temperatures						
		0	2	4	6	8	10	20
	(°C)	(percent)						
Total	13	89.4	88.3	83.1	88.8	90.3	90.3	91.1
	24	89.4	92.2	92.8	93.0	93.6	93.1	91.3
Leaf	13	82.6	83.4	85.9	86.9	89.9	88.2	87.8
	24	82.6	90.2	90.6	90.8	92.4	91.5	89.4
Stem	13	89.2	86.8	85.8	85.0	87.0	87.8	88.2
	24	89.2	91.6	91.3	91.4	91.2	91.8	88.4
Root	13	94.2	93.8	93.3	94.0	93.8	94.2	94.6
	24	94.2	94.7	95.5	95.8	96.0	95.9	95.9

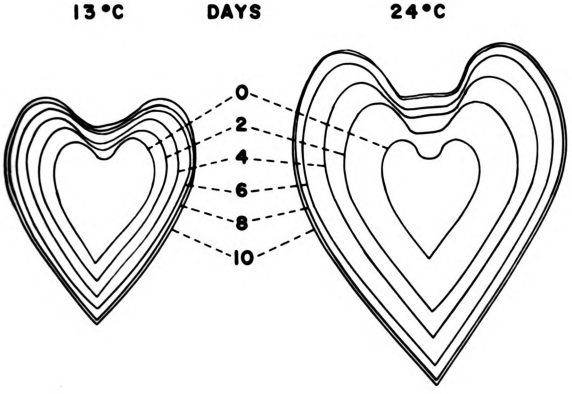
[1] Main effects of temperature, time and interaction between time and temperature were significantly different at P=.01. All differences between temperatures significant at P=.01 with exception of 20 day harvest and root.

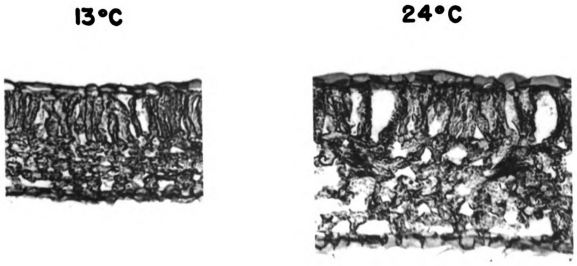
Figure 10

Leaf tracings at 2 day intervals illustrating differential expansion of primary leaves of bean plants grown at root temperatures of 13° (left) and 24°C. (right).

Figure 11

Transverse sections of the blade near the center of primary leaves of bean plants grown for 6 days at root temperatures of 13° (left) and 24°C. (right). Both at 125 X.


13°C
DAYS
24°C
0
2
4
6
8
10


13°C
24°C

for two day intervals illustrate marked differences in expansion rates between primary leaves of bean plants grown at 13° and 24°C. root temperatures. Expansion was more rapid at the higher temperature and, by the end of 10 days, the leaves of the plants grown at the 24°C. root temperature were approximately twice the size of those from plants grown at 13°C. (Appendix I). Initial expansion was greater than later in the period, especially for the 24°C. treatment. Very little leaf expansion occurred after 10 days with plants grown at the 24°C. root temperature but continued at a reduced rate for a few days longer at the 13°C. root temperature.

Transverse sections of the blade near the center of bean leaves from plants grown for 6 days at root temperatures of 13° and 24°C. revealed differences not only in measurements of the various components but also structure (Fig. 11). The dimensions of the epidermal, palisade and spongy parenchyma cells were significantly greater at the higher root temperature but not the cuticle (Table 8). Much of the difference in leaf thickness is found in the spongy parenchyma where intercellular spaces are much greater with plants grown at the higher root temperature. Accurate measurements of cuticle thickness were difficult with the light microscope because it is so thin on bean leaves.

Examination of cellulose acetate replicas of the upper surfaces of leaves of bean plants grown for 8 days at 13° and 24°C. root temperatures revealed approximately twice the number of epidermal cells, stomata and leaf hairs per unit area on leaves of plants grown at the 13°C. root temperature than at 24°C. (Appendix II). This would be expected since

Table 8. Some measurements (transverse section) of the components of primary leaves of bean plants grown for ten days at specified root temperatures.

Components	Root Temperatures 13°C	24°C
	(microns)	
Lamina	307.3	403.2
Upper Cuticle	1.2	1.1
Upper Epidermis	18.5	23.3
Palisade Parenchyma	106.9	136.7
Spongy Parenchyma	165.5	220.2
Lower Epidermis	13.5	19.6

All differences between temperatures significant at P=.01 except for cuticle.

the leaves of plants grown at the higher temperature had expanded to approximately twice the area.

Leaf Surface Characteristics

Surface Wax - - Light microscope studies of cellulose acetate replicas of bean and pea leaf surfaces revealed the existence of minute structures, probably deposits of wax (Fig. 12, A-H). The amount of wax deposited on the leaf surface decreased with increasing root temperatures.

Electron micrographs of aluminum replicas of bean and pea leaf surfaces present a more detailed view of these structures and their pattern (Fig. 13, A-D). The area bordering the anticlinal walls of epidermal cells of expanding bean leaves appeared to be relatively free of surface wax when compared to the area over the central part of the outer periclinal walls. With pea leaves there was no difference in wax accumulation between these two areas.

Marked differences were apparent in the surface above and near anticlinal walls of very young (primary leaf 10%expanded, Fig. 14, A) and a more mature (80% expanded) leaf (Fig. 14, B). The surface of the younger leaf was more irregular in this area, possibly a result of greater stresses on a more fragile cuticle. The area above the anticlinal walls of more mature leaves appeared smoother and perhaps firmer. Some wax deposits were evident in this area but they were neither as large nor as abundant as in the central surface area of the cell.

At higher magnification (18,000 X) it was apparent that wax de-

Figure 12

Photomicrographs of cellulose acetate replicas of the upper surfaces of bean and pea leaves of plants grown at different root temperatures.

Top - From bean plants grown at: 7°(A), 13°(B), 18°(C) and 24°C.(D).

Bottom - From pea plants grown at 7°(E), 13°(F), 18°(G) and 24°C.(H).

All at 500 X.

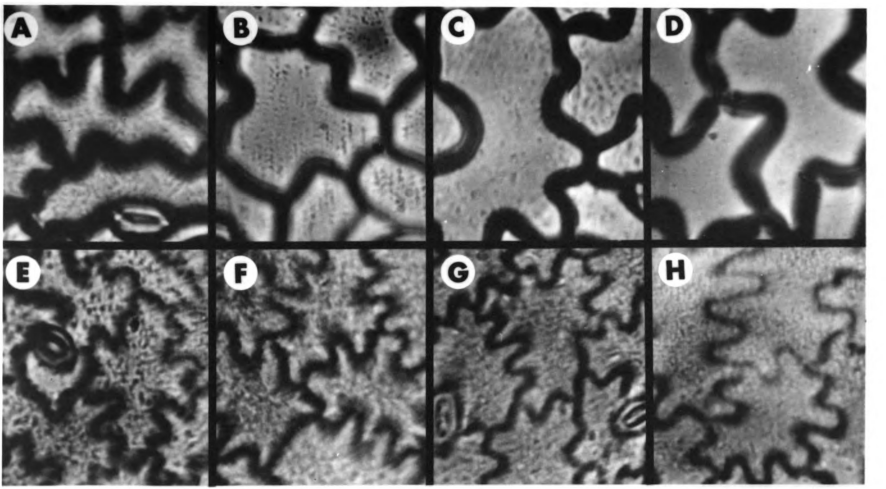

A
B
C
D
E
F
G
H

Figure 13

Electron micrographs of aluminum replicas of upper leaf surfaces of bean and pea plants grown at 13° and 24°C. depicting areas above and adjacent to anticlinal walls (aw) of epidermal cells. All at 3000 X.

Top - Bean at 13°(A) and 24°C. (B).

Bottom - Pea at 13°(C) and 24°C. (D).

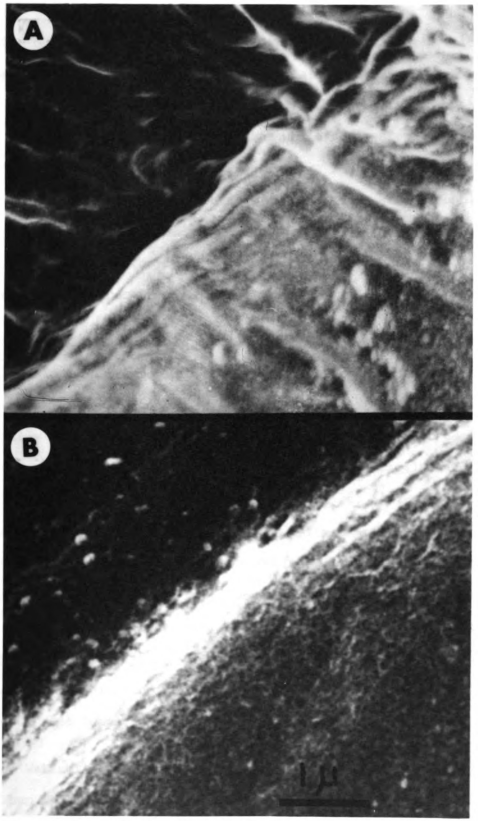
A
GW
B
GW
C
GW
D
GW
5 μ

Figure 14

Electron micrographs of aluminum replicas of the upper leaf surfaces above and near the anticlinal walls of primary bean leaves. Both at 18,000 X.

A. A very young leaf (10 percent expanded) from a seedling at time of transferral to designated root temperature.

B. A more mature leaf (80 percent expanded) after the parent plant had been grown at a 13°C. root temperature for 7 days.


A
B
1 μ

position on the surface above the epidermal cells decreased with an increase in root temperature (Fig. 15). Leaf surfaces of bean plants grown for 6 days at a root temperature of 7°C. were covered with a dense deposition of rod-like structures while these structures were less frequent as temperatures increased (Fig. 15, A-D). Unlike the bean, the wax deposits on the surface of pea leaves were ribbon-like in appearance although some rod-like structures were apparent at the lower temperatures. The density of surface wax decreased with an increase in root temperatures (Fig. 15, E-H).

Cuticle - - In an attempt to define the fine structure of the cuticle, electron microscopic studies of transverse sections of bean and pea cuticles were conducted. Considerable difficulty was encountered, especially with leaves from plants grown at the higher root temperatures. This was probably due in part to the very thin and fragile nature of these cuticles. The cuticle of the bean (Fig. 16, A) appeared to be thinner than that of the pea (Fig. 16, B) from plants grown at the same root temperature. Further, the cuticle from pea plants grown at a root temperature of 18°C. (Fig. 16, C) appeared thinner than from comparable plants grown at a 7°C. root temperature. There was some impregnation of the epidermal cell wall by the cutin at the lower temperature while this was not evident with the bean at the same temperature or with the pea at the 18°C. root temperature.

Surface Wettability

When measured drops of water (10 ul) were placed on excised primary leaves of bean plants previously grown for six days at root temperatures

Figure 15

Electron micrographs of aluminum replicas of surfaces of bean and pea leaves obtained from plants grown at different root temperatures depicting deposits of surface wax above the central part of the outer periclinal walls of upper epidermal cells. All at 18,000 X.

Top - From bean plants grown at 7°(A), 13°(B), 18°(C) and 24°C.(D).

Bottom - From pea plants grown at 7°(E), 13°(F), 18°(C) and 24°C.(H).

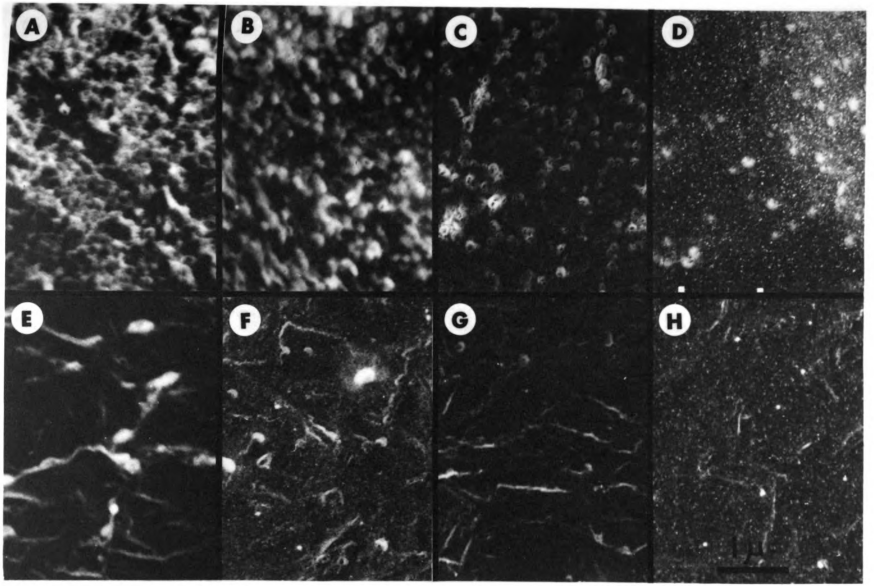

A
B
C
D
E
F
G
H

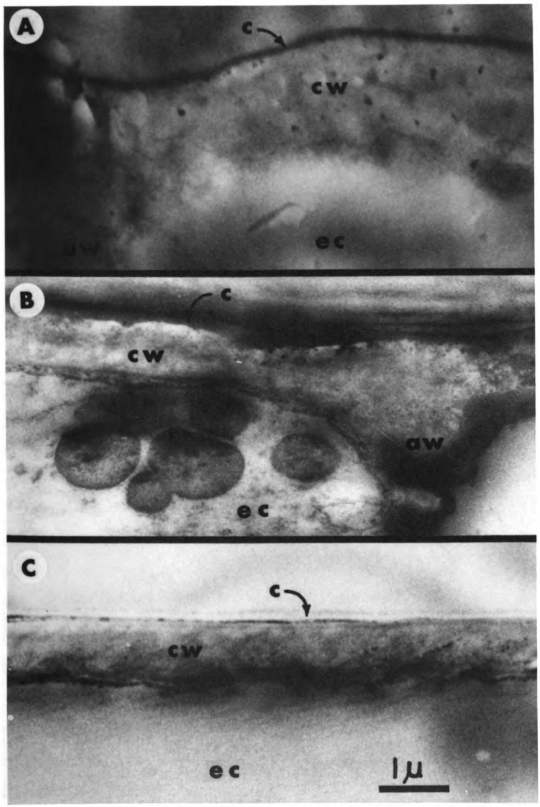
Figure 16

Electron micrographs of transverse sections of bean and pea leaves depicting the cuticle (c), cell wall (cw), anticlinal wall (aw) and epidermal cell (ec). All at 16,000 X.

A. Bean leaf from a plant grown for 10 days at a root temperature of 7°C.

B. Pea leaf from a plant grown for 10 days at a root temperature of 7°C.

C. Pea leaf from a plant grown for 10 days at a root temperature of 18°C.


A
c
cw
ec
B
c
cw
aw
ec
C
c
cw
ec
1μ

of 7°, 13°, 18° and 24°C., the contact area of the drop increased with an increase in root temperature (Table 9). This indicated greater wettability of the leaf surface of plants grown at higher root temperatures as a result of decreasing surface tension. When a surfactant was added to the water droplets, the contact area on leaves of low temperature grown plants was equal to that on high temperature grown plants, but greater than the contact area of a droplet on high temperature grown plants in the absence of a surfactant (Table 9).

Transpiration

Plants previously grown at a root temperature of 13°C. transpired ($ul/cm^2/hr$) less water than those grown at 24°C. when subjected to a uniform environmental stress (Table 10). There was no significant difference in transpiration rate in the presence or absence of roots.

A study of relative transpiration rates between plants previously grown at root temperatures of 13° and 24°C. indicated that this relationship varied with time (Fig. 17). After 2 days growth at these temperatures, transpiration by plants grown at 13°C. was reduced to 90.6 percent of that by plants grown at the 24°C. root temperature on a unit area basis. At 4 days this was decreased to 77.8 percent. A slight increase in this ratio was detected at the end of eight days.

Treatment with phenylmercuric acetate effectively closed most stomata and prevented them from opening during transpiration studies in light. With the stomata closed, transpiration should occur mostly through the cuticle. Transpiration was markedly decreased in the presence of phenylmercuric acetate, being 30 percent of the control with

Table 9. Contact area of a 0.01 ml droplet of water containing India ink after drying on primary leaves of bean plants grown for six days at different root temperatures.

Root Temperature	Contact Area of Droplet[1/]	
	Minus Surfactant	Plus Surfactant
(°C)	(mm^2)	
7	4.1[a]	9.5[e]
13	5.0[b]	9.3[e]
18	5.9[c]	9.3[e]
24	6.5[d]	9.2[e]

[1/] All means not followed by the same letter are significantly different at P=.01.

Table 10. Effect of pre-treatment at given root temperatures for 6 days on transpiration of bean plants.

Root Temperature	Transpiration	
	Intact Plants	Roots Removed
(°C)	(ul/cm²/hr)	
13	18.7[a]	15.9[a]
24	24.3[b]	29.0[b]

Means followed by different letters are significantly different at P=.05.

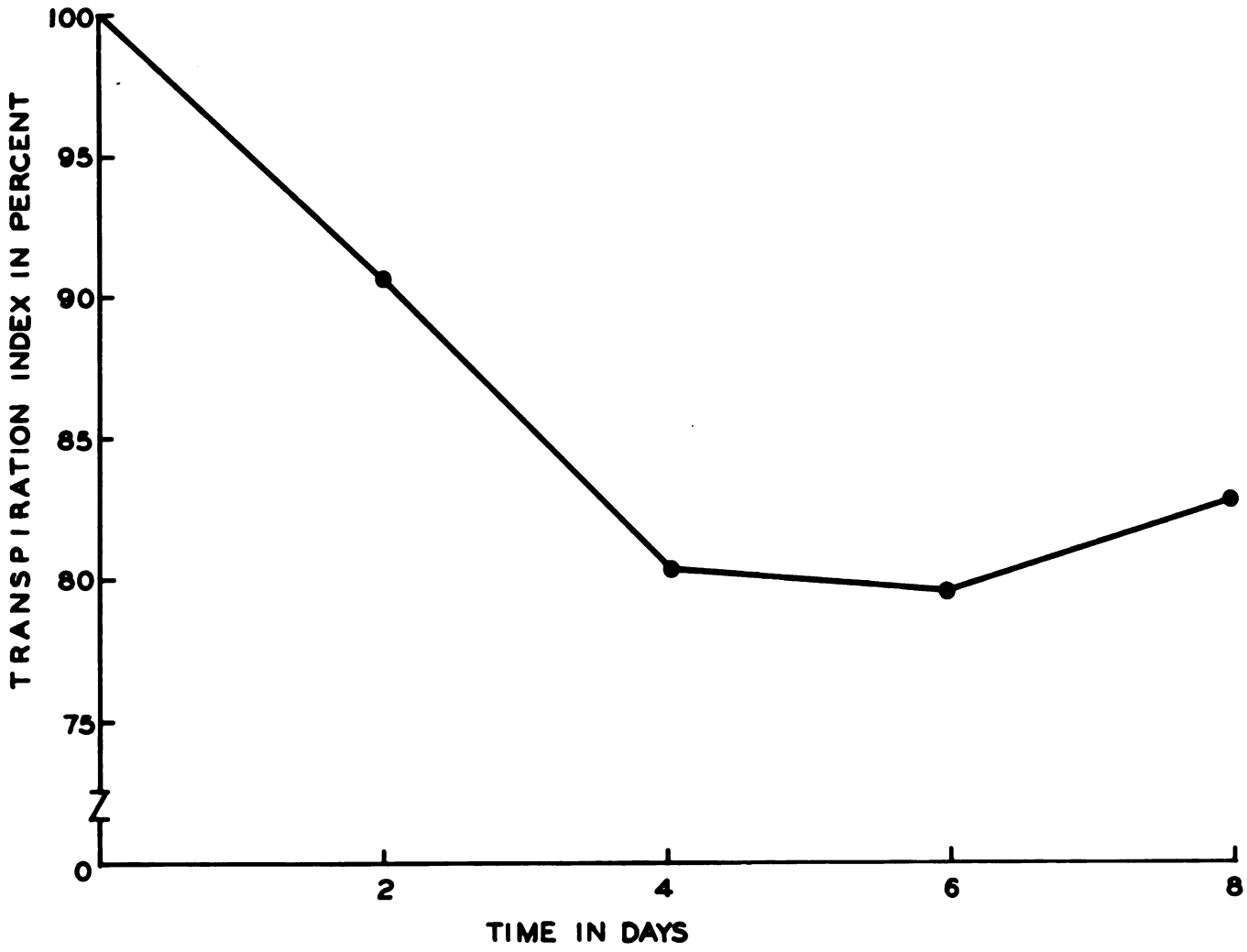
Table 11. Effect of stomatal closure with phenylmercuric acetate (PMA) on transpiration by bean plants grown for 10 days at specified root temperatures.

Root Temperature	Transpiration		Calculated Stomatal Transpiration
	-PMA	+PMA	
(°C)	(ul/cm²/hr)		
13	15.6[a]	4.7[c]	10.9
24	20.1[b]	6.6[d]	13.5

Means followed by different letters are significantly different at P=.05.

Figure 17

Effect of pre-treatment for varying times at 13° and 24°C. root temperatures on the transpiration of the primary leaves of the bean plant under a comparable environment. Transpiration index was calculated by dividing the amount ($ul/cm^2/hr$) transpired by plants grown at the 24°C. temperature into amount transpired by plants grown at the 13°C. root temperature.


TRANSPIRATION INDEX IN PERCENT
100
95
90
85
80
75
0
2
4
6
8
TIME IN DAYS

plants previously grown at the 13°C. and 33 percent of the control with plants previously grown at the 24°C. root temperature (Table 11). Transpiration by plants previously grown at the higher root temperature continued to be significantly greater per unit area than by plants grown at the 13°C. root temperature after both were treated with this chemical. These results suggest greater cuticular transpiration by bean plants grown at a higher root temperature. Calculated stomatal transpiration was also greater for plants grown at the higher root temperature.

Concomitant with transpiration studies, stomatal measurements and observations on the degree of wilting were made at various times of the day. Dimensions of the stomatal opening at 10 A.M. and 2 P.M. are given in Appendix II. Many stomata were at least partially open at 10 A.M. at the 13°C. root temperature but very few were open at 2 P.M. In contrast, most stomata of plants grown at 24°C. were open at both times. The greatest amount of wilting occurred at the lower root temperatures at 2 P.M. while no wilting was observed at any time with plants grown at the 24°C. root temperature (Appendix III).

DISCUSSION

Influence of Root Temperature on Foliar Absorption of P^{32} and Ca^{45}

Intact Bean and Pea Plants - - Results of the preceding experiments clearly indicate that the temperature of the root environment has a pronounced influence on the absorption and translocation of foliar applied phosphorus and calcium. Absorption of P^{32} and Ca^{45} increased with increasing root temperature and time with intact bean and pea plants. Although this increase in absorption with temperature followed a straight line relationship rather well, there was an equal tendency for a logarithmic relationship in some cases (Fig. 3, A and B).

The bean and pea were chosen to provide a comparison in response to root temperature between a thermophilic plant such as the bean and a more cold resistant plant such as the pea. The bean responded to differences in root temperature more than the pea in regard to both absorption and growth. This may be related to the finding of Barskaya (68) that a lowering of the root temperature decreased root respiration in thermophilic plants more than in cold resistant plants.

As with absorption, translocation of the applied P^{32} from the site of treatment to the roots was also greatly influenced by root temperature. Low root temperatures might retard movement in the phloem resulting in congestion of substances in the shoot. The fact that the mean kinetic energy of the molecules and ions is a function of temperature makes it self evident that all permeation and transport processes must of necessity be influenced by temperature.

Distribution of P^{32} applied to the primary leaves of bean plants

growing at different air-root temperature combinations seemed to depend upon the temperature of each plant part, the greater amount being located where the temperature was higher. It appears from these findings that the distribution of phosphorus within the plant is proportional to the growth and metabolic activity of the various plant tissues as they are affected by temperature.

Since root temperature before absorption affected subsequent absorption while the air temperature did not, perhaps root temperature affects growth, anatomy and hydration of leaves to a greater extent than does air temperature. These factors could significantly affect absorption.

Greater absorption of phosphorus is known to occur in younger leaves (120) and it is more abundant in other meristematic tissues of growing plants (86, 87). Therefore this nutrient may be absorbed and translocated more readily in rapidly growing plants such as those growing at an optimum root temperature. Processes such as growth and the accumulation of ions require the expenditure of energy by the cells. Adenosine triphosphate (ATP) is regarded as the primary source of cellular energy. ATP is generally produced in the leaves through photosynthetic phosphorylation or in various plant parts through oxidative phosphorylation. These processes require inorganic phosphorus. ATP is utilized in the leaf to make sugars for growth and in other active metabolic areas for energy and growth. Therefore we may conclude that phosphorus is needed and used to a greater extent in the more actively growing and metabolizing plant parts. Phosphorus preferentially moves to areas of high metabolic activity and, since an increase

in respiration rate generally results from an increase in temperature, this could help explain greater translocation to the roots of plants grown at the higher root temperature. Translocation of phosphorus is downward in the phloem (12, 13, 14) and its movement is associated with the movement of sugars in a mass flow process (18). Therefore the greater photosynthetic accumulation of sugars in the leaf and their movement to the roots of actively growing plants should tend to increase absorption and translocation of phosphorus.

Calcium is also readily absorbed but its movement in the plant is greatly limited (15, 23, 98, Fig. 5). Since calcium enters into the structure of plant cells and is an activator of several enzymes, it too may be absorbed in greater quantity by actively growing and metabolizing tissue. Minute quantities of the applied Ca^{45} were found in the stem and root of treated plants. This bears out the statement of Biddulph et al. that calcium is not entirely immobile (16). The phloem is a living tissue while the xylem is not. Perhaps this is a reason why phosphorus will move in both while calcium moves only to a very limited extent, if at all, in the phloem. This could be due to the selective permeability of the membranes of the phloem sieve tubes. Phosphorus may be more lipid soluble than calcium.

Although foliar absorption and translocation are different processes, it is questionable that they can be separated one from the other because they are interdependent. Not only is a greater quantity of the nutrient translocated when more is absorbed but we could expect greater absorption when more is translocated from the leaf. Translocation of foliar applied phosphorus is an active process (60). If diffusion is a

factor in foliar absorption then we might partially explain the phenomenon by stating that as the nutrient is transported from the site of application the diffusion pressure deficit is increased thus facilitating further absorption.

Excised Leaves - - It is possible to separate foliar absorption from translocation by using detached leaves. If maintained in a turgid condition they are suitable for foliar absorption studies. The method which involved applying a droplet of the treating solution to a bean leaf with its petiole in water shows absorption under otherwise fairly natural conditions since they are exposed to the air (Fig. 8). By using the leaf immersion method the difficulties of drying, concentration and crystallization of the treating solution on the leaf surface were avoided. Since absorption by leaves from plants grown at a root temperature of 7°C. decreased with time of exposure to this temperature (Table 5), perhaps factors inhibiting absorption increased with time. Because the difference in absorption persisted with both methods using excised leaves treated in a uniform environment, it was hypothesized that induced anatomical differences may modify absorption.

Growth Responses

Growth in general should influence absorption since dividing and enlarging cells such as are found in the younger parts of all growing regions have an especially high capacity for the accumulation of ions. Overall growth was greater in plants grown at the higher root temperature (Fig. 9). The greatest difference occurred in the leaves and this may have resulted in structural differences in that organ. The much greater

root growth in the early stages by plants grown at the higher root temperature may act as a phosphate sink and direct translocation to that organ. The dry weight of the stem, however, appeared to be slightly greater in the early stages in plants grown at the lower root temperature. Since some congestion of the applied nutrients appeared to take place in the stem, this result may be explained on the same basis. In as much as both calcium and phosphorus are involved in the growth process a direct relationship was hypothesized and did occur.

Leaf expansion varied with root temperature and we might expect surface and anatomical characteristics to vary accordingly. Larger epidermal and parenchyma cells were apparent in leaves of plants grown at 24° compared to those grown at 13°C. The difference in leaf thickness was mostly due to the greater intercellular spaces in the spongy parenchyma.

Surface Morphology

In addition to greater utilization of phosphorus and calcium by a rapidly expanding leaf, the greater distance between such structures as leaf hairs may result in greater contact between the applied solution and the leaf since these structures may limit contact with the leaf surface. This may be one reason that the contact area of water droplets increased with root temperature (Table 9).

The existence of wax deposits on leaf surfaces has been related to wettability (10). The greater concentration of wax deposits on leaves of plants grown at the lower root temperatures (Fig. 15) might explain the reduction in wettability and subsequent absorption by the leaves of

such plants. Perhaps the wax deposits are spread out more by the more rapid leaf expansion of plants grown at the higher root temperature or less is deposited. If the wax were more volatile or water soluble at the higher temperature, some could be carried off in cuticular transpiration. Also, if it is more fluid it would tend to flow over the surface more and therefore not result in such distinct structures.

The surface area adjacent to the anticlinal walls of bean leaves was found to have less surface wax (Figs. 13 and 14). This is the main area of new cell growth and therefore wax accumulation would not be as great. Since greater cell expansion occurred in leaves of plants grown at the higher root temperature, this area of less surface wax would be greater. The presence of less surface wax and a more fragile cuticle in this area may be related to absorption since this has been shown to be a preferential site of absorption (39). This could also help explain the greater contact area of droplets applied to the leaf surface of plants grown at a higher root temperature since wettability would be enhanced by greater areas of less surface wax. With the pea this area does not appear to contain less surface wax and perhaps this is because the pea leaf does not expand as much as the bean leaf.

Cuticle

Accurate measurements of the cuticle of bean and pea leaves with the light microscope were difficult because the cuticle is so thin. However, there was some indication of a thicker cuticle on leaves of plants grown at a low root temperature. This would agree with findings that water stress may result in greater cuticle deposition (115).

Electron micrographs of transverse sections of the very thin cuticles of bean and pea leaves did not provide adequate detail for determination of structural differences. Although a difference in cuticle thickness is still subject to question, there were indications that at low root temperatures the cuticles were thicker and impregnated the epidermal walls to a greater extent.

Since the leaves of plants grown at a higher root temperature expand at a greater rate, it is possible that the overlying cuticle could be stretched, making it thinner and more likely to have imperfections. A marked difference in cuticle appearance was observed at the margins of epidermal cells between very young, rapidly expanding leaves and older leaves from plants grown at a low root temperature (Fig. 14). This is the area of greatest epidermal cell growth. The more irregular surface on the younger leaf may result from greater stresses on a more fragile cuticle. These factors could help explain greater permeability of the cuticle to aqueous solutions at high root temperatures.

Attempts to isolate the cuticle of bean and pea leaves met with limited success. The fragile cuticle of the bean leaf broke into fragments, particularly from those plants grown at the higher root temperatures. Since evidence of pectinaceous materials in the cuticle has been reported (94, 104), perhaps the cuticle of bean leaves of plants grown at the higher root temperature contains more pectinaceous material which may be acted upon by the pectic enzymes. This could further explain greater absorption since pectinaceous materials have been suggested as pathways through the cuticle since they connect with similar layers in the walls of epidermal cells (104). Attempts to isolate the upper

cuticle of the pea were not successful although it was possible with the lower cuticle. This could be due to impregnation of the upper epidermal cell wall by cutin but not the lower.

A difference in chemical composition of the cuticle may be related to its penetration by aqueous solutions. Long chain fatty acids, alcohols, ketones and paraffins (28, 100) have been detected in the cuticle. If the more hydrophilic substances predominated, cuticular penetration would be facilitated. Skoss (115) observed parallel changes in the wax content of cuticles and wettability of leaves grown under different conditions and concluded that the amount of wax in the cuticle largely controls the movement of water into leaves.

Cell Wall

Substantial evidence has related ectodesmata to foliar absorption and cuticular transpiration (45, 46, 47). Conditions in the leaves of plants grown at a root temperature of 24°C. are more conducive to their presence than are conditions in leaves of plants grown at a 13°C. root temperature. It is believed that ectodesmata may result from forces and tension which broaden fibrillar openings and that these spaces are filled with a reducing substance. Perhaps the more rapid growth by the leaves of plants growing at the higher root temperature creates greater stresses which results in a greater number of ectodesmata. Also, fewer ectodesmata have been detected in leaves which are wilted or which possess a thick cuticle.

Water Relations

It was determined that the moisture content of bean plants was

significantly influenced by root temperature (Table 7). This probably results from a lowered capacity of roots maintained at a lower root temperature to absorb water and transport it to other parts of the plant. Results of others have shown that a soil moisture stress retards the absorption and translocation of foliar applied phosphorus (96).

One of the more obvious effects of lowered water absorption is the wilting of leaves on sunny days (Appendix III). A decreased water content of the aerial portions of the plant may affect several factors which influence foliar absorption and translocation. The wettability of wilted leaves tends to be reduced (1). A reduction in water content of the leaves may influence the rate of photosynthesis by (1) lowering the availability of water for the process, (2) reducing the diffusive capacity of the stomates, and (3) decreasing the hydration of the chloroplasts and other parts of the protoplasm which in some manner diminishes the effectiveness of the photosynthetic mechanism (86). As discussed earlier, photosynthesis may play an important role in the absorption of phosphorus and calcium.

Dehydration increases the concentration of solutes within the cell thereby decreasing the gradient between the solution outside and inside the cell. This could reduce the ease of penetration. Dehydration also increases the viscosity of the cytoplasm which could affect translocation. Greater hydration of the cuticle which might result from greater hydration of the leaf may also affect absorption. When the cuticle is hydrated, a swelling of pectinaceous material may spread the wax components further apart thus facilitating absorption (124).

A relationship between transpiration and water absorption by roots

is proposed by the transpiration-cohesion-tension theory (18). The outward diffusion of water vapor depends principally upon the excess of vapor pressure of the leaf over that of the atmosphere. Therefore greater transpiration in a uniform environment should result from greater hydration of the leaf and its cuticle. Greater transpiration, particularly cuticular, might result in moist conditions at and immediately above the leaf surface. This could result in retarding drying of a solution applied to this area. It has been shown that foliar absorption is influenced by humidity (120).

Since transpiration by plants previously grown at a 24°C. root temperature was greater per unit area than by plants previously grown at a 13°C. root temperature when both were studied in a uniform environment (Table 10), anatomical characteristics may be influencing transpiration. In addition to the thickness and composition of the cuticle, the fact that greater intercellular spaces are present in leaves of plants grown at the higher root temperature should serve to enhance transpiration. In as much as no significant difference in transpiration occurred in the presence or absence of roots, one may assume that pretreatment with varying root temperature did not alter membrane permeability of the root.

The finding that transpiration by plants previously grown at a 24°C. root temperature continued to be greater than transpiration by those grown at 13°C. after the stomata of both were closed with phenylmercuric acetate indicated greater cuticular transpiration by plants previously grown at the higher root temperature. Therefore a difference in permeability and hydration of the cuticle probably existed and this

would have a definite effect on absorption.

Cuticular transpiration was calculated at approximately 30 percent. Although this appears high it can be explained since not all of the stomata were completely closed and this amount included some transpiration by the stem where the stomata were not closed with phenylmercuric acetate. Also, we might expect transpiration through such a thin cuticle to be higher. Sitte and Rennier (114) suggest, however, that no direct relationship exists between the thickness of the cuticle and cuticular transpiration. If this is the case then we must assume that differences in cuticular transpiration and probably absorption result more directly from differences in chemical composition of the cuticle.

General

It has been shown that the temperature of the root environment influences a number of factors which, in turn, influence absorption of nutrients applied to the leaf. The nutrition of plants through the leaves is closely interrelated with the entire complex of major physiological processes, including photosynthesis, respiration, enzyme activity and the root nutrition of plants (8, 37, 56). Since practically all processes are influenced simultaneously by temperature, we are dealing with complicated interreactions.

This relationship is largely due to an inseparable interdependence between the activity in the root and in the aerial portion of the plant. Foliar absorption of phosphorus and calcium was closely related to differences in growth of the various plant parts resulting from different

root temperatures. An unfavorable soil temperature may result in a limitation of the activities in the aerial parts because of restricted absorption of water or nutrients by the roots and this, in turn, affects root growth. Water relations were significantly influenced by root temperature and this factor is very important because of its influence on various other factors.

The growth of plants is controlled and integrated by many growth factors, each produced in particular organs and translocated to other organs. Adenine, which is required for leaf growth, is synthesized in mature leaves, roots and possibly other tissues but only to a small extent, if at all, in developing leaves (78). Davis and Lingle have suggested that differences in soil temperature may result in a differential production of root-produced substances having shoot regulatory activity (36). Thiamine and pyridoxine, which are components of important enzymes involved in such processes as carbon dioxide evolution, cell division and synthesis of amino acids, are synthesized in green leaves and translocated to roots. It is small wonder that the temperature of one plant part should have an influence on the response of another.

SUMMARY

The temperature of the root environment has a pronounced influence on the absorption and translocation of foliar applied nutrients. Experiments using P^{32} applied to one of the primary leaves of bean seedlings growing at root temperatures of 7°, 13°, 18° and 24°C. indicated that absorption and translocation of P^{32} increased with an increase in root temperature. Similar results were obtained with the pea but absorption was affected slightly less by temperature.

The absorption of calcium by the leaves of intact bean and pea plants also increased with increasing root temperatures but its translocation from the treated leaf was negligible.

Air and root temperatures had a comparable influence on foliar absorption by young bean seedlings. Translocation of the absorbed phosphate to the root, however, was greater at higher root temperatures, regardless of the air temperature. By comparing the effects of root and air temperatures before and during absorption, it was found that the root temperature before treatment affected subsequent absorption of P^{32} while the pre-absorption air temperature did not. Both had a significant effect during absorption.

Excised primary leaves of bean plants previously grown at root temperatures of 13° and 24°C. continued to absorb P^{32} and Ca^{45} at different rates when cultured in a uniform environment. Initial absorption was greater by leaves of plants grown at the higher root temperature

than at 13°C. This difference persisted with P^{32} but not with Ca^{45}. Immersion of excised primary leaves from bean plants grown at root temperatures of 7°, 13°, 18° and 24°C. into a solution containing a known concentration of phosphate resulted in an increase in absorption with an increase in the root temperature of the parent plant.

Growth and anatomical modifications were observed with bean plants grown at root temperatures of 13° and 24°C. which may account in part for the differential absorption. The higher root temperature resulted in a greater increase in dry weight and moisture content of the various plant parts.

The surface area of the primary leaves of bean plants grown at the 24°C. root temperature was approximately twice that of plants grown at the 13°C. root temperature after several days at these temperatures. Transverse sections revealed that the dimensions of the various leaf components were significantly greater at the higher root temperature except for the cuticle. Electron micrographs indicated the possibility that low root temperatures result in thicker cuticles which impregnate the epidermal walls to a greater extent.

Light microscopic studies with cellulose acetate replicas of leaf surfaces of bean and pea plants revealed the existence of minute structures, probably surface wax. More detailed studies of aluminum replicas of the leaf surface with an electron microscope indicate that these deposits vary according to root temperature and plant species. The amount of wax deposited on the leaf surface appears to decrease with increasing root temperatures. Surface wax appeared as rod-like deposits on bean leaves while pea leaves had ribbon-like deposits. The area

bordering the anticlinal walls of epidermal cells of expanding bean leaves had less surface wax than in the central part of the outer cell surface but little or no difference occurred with the pea.

The contact area of measured drops of water placed on the primary leaves of bean plants increased with increasing root temperatures. The addition of a surfactant increased the area of all drops and eliminated this difference.

Bean plants previously grown at root temperatures of 13° and 24°C. transpired at different rates when studied in a uniform environment. Transpiration by plants previously grown at 24°C. was more than 80 percent greater per unit area than that of plants grown at the lower root temperature. Transpiration by plants grown at the higher root temperature continued to be greater than by plants grown at the 13°C. root temperature after the stomata on the leaves of both were closed with phenylmercuric acetate.

There is a definite interrelationship between the temperature of the root environment and the absorption of nutrients by foliar organs. Root temperature appears to influence both physiological and anatomical modifications of leaves and leaf surfaces that, in turn, alter foliar absorption and subsequent transport.

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APPENDIX

I. Expansion of Primary Leaves of Bean Plants Grown for 10 Days at Different Root Temperatures (see Fig. 12).

Root Temperature	Days at Specified Temperatures					
	0	2	4	6	8	10
(°C)	(cm^2)					
13	5.7	10.0	13.2	17.9	20.0	22.5
24	5.7	17.8	27.9	38.5	43.5	44.9

II. Some Characteristics of the Upper Surface of Bean Leaves from Plants Grown for Eight Days at Different Root Temperatures.

	Root Temperature	
	13°C.	24°C.
Epidermal cells per mm^2	453	235
Leaf hairs per mm^2	4.3	2.2
Stomata per mm^2	97	46
Stomatal opening at 10 A.M.		
length	17.7	21.2
width	1.9	8.3
Stomatal opening at 2 P.M.		
length	18.4	21.6
width	.7	6.6

III. Average Degree of Wilting at Various Times of the Day over a Two-Week Period

Root Temperature	8 A.M.	10 A.M.	12 A.M.	2 P.M.	4 P.M.
7	1.1	1.4	2.0	4.3	2.7
13	0.0	0.0	1.3	1.4	0.0
18	0.0	0.0	0.1	0.1	0.0
24	0.0	0.0	0.0	0.0	0.0

0 = no wilt, 1 = very slight wilt, 2 = slight wilt, 3 = moderate wilt, 4 = much wilt, 5 = very much wilt, 6 = extreme wilt.