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GROWTH, DEVELOPMENT AND POSTHARVEST PHYSIOLOGY OF GREENHOUSE CUCUMBER (*Cucumis sativus* L.) AS AFFECTED BY PHOSPHORUS NUTRITION AND VESICULAR-ARBUSCULAR MYCORRHIZAE

BY

MARGARET RAE TRIMBLE

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

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IN

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **GROWTH, DEVELOPMENT AND POSTHARVEST PHYSIOLOGY OF GREENHOUSE CUCUMBER (*Cucumis sativus* L.) AS AFFECTED BY PHOSPHORUS NUTRITION AND VESICULAR-ARBUSCULAR MYCORRHIZAE** submitted by **Margaret Rae Trimble** in partial fulfillment of the requirements for the degree of **Master of Science** in **Horticulture**.

N. R. Knowles, Supervisor

J. Hoddinott

P. Blenis

M. Mirza

DATE: July 16/93

DEDICATION

To my parents for their continuous support and love.

ABSTRACT

The growth response of greenhouse cucumbers (*Cucumis sativus*) to infection by vesicular-arbuscular mycorrhizal (VAM) fungi was determined from plant establishment to full maturity, under varying levels of P nutrition. Infection of plants by VAM species (*Glomus mosseae*, *G. dimorphicum* and *G. intraradices*) decreased as the level of P nutrition increased. Other than the stunting of plant growth at low levels of P nutrition, visual symptoms of P deficiency were not apparent during plant establishment (to 38 DAP). However, leaves of P-deficient mature plants had localized, desiccated areas in the interveinal regions. Leaf area and plant dry weight components were greatly increased by VAM infection and increasing P nutrition during plant establishment. Moreover, from seeding to 38 DAP, VAM stimulated plant growth at all levels of P nutrition. During establishment, plant P concentration increased with P nutrition and decreased with time. VAM-infected plants accumulated more total P in roots, stems and leaves than nonmycorrhizal plants. The concentration of soluble nitrogen in plant tissues decreased with increasing P nutrition and time, and VAM-infection influenced total nitrogen accumulation and the rate of change in soluble nitrogen pools over time. Leaf and root total soluble carbohydrate concentration increased faster during growth of control plants, as compared with VAM plants.

VAM did not enhance total fruit yield per plant at any level of P nutrition. However, VAM-infected plants produced more fruit than nonmycorrhizal plants during the first two weeks of production, resulting in higher early yields from VAM plants. Total yield increased significantly with increasing P nutrition. Phosphorus nutrition affected the postharvest respiration rates of fruit such that low P plants produced fruit with higher respiration rates than those from high P plants. Fruit P concentration was also lower in fruit from low P plants. VAM infection resulted in higher fruit respiration rates. VAM could be used in the greenhouse cucumber industry to enhance establishment of transplants and stimulate a greater quantity of high quality early fruit, but may be of little use in increasing overall fruit yield. P nutrition was shown to be very important in all aspects of cucumber production.

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LIST OF ABBREVIATIONS

a.i.	active ingredient
ANOVA	analysis of variance
BSA	bovine serum albumin
C	control
d	day
D	*Glomus dimorphicum*
DAP	days after planting
HI	harvest index
I	*Glomus intraradices*
LA/LF	leaf area per leaf
LAR	leaf area ratio
Lw/W	leaf weight ratio
M	*Glomus mosseae*
N	nitrogen
P	phosphorus
RS	reducing sugars
Rw/W	root weight ratio
SEM	scanning electron microscopy
SLA	specific leaf area
Sw/W	stem weight ratio
TSC	total soluble carbohydrates
TBO	toluidine blue O
VAM	vesicular-arbuscular mycorrhizae
v/v	volume/volume
wt	weight

Chapter I

BACKGROUND AND OBJECTIVES

SOIL PHOSPHORUS

The amount of phosphorus (P) available for use by plants (i.e. in the soil solution) is generally very low, only about 0.01% of total soil P (Brady, 1984; Bolan, 1991). Phosphorus occurs as organic and inorganic forms in the soil, and the ratio of these two forms varies depending upon soil type. Inorganic P occurs in solution, can be bound by silicate clays or hydrous oxides of iron and aluminum, and can form insoluble mineral compounds with calcium, iron and aluminum. Organic P is also present in solution, is tied up in soil organic matter, and is found adsorbed to soil aggregates (Brady, 1984; Bolan, 1991). However, organic P is generally mineralized to the inorganic form for plant uptake (Bolan, 1991). The availability of inorganic P to plants is determined largely by soil pH, although other factors such as soil moisture play a part. At low pH, inorganic P is strongly 'fixed' or tied up as insoluble iron and aluminum phosphates. As pH increases, the fixing capacity of the soil is reduced and P is released. Maximum P availability occurs in soils with a pH between 6.0 and 7.0. At higher pH, P availability is once again reduced, due to precipitation with insoluble calcium compounds (Brady, 1984). Thus, plant P nutrition is affected by availability , not quantity, of P in the soil. Addition of P fertilizers is not very efficient, due to rapid 'fixation' to insoluble forms, resulting in low recovery (10-30%) of applied P in a given cropping season (Brady, 1984).

PLANT PHOSPHORUS

Plant nutrients reach the surface of roots by mass flow, diffusion, and root interception (Barber, 1984). Of these three mechanisms, mass flow provides relatively little P to the plant, since the concentration of soil P in solution is quite low (0.05 to 0.3 μg P mL^{-1}; Ozane, 1980). Similarly, root interception does not result in a large amount of P uptake, since plant roots occupy

less than 1% of soil volume in the root zone and hence, only a small portion of available soil P is accessed by the plant (Bolan, 1991). Most P required by plants is obtained by diffusion. Diffusion of P toward the root surface, however, is slow and is not usually enough to maintain the maximum growth rate of the plant. Therefore, plants with a high root length density and long root hairs are most efficient at scavenging P (Koide, 1991), since their roots are able to extend beyond P deficient areas surrounding root surfaces. Some plant species may chemically alter their surrounding rhizophere in order to solubilize more P for uptake. This is accomplished by acidification of the rhizosphere, increased production of phosphatases, or production of iron chelators (Koide, 1991).

Phosphorus for plant growth must come from organic and inorganic soil reserves, or from additives such as fertilizers and plant and animal residues (Brady, 1984). The plant uses P as a component of adenylate nucleotides, which are involved in the energy transfomations required to drive life-sustaining metabolic processes. Phosphorus is thus necessary in all aspects of plant growth, including cell division, seed formation and crop maturation, root development, and crop quality (Brady, 1984).

VESICULAR-ARBUSCULAR MYCORRHIZAE

Vesicular-arbuscular mycorrhizal (VAM) fungi form mutualistic symbioses with plants. These fungal associations with plant roots are widespread throughout the plant kingdom, occuring in a variety of geographical and ecological areas (Powell and Bagyaraj, 1984; Koske and Gemma, 1992). The exchange of ^{32}P- and ^{14}C-labelled compounds between the VAM fungus and plant host is evidence of the mutualistic association (Cox *et al.*, 1975). VAM fungi cannot be grown in axenic culture, and thus, they are considered to be obligate biotrophs (Hepper, 1984). The additional volume of soil scavenged by the abundant external hyphae of VAM fungi results in increased uptake of P and other nutrients which are relatively immobile (eg. zinc and copper) (Smith and Gianinazzi-Pearson, 1988; Hayman, 1983).

The VAM fungi are classified as Zygomycetes of the order Glomales (Morton and Benny, 1990) and are found in the genera *Gigaspora*, *Glomus*, *Acaulospora* and *Sclerocytis* (Powell and Bagyaraj, 1984). *Glomus* species have been the most widely studied of the VAM fungi and are characterized by the presence of arbuscules and vesicles, and 'chlamydospores' produced apically on hyphae (no auxilliary cells), either singly or in loose aggregates in the soil (Morton and Benny, 1990).

The VAM infection occurs solely in epidermal and cortical parenchyma cells of primary roots. Infection does not penetrate the endodermis and thus is not found in the vascular tissues; nor is infection found in the apical regions of roots (Bonfante-Fasolo, 1984). Although VAM infection of roots may be quite extensive, there are usually no recognizable root surface alterations. However, mycorrhizal roots can sometimes appear yellow when compared to nonmycorrhizal (white) roots (Carling and Brown, 1982). Also, plant species with coarse root systems and little root hair development are frequently mycorrhizal-dependent (Bonfante-Fasolo, 1984).

Structural characteristics of VAM fungi in the plant host vary somewhat, depending upon the species of VAM; however, a general description is possible. VAM fungi have both external and internal root components. Globose to ovate spores, usually borne terminally or on short branches, are associated with the external hyphae. Spores can vary in size from 20 to 150 μm in diameter and are rich in oil globules. When an external hypha comes in contact with a root surface, the hypha produces an appressorium-like structure on the epidermis. Penetration of the root by hyphae can occur through or between epidermal cells, before reaching the cortex. Once the hypha has penetrated the root, the infection is considered to be internal and may spread along the root either intercellularly or intracellularly. Often, outer cortical layers are colonized by intracellular hyphae which coil inside the cells, whereas intercellular hyphae are found in inner cortical layers. Vesicles are formed by a swelling of the hyphae and may be inter- or intracellular. The vesicles are thought to be mainly resting or storage organs for the fungus, since they are rich in lipids and their numbers often increase in old or dead roots. Intercellular hyphae penetrate the cortical cells giving rise to arbuscules, which occur from multiple dichtomous branching of the

pentrating hypha (Bonfante-Fasolo, 1984). As arbuscules develop, the volume of cytoplasm in host cells increases, and the host nuclei enlarge. The arbuscule itself occupies a large portion of the host cell volume. The arbuscule has a life span of only 4-15 days (Carling and Brown, 1982; Cox and Tinker, 1976) before the branches begin to collapse. The plant plasmalemma and tonoplast invaginate around fungal tissues (intracellular hyphae, arbusucles, vesicles) that penetrate the cell wall.

The arbuscule is considered to be the site for fungus/plant metabolite exchange. The fungus takes up P from the soil and accumulates it as polyphosphate granules for transportation to arbuscules, where it is broken down for release to the plant host (Callow *et al.*, 1978). In exchange for P nutrition, the plant provides the fungus with carbohydrate nutrition (Miller *et al.*, 1986; Gianinazzi-Pearson and Gianinazzi, 1983).

HOST RESPONSE

The rate of plant growth is closely coorelated with the nutrient demand of the plant. The difference between P supply and the plant's requirement for P to maintain growth therefore dictates the level of P deficiency (Koide, 1991). Low P nutrition often limits shoot growth (McArthur and Knowles, 1993; Rufty *et al.*, 1990) and leaf area development. For example, Fredeen *et al.* (1989) showed that the rate of leaf expansion of soybeans grown with low-P was 85% lower than that of plants grown with high-P.

VAM-induced stimulation of plant growth is mainly due to improved nutritional status afforded by enhanced efficiency of P uptake from the soil. Two factors which affect a plant's response to VAM infection are growth rate and the minimum P content required to maintain healthy foliage (Hayman, 1983). In soils with high P concentration, the level of root infection by VAM fungi is generally suppressed or eliminated. This inability of VAM to infect plants grown with sufficient P has been attributed to a greater defense response by the plant (Schwab *et al.*, 1991), which is somehow manifested by a relatively high concentration of P within the plant's tissues (Carling and Browm, 1982).

Phosphorus deficiency can affect the ability of plants to assimilate other nutrients. In particular, low P nutrition has been associated with accumulations of nitrate and free amino acids in shoot tissue (Rufty *et al.*, 1990). In low-P plants, high nitrate levels in leaves were associated with low nitrate reductase activity (McArthur and Knowles, 1993), whereas free amino acid accumulation was associated with enhanced protein degradation (rather than inhibition of protein synthesis) (Rufty *et al.*, 1990). Mycorrhizal effects on the assimilation, incorporation and metabolism of nitrogen are considered indirect, and are thought to be mediated through improved P nutrition provided by the infection (Oliver *et al.*, 1983).

THE CUCUMBER

The greenhouse cucumber (*Cucumis sativus* L.), often termed the long-English or European seedless cucumber, is a semi-tropical vegetable which grows best under conditions of high soil moisture, fertilizer, temperature, relative humidity and light (Wittwer and Honma, 1979). The indeterminate growth habit requires pruning and training of the plant on wires to facilitate heavy fruit production. Fruit are parthenocarpic, 30 to 50 cm long, uniformly green and relatively thin skinned. The thin epidermis provides little resistance to moisture loss and thus fruit are shrink-wrapped after harvesting (Wittwer and Honma, 1979; Mirza, 1990).

To sustain high rates of growth, cucumbers require large quantities of fertilizer, especially nitrogen, potassium, calcium and phosphorus. Deficiencies of any of these nutrients will result in significant yield loss (Adams, 1978). Since cucumbers are often grown in soilless culture systems, the precise control of nutrients is of utmost importance to the crop. Mycorrhizal fungi have been shown to enhance the growth of many crops. There is no information, however, on the effects of VAM infection and P nutrition on growth and development of greenhouse cucumbers. This is surprising since the cucumber is a relatively major greenhouse crop that requires large nutrient inputs to produce high yields of good quality fruit. This study was undertaken to characterize the growth responses of greenhouse cucumber to VAM infection under varying levels of P nutrition. In particular, we wanted to determine whether any potential

exists for using VAM fungi to enhance growth of greenhouse cucumber for the commercial industry. Cucumber response to VAM infection and P nutrition was investigated at two growth stages to determine: 1) the effects of VAM and P on the establishment and early growth of cucumber plants, and 2) the effects of VAM and P on the mature, fruit-producing plant in terms of shoot growth, fruit production and fruit quality.

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Chapter II

EFFECTS OF PHOSPHORUS NUTRITION AND MYCORRHIZAE ON PLANT ESTABLISHMENT AND EARLY DEVELOPMENT

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi form mutualistic relationships with many plant species. These relationships often result in enhanced growth due to increased uptake of phosphorus (P) and other mineral nutrients with poor soil mobility (eg. zinc and copper) (Hayman, 1983; Brady, 1984; Gianinazzi-Pearson and Gianinazzi, 1983). Enhanced nutrient uptake by mycorrhizal plants is facilitated by external hyphae that grow beyond the nutrient depletion zone surrounding roots (Brady, 1984). In many cases, plant growth response to a particular VAM species depends upon both plant species and cultivar (Jakobsen *et al.*, 1992; Schubert and Hayman, 1986; Thomson *et al.*, 1990; Bryla and Koide, 1990). Other possible benefits to the plant afforded by VAM colonization include increased tolerance of roots to soil-borne pathogens (Gianinazzi-Pearson and Gianinazzi, 1983; Heald *et al.*, 1989), highly acidic soils and drought stress (Raven *et al.*, 1986; Davies *et al.*, 1992), and increased protection from salt stress (Rosendahl and Rosendahl, 1991).

European seedless cucumbers (*Cucumis sativus* L.) are grown under greenhouse conditions, often in soilless culture systems. Thus, the precise control of nutrients is extremely important to the establishment and growth of a marketable cucumber crop (Mirza, 1990). In commercial production, cucumbers are transplanted at the fourth to fifth leaf stage to the chosen growth medium, where they remain for the rest of the cropping season (Mirza, 1990; Adamson and Maas, 1981). A high percentage of good-quality transplants is desirable to facilitate sequence cropping (up to 6 crops per year), which requires several sowings to maintain constant fruit production (Adamson and Maas, 1981).

Phosphorus is a major nutrient required by plants, being an integral component of adenosine nucleotides and nucleic acids. Thus, P deficiency, which can be quite common, is very detrimental to the plant (Brady, 1984). Symptoms of P deficiency include plant stunting due to restricted cell division, cell expansion, photosynthesis and respiration, death of older leaves, accumulation of anthocyanin pigments, impaired uptake and transport of other nutrients such as nitrate, and delayed plant maturity (Salisbury and Ross, 1985; Fredeen *et al.*, 1989; Rufty *et al.*, 1990). Cucumber plants under P-deficient conditions show symptoms of plant stunting and, in more severe cases, desiccation of older leaves (Roorda van Eysinga and Smilde, 1981; Mirza, 1990). Thus, P deficiency in a young cucumber crop will have drastic effects upon the rate of growth and, therefore, the time to fruit production.

Addition of VAM fungi to cucumber plants during the initial stages of growth may be beneficial for producing faster growing transplants that are vigorous (due to improved P nutrition, root pathogen resistance, etc.) and uniform in size, to enable more efficient sequence cropping throughout the year. Lack of literature on the value of VAM in greenhouse cucumber production is surprising, given the importance of this crop to the greenhouse vegetable industry and our knowledge of the growth enhancing effects of VAM in general. The potential use of VAM to enhance growth of greenhouse cucumber from seeding through fruit production thus warrants investigation. The objective of this study was to characterize the relationship between P nutrition and VAM-enhanced growth during plant establishment. The effects of three species of VAM fungi and three levels of P nutrition on growth and mineral nutrient (P, soluble nitrogen) accumulation in cucumber plants were characterized at 7-day intervals from 24 to 38 days after planting (DAP). This time course covered the growth period from the second to the tenth true leaf stage, where exponential growth began.

MATERIALS AND METHODS

Inocula Preparation. Inocula for control and VAM treatments consisted of a mixture of soil and roots from two-month-old clover (*Trifolium repens* L., cv. Altaswede) plants infected with

Glomus mosseae [Nicol. and Gerd.] Gerdemann and Trappe, *G. dimorphicum* Boyetchko and Tewari, or *G. intraradices* Schenck and Smith, and non mycorrhizal (control) clover grown in an autoclaved sand:soil (3:1 v/v) medium. *G. dimorphicum* was originally obtained as reported by Boyetchko and Tewari (1986). *G. mosseae* and *G. intraradices* were originally obtained from the International Culture Collection of VA Mycorrhizal Fungi at the University of Florida, Gainesville. Plants were grown in 6 inch (1.3 L) plastic pots and were fertilized sequentially with 100 ml each of Peters 15-0-15 (1 g/l) and 15-15-15 (1 g/l) over the two-month interval. Percent infection of the clover pot culture was determined at the time of seeding. To measure percent infection level, a washed root sample from each pot culture was stored in FAA (5% formalin, 5% acetic acid, 45% ethanol) until clearing and staining (Phillips and Hayman, 1970). Percent infection of the roots was determined using the gridline intersect method (Giovanetti and Mosse, 1980) on 4 slides per sample. *G. mosseae* pot culture was 57% infected, *G. dimorphicum* culture was 47% infected, and *G. intraradices* culture was 56% infected. In addition, spore counts were made on 20 g soil/root samples from each of the pot cultures. The root and soil samples were washed through several sieves and spores were collected on 150 µm and smaller sieves. Spores and other debris collected on these sieves were placed in a Waring blender to separate spores from any soil or hyphae. The spores were floated in water and then collected on filter paper to be counted. *G. dimorphicum* and *G. intraradices* pot culture averaged 75 to 100 spores per 20 g sample, while *G. mosseae* averaged 50 spores per 20 g sample. Cucumber pots were inoculated with 125 g of pot culture (clover roots and soil). This quantity of VAM provided enough infection potential to offset the slight differences in pot culture infection level and spore number. The clover pot culture was provided by one pot of clover per VAM species.

Plant Growth. Seeds of greenhouse cucumbers (*Cucumis sativus* L., cv. Corona) were directly seeded into 1.3 L plastic pots (2 seeds/pot) containing an autoclaved mixture (2:2:1 v/v/v) of coarse sand, fine sand, and loam (6 ppm P in 1:1 (v/v) water/medium extract). At seeding, roots and soil from the clover pot culture (125 g) were placed 2.5 cm below the soil surface. Seeds were sown 1.5 cm deep. The three P fertilization treatments (4, 12, or 20 ppm P) were applied three

times per week through an automated fertilization system (Harrow Fertigation Manager, Labbate Control Systems, Leamington, ON), along with other nutrients (pH 6.0) at levels sufficient to support the initial stages of growth (100 ppm NO_3-N, 10 ppm NH_4-N, 150 ppm K, 80 ppm Ca, 60 ppm Mg, 400 ppm (max) S, 0.850 ppm Fe, 0.550 ppm Mn, 0.165 ppm Zn, 0.201 ppm B, 0.102 ppm Cu, and 0.072 ppm Mo) (Adamson and Maas, 1981). Nutrient additions were started at 17 DAP. From 17 to 24 DAP, plants received 1.61, 4.82, or 8.04 mg P/week (134 ml/pot/fertigation). This was increased to 3.22, 9.65, or 16.08 mg P/week for the rest of the experimental period (268 ml/pot/fertigation). Plants were watered on non-fertigation days by hand. Plants were maintained under long-day conditions (16 hrs supplemental light from HID lamps with high pressure sodium bulbs, Sylvania 400 watt) and temperatures of 21 to 25 ^{o}C in the greenhouse. A thrips predator, *Amblesius cucumeris*, was applied twice over the 38 day growth period for biological control of western flower thrips (*Frankliniella occidentalis*).

The experiment was constructed in a randomized complete block design with three replications of all combinations of four inoculation treatments (three VAM species plus a nonmycorrhizal control), three concentrations of P and three harvest dates (24, 31, and 38 DAP). The experiment, depicting the trickle irrigation system and the plants at 21 days from seeding, is shown in Figure II-1. At each harvest, leaf area was measured using a LI-3100 Area Meter (LiCor Inc., Lincoln, Nebraska) and the leaves and stem of each plant were frozen at -20 ^{o}C. Soil was washed from the roots and a small sample of roots was stored in FAA solution for determination of infection level as described above. The rest of the root mass was frozen. The frozen plant material was lyophilized and dry weights of each component were determined. The dried plant materials were ground with a Wiley mill through a 40 mesh screen and stored for further analysis. Growth indices (LA/LF, LAR, SLA, Rw/W, Sw/W, Lw/W) were calculated from component dry weights and leaf areas. Growth and physiological data were subjected to ANOVA and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Based on the ANOVA results, regression analysis was used to

Figure II-1. 'Corona" cucumber plants (VAM-infected and noninfected) at 21 DAP. Plants were grown in 1.3 L pots (two plants per pot) and each pot received P and other nutrients via a trickle irrigation system (note tubing leading to each of the pots). The pots were arranged in a randomized complete block design. Treatments consisted of three levels of phosphorus nutrition and three VAM species plus control. The bran-like material apparent on some of the leaves was the medium in which thrips predator (*Ambleisus cucumeris*) was applied.


DO NOT
WATER

derive growth rates describing the various relationships. Only significant results are reported in this document.

Analytical Procedures. The lyophilized and ground leaves, stems and roots were analyzed for total P by ashing 50 to 100 mg of plant tissue overnight in a muffle furnace (550 ^{o}C), digesting the ash in 1 mL of HCl for 20 minutes, followed by the addition of 9 mL of 0.72 N H_2SO_4. The mixture was then centrifuged (1640 g, 20 ^{o}C) for 30 minutes to settle out any undigested components. Phosphorus was determined on a 200 µL aliquot of the supernatant by the methods of Serrano *et al.* (1976), using sodium phosphate as the standard. Soluble nitrogen (N) and carbohydrate pools were determined by grinding (mortar and pestle, 4 ^{o}C, 2 minutes) 50 mg of lyophilized leaves or roots in 5 mL of cold (4 ^{o}C) Hepes buffer (50 mM, pH 7.4). Stems were not analyzed due to a limited amount of dry matter in some treatments. The crude homogenate was centrifuged at 1640 g (4 ^{o}C) for 30 minutes, and the supernatant was analyzed for soluble carbohydrates and nitrogen. Soluble protein-N was determined using a BSA (15.6% N) standard and a 100 µL (leaf) or 200 µL (root) sample of the supernatant in a modified Lowry procedure (Bensadoun and Weinstein, 1976). Free amino-N was determined on a 50 µL sample of the supernatant using a leucine (10.7% N) standard in a ninhydrin assay (Rosen, 1956). Nitrate-N was assayed by the methods of Cataldo *et al.* (1975) with a KNO_3 standard and a 50 µL (leaf) or 100 µL (root) sample of the supernatant. Reducing sugars (RS) were measured using the methods of Nelson (1944) and Somogyi (1952) with a glucose standard and a 50 µL (leaf) or 100 µL (root) sample of the supernatant. Total soluble carbohydrate (TSC) determination followed the methods of Dubois *et al.* (1956) using a glucose standard and a 50 µL sample of the supernatant.

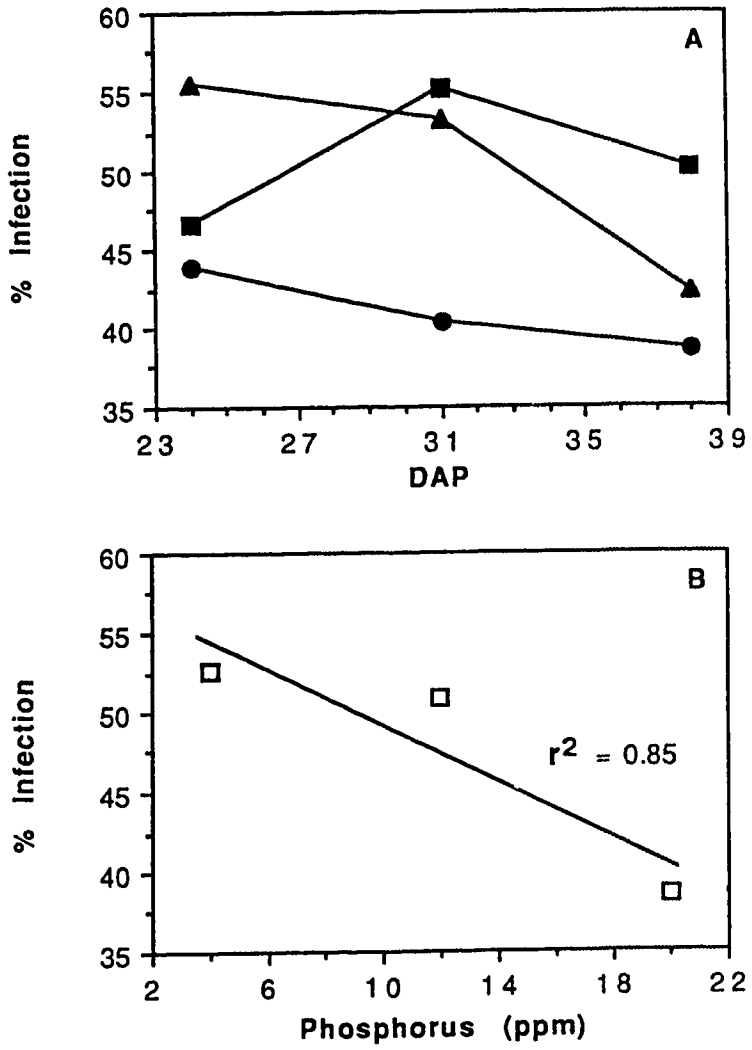
RESULTS

Root Infection. VAM infection became well established during the early stages of cucumber growth. Infection by the VAM species resulted in abundant hyphae, arbuscules, and vesicles, with some external spores developing by 38 DAP (Fig. II-2). Figure II-3(A) characterizes trends in infection levels of the three VAM species over the experimental period. By 38 DAP, *G*

Figure II-2. Bright-field microscopy of VAM fungal (*Glomus intraradices*) infection of 'Corona' cucumber roots at 38 DAP. (A) VAM spores (s) with extraradical hyphae (h). Note the spore wall layers characteristic of this particular VAM species (mag. 1,560x). (B) The appressorium (ap) initiates internal root infection, resulting in intraradical hyphae (h) and arbuscules (a) (mag. 1600x).


A
s
h
ap
a
h

Figure II-3. (A) Time course of the percent infection of cucumber roots with *G. mosseae* (■), *G. dimorphicum* (●), and *G. intraradices* (▲). Data was averaged for three P fertilization levels. F-values for the main effect of *G. dimorphicum* versus *G. intraradices*, and the interaction of *G. mosseae* versus the average of *G. dimorphicum* and *G. intraradices* x DAP_{linear}, were significant at the 0.01 and 0.05 levels, respectively. (B) Effect of P nutrition on percent infection of roots by VAM fungi. Data was averaged for the three VAM species and harvest dates. F-values for linear and deviations trends were significant at the 0.01 and 0.05 levels, respectively.


A
% Infection
60
55
50
45
40
35
23
27
31
35
39
DAP
B
% Infection
60
55
50
45
40
35
2
6
10
14
18
22
r^2 = 0.85
Phosphorus (ppm)

mosseae had established a percent infection level that was 19% higher than *G. intraradices* and 30% higher than *G. dimorphicum*. The general decline in percent infection by *G. dimorphicum* and *G. intraradices* with successive harvest date was most likely due to a higher root growth rate than fungal infection rate (Hayman, 1983). Increasing the level of P nutrition resulted in a linear decrease in percent infection by all VAM species (Fig II-3B). The linear model indicated that the percent infection declined by 1.8% for every 2 ppm increase in P nutrition ($P < 0.01$). Hence, plants grown on 20 ppm P were 35% less infected than those grown on 4 ppm P.

Plant Growth and Development. Increasing P fertilization level greatly enhanced the growth rates of cucumber plants over time. Root and stem dry weights were especially responsive to improved P nutrition, with high-P (20 ppm P) plants growing 84% and 86% faster, respectively, than low-P (4 ppm P) plants (Fig. II-4AB). Leaf dry weight also responded to P level, with high-P plants growing 31% faster than low-P plants (Fig. II-4C).

VAM infection significantly influenced the absolute growth rates of the primary plant yield components over the experimental period (Table II-1). Roots of control (nonmycorrhizal) and *G. mosseae*-infected plants grew 9% faster than roots of *G. dimorphicum* and *G. intraradices*-infected plants. Control plants, however, had 18% and 11% lower stem and leaf growth rates, respectively, than VAM-infected plants. Thus, it would appear that control plants partition more of their nutritional resources toward root growth in order to more effectively scavenge the soil for available P. The lower root dry weights of *G. dimorphicum* and *G. intraradices*-infected plants (699 mg and 681 mg at 38 DAP, respectively), as compared with control plants (722 mg at 38 DAP), may indicate that the VAM-infected plants were more efficient at P uptake, and therefore allocated less plant reserves for root growth and more toward foliar development. *G. mosseae*-infected plants had higher root dry weights (780 mg at 38 DAP) than all other plants in the experiment. In addition, *G. mosseae*-infected plants had the highest shoot and root growth rates (Table II-1). *G. dimorphicum*-infected plants had almost the same shoot growth rates as plants infected with *G. mosseae*, yet *G. dimorphicum*-infected plants had a much lower root growth rate and a significantly lower percent infection. It therefore appears that *G. dimorphicum* may be more efficient at

Figure II-4. Effect of P nutrition on the rates of dry matter accumulation in cucumber roots (A), stems (B), and leaves (C). Data were averaged for VAM-infected and nonmycorrhizal plants. Linear regression coefficients and coefficients of determination for each level of P nutrition are shown in the insets. For dry matter accumulation of roots, F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interaction of $[P]_{linear}$ x DAP_{linear} were all significant at the 0.01 level. For dry matter accumulation of stems , F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interactions $[P]_{linear}$ x DAP_{linear} and $[P]_{linear}$ x $DAP_{deviations}$ were all significant at the 0.01 level. For dry matter accumulation of leaves, F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interaction of $[P]_{linear}$ x DAP_{linear} were all significant at the 0.01 level.

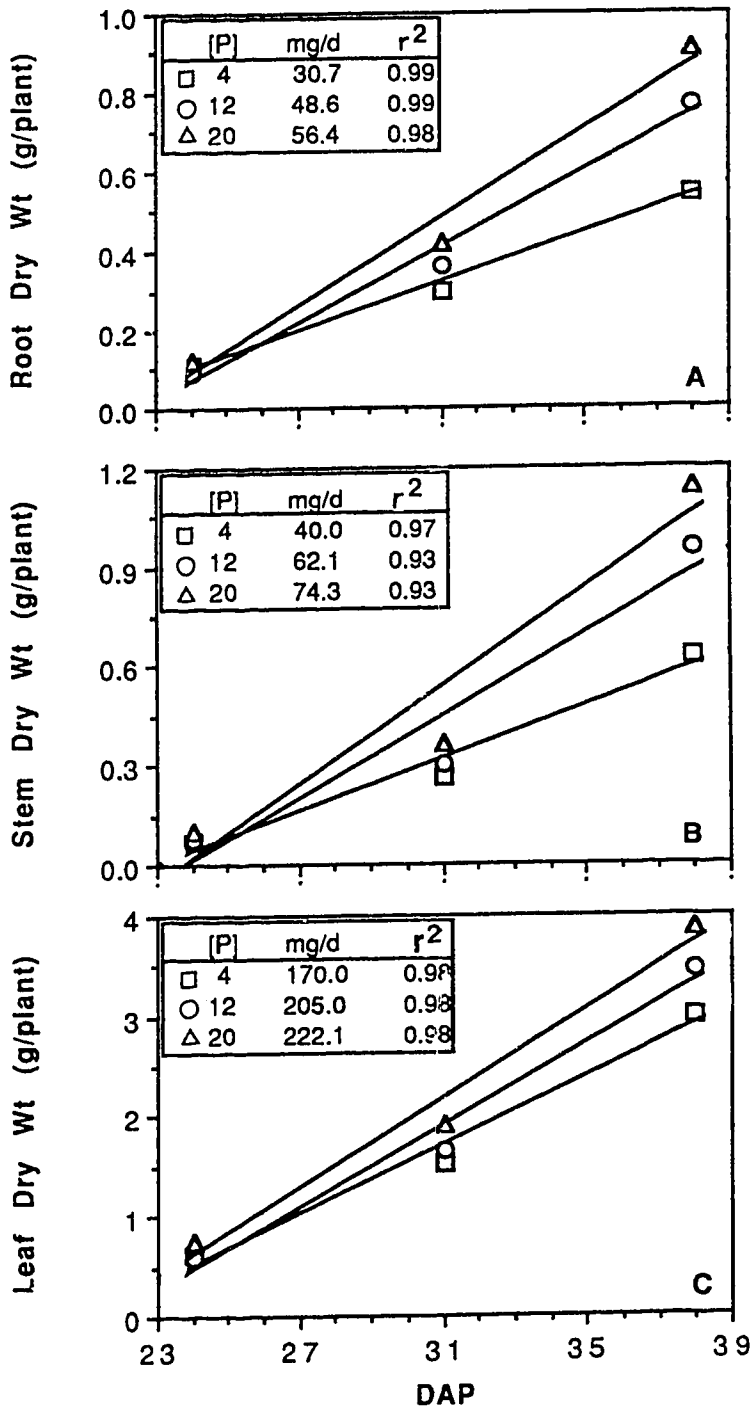

Root Dry Wt (g/plant)
[P] mg/d r2
4 30.7 0.99
12 48.6 0.99
20 56.4 0.98
A
Stem Dry Wt (g/plant)
[P] mg/d r2
4 40.0 0.97
12 62.1 0.93
20 74.3 0.93
B
Leaf Dry Wt (g/plant)
[P] mg/d r2
4 170.0 0.98
12 205.0 0.98
20 222.1 0.98
C
DAP

Table II-1: Absolute growth rates of primary yield components of cucumber plants as affected by infection by three species of VAM fungi. Data was averaged over three P levels and regressed against time from 24 to 38 DAP. Values are the linear regression coefficients and the coefficients of determination.

	Dry Weights								Leaf Area	
	Root		Stem		Leaf		Plant			
Treatment	mg d^{-1}	r^2	mg d^{-1}	r^2	mg d^{-1}	r^2	mg d^{-1}	r^2	cm^2 d^{-1}	r^2
Control (C)	46.5	0.98	50.3	0.95	181.4	0.97	278.1	0.97	52.5	0.99
G. mosseae (M)	47.9	0.99	65.0	0.94	219.1	0.98	338.2	0.97	60.6	0.99
G. dimorphicum (D)	42.8	0.99	63.1	0.94	208.6	0.99	314.6	0.98	61.0	0.99
G. intraradices (I)	43.8	0.98	56.4	0.91	187.0	0.97	286.8	0.96	59.0	0.99
C/MDI x DAP [a]	ns [b]		0.01		0.05		0.05		0.01	
M/DI x DAP	0.01		----		----		0.05		ns	
I/MD x DAP	----		ns		0.05		----		----	
D/I x DAP	ns		----		----		ns		0.05	
M/D x DAP	----		ns		ns		----		----	

[a] sources of variation (DAP, days after planting)

[b] significance levels for indicated sources of variation (ns, not significant)

enhancing P uptake than *G. mosseae*. *G. intraradices* had the lowest growth-enhancing ability of the VAM species, despite an intermediate infection level (Fig. II-3, Table II-1).

All VAM species stimulated plant dry matter accumulation relative to control (nonmycorrhizal) plants. The VAM-enhanced growth was visually apparent prior to the first harvest date (24 DAP), as evident by *G. mosseae* and control plants at 21 DAP depicted in Figure II-5. By 38 DAP, *G. mosseae*-infected plants had the greatest stem and leaf dry weights (942 mg and 3.70 g, respectively), followed by *G. dimorphicum* (921 mg and 3.53 g, respectively) and *G. intraradices*-infected plants (804 mg and 3.14 g, respectively), all of which were significantly higher ($P < 0.05$) than control plants (715 mg and 2.91 g, respectively). Even though control plants produced more roots than VAM-infected plants, their foliar growth was limited, as evidenced by low total plant dry weight at each harvest and the slowest plant growth rate relative to VAM-infected plants (Table II-1). In addition, plant leaf area increased 15% faster in VAM-infected plants relative to controls.

The ratios of root, stem and leaf dry weights to total plant dry weight indicate how the plant partitions its resources in response to the various treatments. Since cucumber plants have an indeterminate growth habit and elongate rapidly after they are established, the ratios would be expected to change substantially with successive harvest date and improved P nutrition. Root wt/plant wt ratio (Rw/W) was not significantly affected by increasing P level, even though the rate of root dry weight accumulation increased with improved P nutrition (Figure II-4A). As a proportion of total plant dry weight, root dry weight increased from 24 DAP to 31 DAP and then decreased slightly to 38 DAP (Fig. II-6A). Thus, initially, root establishment was a priority for the plant, and then shoot growth began to comprise a greater proportion of total plant weight when averaged over the growth interval. Rw/W of control (nonmycorrhizal) plants was significantly higher (18%) than that for VAM plants (Table II-2). Moreover, plants infected with *G. mosseae* partitioned significantly more plant dry matter into roots than those infected with *G. dimorphicum* and *G. intraradices*. Stem wt/plant wt ratio (Sw/W) increased with improved P nutrition and harvest date (Fig. II-6B). Plants grown with high-P (20 ppm P) and medium-P (12 ppm P) fertilization levels

Figure II-5. Growth response of 'Corona' cucumber plants to infection with *Glomus mosseae*. Pots were inoculated at seeding with G. mosseae (m) or control (c) (nonmycorrhizal) clover pot culture. The plants (two plants per pot) were grown for 21 days with the lowest level of supplemental P (4 ppm).


m
c

Figure II-6. (A) Main effect of time on cucumber root dry weight ratio (Rw/W), expressed as a fraction of total plant dry weight. Data is the average of P fertilization levels and VAM inoculation treatments. F-values for the main effects of DAP_{linear} and $DAP_{deviations}$ were significant at the 0.01 level. The effects of P nutrition on the rates of change in stem dry weight ratio (Sw/W) and leaf dry weight ratio (Lw/W) are shown in (B) and (C), respectively. Data was averaged for VAM-infected and nonmycorrhizal plants. Linear regression coefficients and coefficients of determination for each level of P nutrition appear in the insets. For Sw/W, F-values for the main effects of $[P]_{linear}$, $[P]_{deviations}$, DAP_{linear}, $DAP_{deviations}$, and the interactions $[P]_{linear} \times DAP_{linear}$, $[P]_{linear} \times DAP_{deviations}$ and $[P]_{deviations} \times DAP_{deviations}$, were all significant at the 0.01 level. For Lw/W, F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interactions $[P]_{linear} \times DAP_{linear}$ and $[P]_{deviations} \times DAP_{linear}$, were significant at the 0.01, 0.01, 0.05, 0.01, and 0.01 levels, respectively.

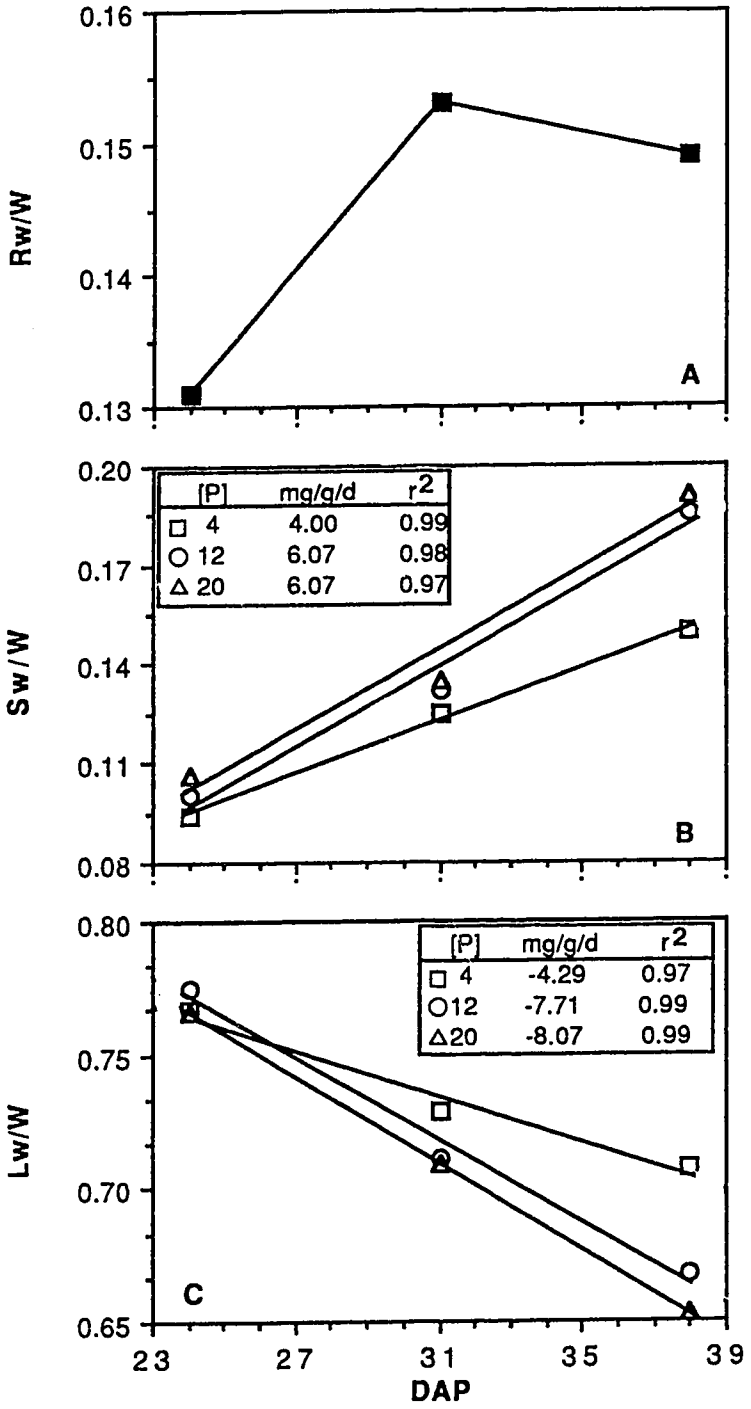

0.16
0.15
0.14
0.13
Rw/W
A
0.20
0.17
0.14
0.11
0.08
Sw/W
[P] mg/g/d r2
□ 4 4.00 0.99
○ 12 6.07 0.98
△ 20 6.07 0.97
B
0.80
0.75
0.70
0.65
Lw/W
[P] mg/g/d r2
□ 4 -4.29 0.97
○ 12 -7.71 0.99
△ 20 -8.07 0.99
C
23
27
31
35
39
DAP

Table II-2. Root (Rw/W), stem (Sw/W) and leaf (Lw/W) dry weight ratios of cucumber plants as affected by infection by three species of VAM fungi. Ratios (g g^{-1}) were averaged over three P levels and three harvest dates (24 to 38 DAP). The rate of change in Lw/W (mg g^{-1} d^{-1}) is the regression coefficient obtained by plotting Lw/W (averaged over P level) against time.

Treatment	Rw/W	Sw/W	Lw/W	
				mg g^{-1} d^{-1}
Control (C)	0.163	0.131	0.707	-5.93
G. mosseae (M)	0.145	0.135	0.720	-7.00
G. dimorphicum (D)	0.133	0.142	0.725	-6.07
G. intraradices (I)	0.136	0.133	0.730	-7.79
C/MDI [a]	0.01[b]	0.01	0.01	—
M/DI	0.01	ns	0.05	—
D/I	ns	0.01	ns	—
C/MDI x DAP_{lin}	—	—	—	0.05
M/DI x DAP_{lin}	—	—	—	ns
D/I x DAP_{lin}	—	—	—	0.05

[a] sources of variation (lin, linear trend)

[b] significance levels for indicated sources of variation (ns, not significant)

partitioned dry matter to stems at a rate 52% higher than low-P (4 ppm P) plants. Low-P conditions thus inhibited the rate of stem growth and elongation. Control plants partitioned significantly less dry weight to stems (Sw/W) than VAM plants (Table II-2). The increased Sw/W ratio of VAM plants may have been due to improved P nutrition provided by the symbiosis. Leaf wt/plant wt ratio (Lw/W) decreased with time (Fig. II-6C), reflecting increased partitioning of dry matter to stems. Moreover, the partitioning of dry matter to leaves was affected by an interaction between P-nutrition and time. The Lw/W of high-P and medium-P plants declined 88% and 80% faster, respectively, than low-P plants. This occured because the partitioning of dry matter to stems was enhanced by increasing P nutrition, thus contributing to a greater proportion of the total plant dry weight over time. Control (nonmycorrhizal) plants had the lowest Lw/W, but this was due in general to greater partitioning of dry matter to roots rather than stems (Table II-2). Control plants also showed the slowest change in Lw/W ratio, indicating less partitioning of reserves toward leaf growth (Table II-2). VAM plants were visually much larger than control plants at all P levels, with *G. intraradices*-infected plants being the smallest of the VAM plants at 38 DAP (Fig. II-7).

VAM plants were more responsive to increased P nutrition than control plants in their rate of leaf production (Fig. II-8). Control plants maintained a relatively constant rate of leaf production (0.35 leaves/plant/day) regardless of P level, while the rate at which VAM plants produced leaves increased linearly with increasing P level. The rate of leaf production for VAM plants grown with 20 ppm P was 36% higher than for those grown with 4 ppm P.

Leaf area increased over time and the rate of increase depended on P fertilization level. Total leaf area of medium-P and high-P plants increased 37% and 50% faster, respectively, than that of low-P plants (Fig. II-9A). Moreover, total leaf area increased 15% faster in VAM-infected plants than in control plants (Table II-1). By 38 DAP, *G. dimorphicum*-infected plants had the greatest total leaf area (1147 cm^2), slightly higher than *G. mosseae* (1119 cm^2) and *G. intraradices*-infected plants (1060 cm^2), and significantly higher ($P < 0.01$) than control plants (912 cm^2). Leaf area per leaf (LA/LF) increased over time and the rate of increase depended on P level

Figure II-7. Growth response of 'Corona' cucumber plants to infection by *Glomus mosseae* (m), *G. dimorphicum* (d), and *G. intraradices* (i) at three levels of P nutrition. Pots were inoculated at seeding with VAM or control (c) clover pot culture. The plants (two plants per pot) were grown for 38 days with 4 ppm P (A), 12 ppm P (B) or 20 ppm P (C).

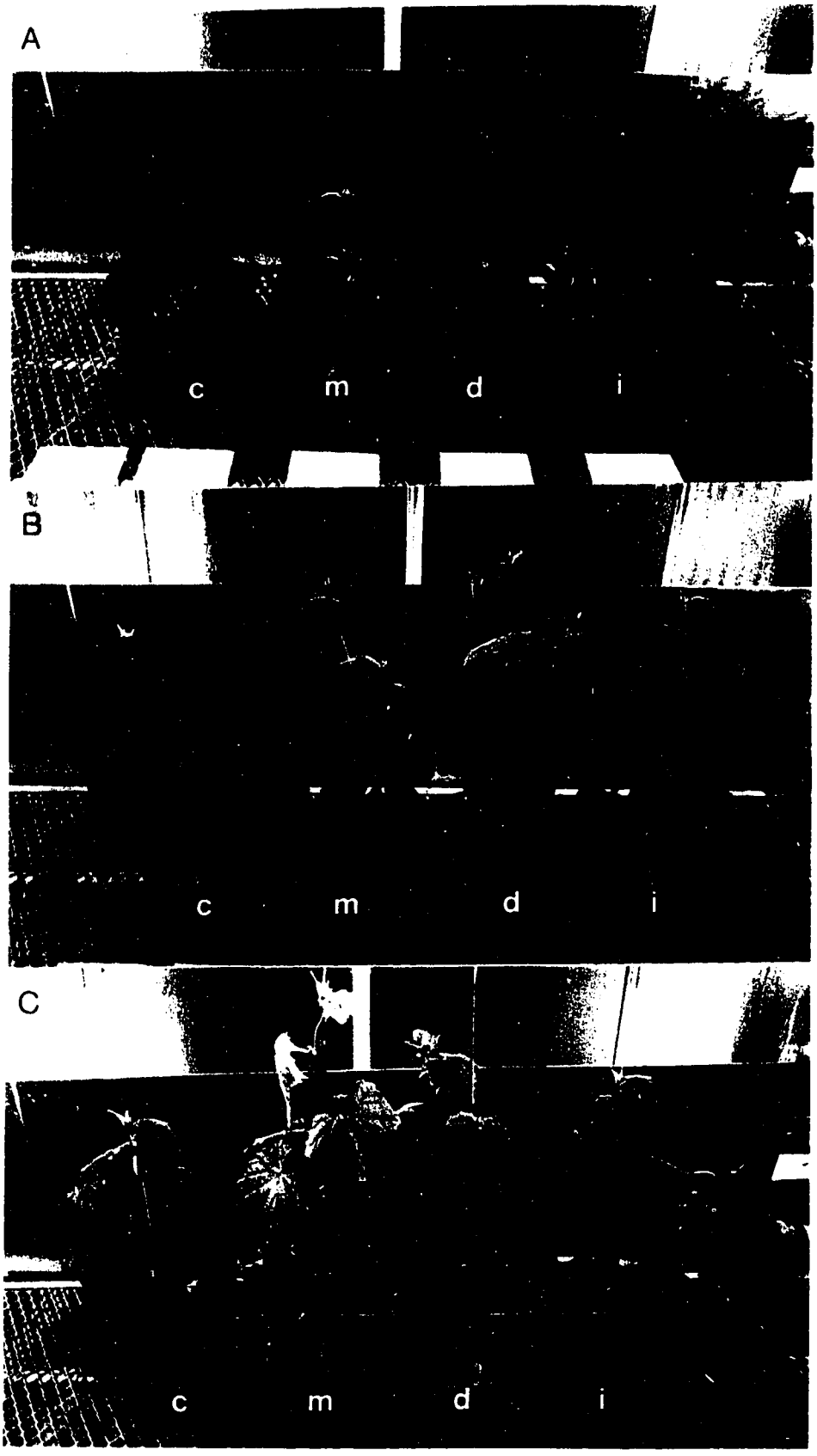

A
c
m
d
i
B
c
m
d
i
C
c
m
d
i

Figure II-8. Effect of P nutrition on the rate of leaf production of VAM-infected (●) and noninfected (□) 'Corona' cucumber plants. Leaf production rates are the linear regression coefficients obtained by plotting leaf number per plant versus time from 24 to 38 DAP (F-values for the linear trends with time were significant at the 0.01 level, and r = 0.99 for both VAM-infected and noninfected plants). F-values for the main effects of VAM and $[P]_{linear}$, and the interaction of VAM x $[P]_{linear}$ were significant at the 0.01 level.

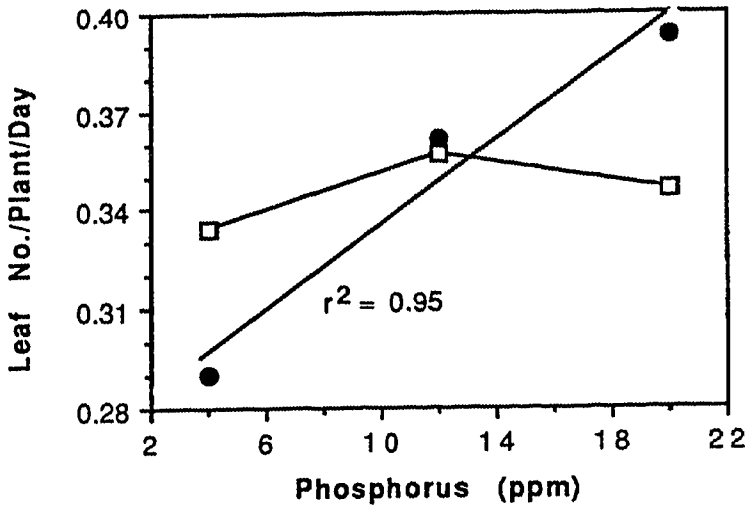
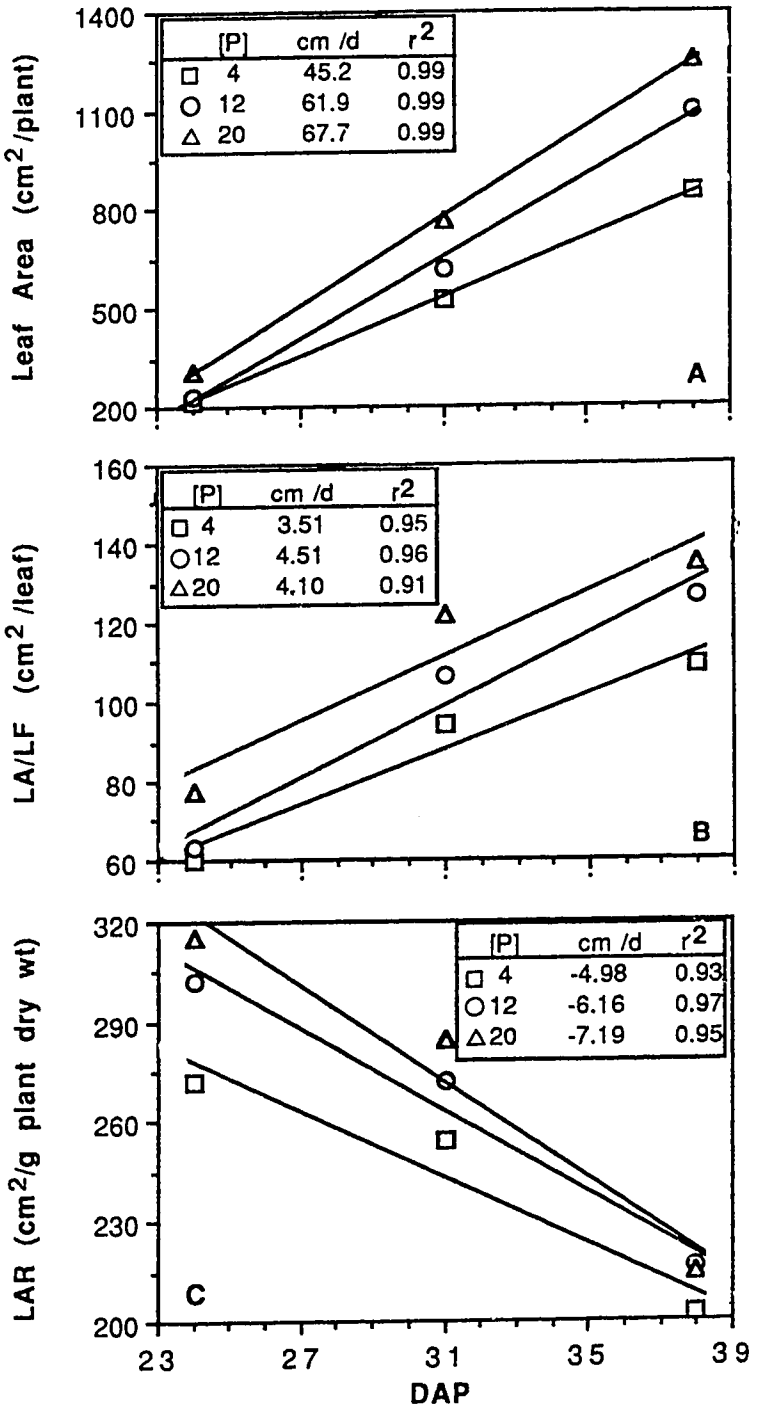

0.40
0.37
0.34
0.31
0.28
Leaf No./Plant/Day
r2 = 0.95
2
6
10
14
18
22
Phosphorus (ppm)

Figure II-9. Effect of P nutrition on the rates of change in leaf area indices during growth of 'Corona' cucumber plants. Data was averaged for VAM-infected and nonmycorrhizal plants. Linear regression coefficients and coefficients of determination for each level of P nutrition are shown in the insets. For leaf area accumulation (A), F-values for the main effects of $[P]_{linear}$ and DAP_{linear}, and the interactions $[P]_{linear} \times DAP_{linear}$ and $[P]_{deviations} \times DAP_{linear}$, were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively. For leaf area per leaf (B), F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interaction of $[P]_{deviations} \times DAP_{linear}$, were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively. For leaf area ratio (C), F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interaction of $[P]_{linear} \times DAP_{linear}$, were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively.


Leaf Area (cm2/plant)
1400
1100
800
500
200
[P] cm /d r2
4 45.2 0.99
12 61.9 0.99
20 67.7 0.99
A
LA/LF (cm2 /leaf)
160
140
120
100
80
60
[P] cm /d r2
4 3.51 0.95
12 4.51 0.96
20 4.10 0.91
B
LAR (cm2/g plant dry wt)
320
290
260
230
200
[P] cm /d r2
4 -4.98 0.93
12 -6.16 0.97
20 -7.19 0.95
C
23
27
31
35
39
DAP

(Fig. II-9B). Leaves of medium-P and high-P plants expanded 28% and 17% faster, respectively, than leaves of low-P plants. Despite the fact that control plants had fewer leaves than VAM plants (averaged over P level and time), they also had significantly smaller leaves relative to VAM plants, as indicated by a 16% lower leaf area per leaf (Table II-3). Thus, VAM infection and increased P nutrition influenced total leaf area to a greater extent than leaf number. Leaf area ratio (LAR) decreased with time and the rate of decrease depended on P nutrition. At 24 DAP, the LAR of medium-P and high-P plants was 7% and 43% higher, respectively, than that of low-P plants; however, LAR of medium-P and high-P plants decreased 24% and 44% faster, respectively, than that of the low-P plants (Fig. II-9C) over the harvest intervals. This was probably due to greater partitioning of dry matter to stems in plants grown with higher P levels. On average, control plants had a significantly lower LAR than VAM plants, and *G. mosseae*-infected plants had a significantly lower LAR than those infected with *G. dimorphicum* and *G. intraradices* (Table II-3). Control and *G. mosseae*-infected plants also had lower specific leaf areas (SLA) than *G. dimorphicum* and *G. intraradices*-infected plants (Table II-3), with *G. mosseae* being significantly lower. SLA decreased with harvest date (SLA = -5.14 (DAP) + 518.2, $r^2 = 0.83$, $P < 0.01$) and increased with increasing P level (SLA = 3.14 [P] + 321.1, $r^2 = 0.94$, $P < 0.01$). Thus, as a fraction of plant and leaf dry weight, control and *G. mosseae*-infected plants had much less leaf area than the other VAM-infected plants (ie. restricted leaf expansion). Since increasing P nutrition greatly enhanced leaf area and plant dry weight components (Figs. II-9 and II-4), control (nonmycorrhizal) plants were probably under some degree of P stress compared with VAM plants at comparable levels of P nutrition. This is supported by the fact that no VAM inoculum by P concentration interactions were apparent for most of the growth parameters measured (except leaf number). Growth enhancement caused by VAM infection thus appeared to be due to enhanced ability of mycorrhizal plants to extract more P for growth. The additional P afforded by the VAM infection (see below) most likely enabled the young cucumber plants to quickly establish an efficient root system leading to rapid shoot growth.

Table II-3. Growth indices of cucumber plants as affected by infection by three species of VAM fungi. Data were averaged over three harvest dates (24 to 38 DAP) and three P levels.

Treatment	LA/LF[a] cm^2 $leaf^{-1}$	LAR cm^2 g^{-1}	SLA cm^2 g^{-1} leaf dry wt
Control (C)	89.0	250.5	353.4
G. mosseae (M)	105.3	247.4	342.7
G. dimorphicum (D)	107.4	269.5	370.6
G. intraradices (I)	96.1	269.7	368.6
C/MDI[b]	0.01[c]	0.05	ns
M/DI	ns	0.01	0.01
D/I	0.01	ns	ns

[a] LA/LF, leaf area per leaf, LAR, leaf area ratio, SLA, specific leaf area

[b] sources of variation

[c] significance levels for indicated sources of variation (ns, not significant)

Plant phosphorus utilization. The partitioning of total plant P among roots, stem and leaves of the young cucumber plants was greatly influenced by P nutrition and time. While high-P (20 ppm P) plants contained 74% more total plant P (7.5 mg) than low-P (4 ppm P) plants (4.3 mg) at 24 DAP, the partitioning of this P among yield components was similar (Fig. II-10). The proportion of plant P allocated to roots remained relatively constant with time, although on average, roots of low-P plants accounted for 2.4% more plant P than roots of high-P plants throughout the study. While leaf P accounted for the greatest percentage of total plant P, it declined faster with time in high-P plants than in low-P plants. In high-P plants, leaf P fell by 16.4% of total plant P from 24 to 38 DAP. In low-P plants, leaf P only declined by 8.3% as a fraction of total plant P over the same time interval. As a percent of total plant P, the decline in leaf P occured with a concomitant increase in stem P. Moreover, the percentage of plant P allocated to stem tissue increased 66% faster in high-P plants (1.18%/day, r^2 = 0.97) than in low-P plants (0.71%/day, r^2 = 0.99). High-P plants contained 140% more total P (38.9 mg) than low-P plants (16.2 mg) at 38 DAP, and the increased rate of allocation of P to the stem reflected the rapid stem growth rate with increasing P fertilization level (Fig. II-4). As expected, the allocation of total P among yield components over the experimental period was similar to the partitioning of total plant dry matter among yield components (Fig. II-6). VAM-infected plants accumulated significantly more total P in roots (2.76 mg), stem (3.61 mg), and leaves (10.60 mg) than control roots (2.29 mg), stem (3.07 mg) and leaves (8.79 mg) ($P< 0.05$). Significant main effects of VAM and P level on total P content of each yield component, along with the absence of significant interactions between P level and VAM, indicates that VAM partially ameliorated the effect of low-P on reduced P accumulation.

On average, the concentration of leaf P in *G. mosseae*-infected plants (5.36 mg g^{-1} dry wt) was 7% lower ($P< 0.05$) than that of the other VAM-infected plants and control combined (5.77 mg g^{-1} dry wt). Leaf P concentration decreased with time and increased with improved P nutrition (Table II-4). Moreover, P nutrition interacted with harvest date to affect the rate of decrease in leaf P concentration. Leaf P concentration of plants grown with 12 ppm P and 20 ppm P decreased

Figure II-10. Partitioning of total plant P between roots (stippled area), stems (striped area) and leaves (clear area) of 'Corona' cucumber plants over time as affected by P nutrition. Data is expressed as the percentage of total plant P allocated to each yield component and is the average of VAM-infected and nonmycorrhizal plants. For clarity, only the low and high P nutrition levels are shown; however, data was analyzed over all three P levels (including 12 ppm P). For roots, F-values for the main effects of $[P]_{linear}$, $DAP_{deviations}$, and the interaction of $[P]_{deviations}$ x DAP_{linear}, were significant at the 0.01, 0.01 and 0.05 levels, respectively. For the stem, F-values for the main effects of $[P]_{linear}$, DAP_{linear}, $DAP_{deviations}$, and the interaction of $[P]_{linear}$ x DAP_{linear}, were all significant at the 0.01 level. For leaves, F-values for the main effects of $[P]_{linear}$, DAP_{linear}, and the interactions of $[P]_{linear}$ x DAP_{linear} and $[P]_{deviations}$ x DAP_{linear}, were all significant at the 0.01 level.

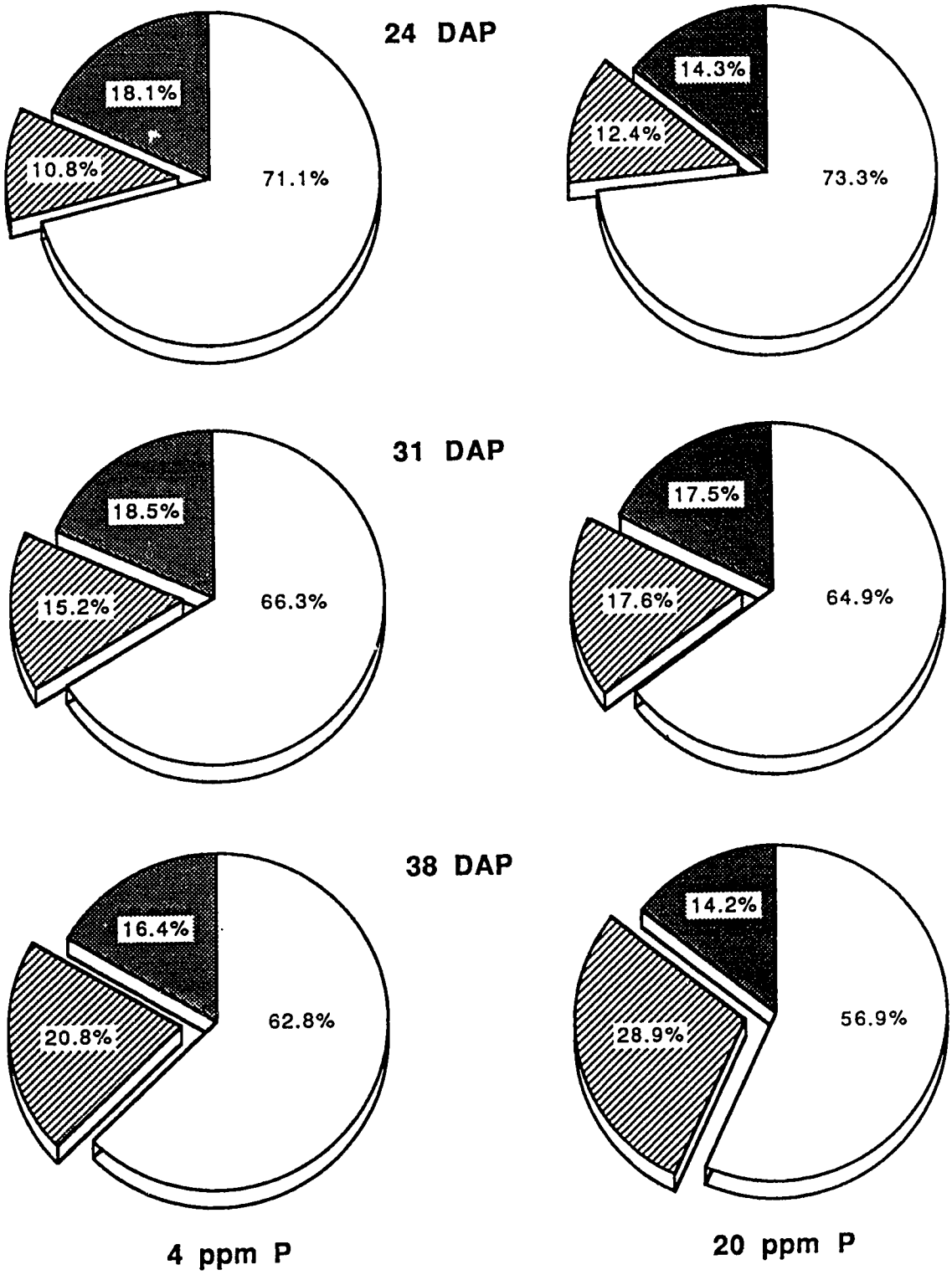

24 DAP
18.1%
10.8%
71.1%
14.3%
12.4%
73.3%
31 DAP
18.5%
15.2%
66.3%
17.5%
17.6%
64.9%
38 DAP
16.4%
20.8%
62.8%
14.2%
28.9%
56.9%
4 ppm P
20 ppm P

Table II-4. Effect of P nutrition on the concentration of P in leaf, stem and root tissue during growth of young cucumber plants. Data is the average for nonmycorrhizal and VAM-infected plants.

Treatment		Leaf	Stem	Root
[P]	DAP		mg P g^{-1} dwt	
4 ppm P	24	4.90	6.01	7.25
	31	4.46	5.66	6.16
	38	3.43	5.31	4.89
12 ppm P	24	6.97	8.00	8.33
	31	6.22	8.70	7.82
	38	4.59	7.07	5.99
20 ppm P	24	7.38	8.89	8.80
	31	7.32	10.41	8.92
	38	5.77	9.94	6.13
$[P]_{lin}$[a]		0.01[b]	0.01	0.01
$[P]_{dev}$		0.01	ns	0.05
DAP_{lin}		0.01	ns	0.01
DAP_{dev}		0.01	0.05	0.01
$[P]_{lin}$ x DAP_{lin}		ns	0.05	ns
$[P]_{lin}$ x DAP_{dev}		0.05	ns	ns
$[P]_{dev}$ x DAP_{lin}		ns	ns	0.01
$[P]_{dev}$ x DAP_{dev}		ns	ns	ns

[a] sources of variation

[b] significance levels for indicated sources of variation (lin, linear trend; dev, deviations from linearity; ns, not significant)

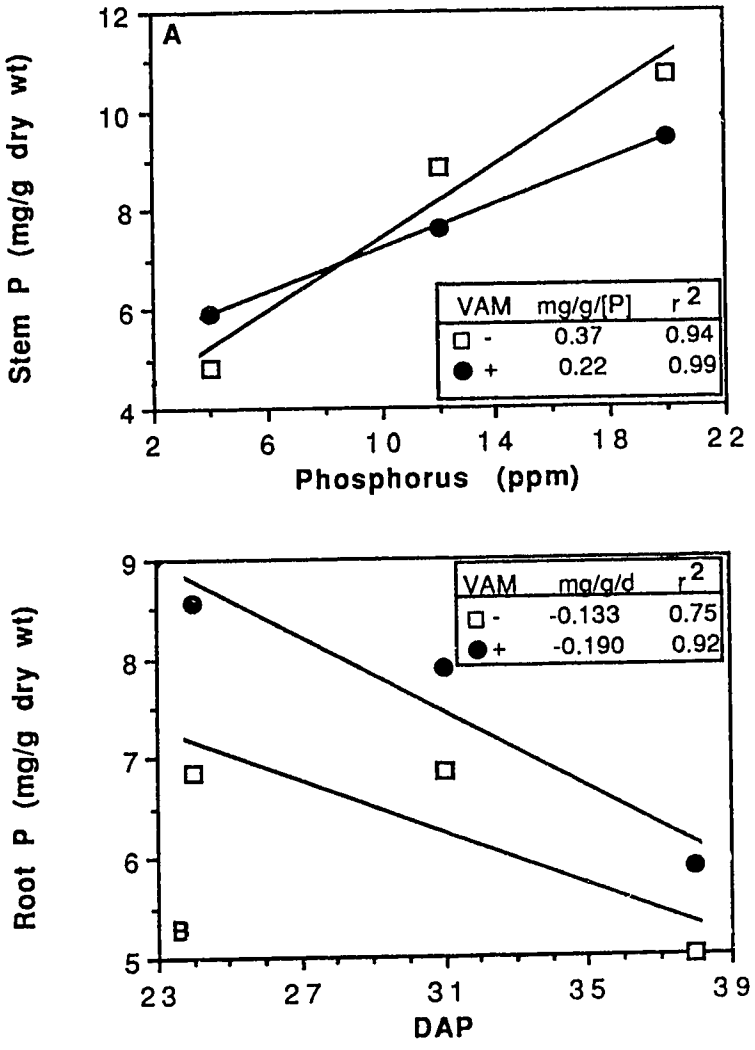
62% (170 μg g^{-1} dry wt d^{-1}) and 10 % (115 μg g^{-1} dry wt d^{-1}) faster, respectively, compared with plants grown with 4 ppm P (105 μg g^{-1} dry wt d^{-1}).

For every ppm increase in available P, stem P of control (nonmycorrhizal) plants increased by 370 μg g^{-1} dry wt, compared with only 220 μg g^{-1} dry wt for stem tissue of VAM-infected plants (Fig. II-11A). Stem P concentration was also affected by an interaction between P nutrition and time (Table II-4). At 4 ppm P, stem P concentration declined linearly by 50 μg g^{-1} dry wt d^{-1}. At 12 ppm and 20 ppm P, stem P concentration increased 9 % and 17 %, respectively, from 24 to 31 DAP, and then declined. Harvest date, P nutrition and VAM infection interacted (DAP_{linear} x $P_{deviations}$ x C/MDI, $P<0.05$) such that control plants had a higher concentration of stem P than VAM plants (except when grown with 4 ppm P), and differences in the rates of increase in stem P with improved P nutrition decreased with successive harvest date (data not shown).

When averaged over time and level of P-nutrition, the concentration of root P was 16% lower ($P< 0.01$) in nonmycorrhizal plants (6.25 mg g^{-1} dry wt) than in plants infected with mycorrhizae (7.44 mg g^{-1} dry wt). Roots of plants infected with *G. intraradices* contained the highest concentration ($P< 0.01$) of root P (7.90 mg g^{-1} dry wt) among the VAM-infected plants (avg. 7.21 mg g^{-1} dry wt). Furthermore, the concentration of root P was affected by an interaction between VAM and time (Fig. II-11B). While roots of VAM-infected plants were 24% more concentrated in P (8.55 mg g^{-1} dry wt) than non-infected plants (6.87 mg g^{-1} dry wt) at 24 DAP, P concentration decreased 43% faster in the roots of VAM plants with further growth. Hence, by 38 DAP the difference in root P concentration between VAM and control plants had decreased to about 17%. The concentration of root P was also affected by an interaction between P nutrition and time (Table II-4). While root P concentration increased with increasing level of supplemental P (root mg P g^{-1} dry wt = 0.116 (ppmP) + 5.76, $r^2 = 0.96$, $P<0.01$), the rate of decrease with time was 14% greater at 20 ppm P (191 μg P g^{-1} dry wt d^{-1}), than at 4 ppm (169 μg P g^{-1} dry wt d^{-1}) or 12 ppm P (167 μg P g^{-1} dry wt d^{-1}).

Plant nitrogen utilization. The concentrations of nitrate-nitrogen (N), free amino-N and soluble protein-N decreased in leaf and root tissues from 24 to 38 DAP, and P nutrition

Figure II-11. (A) Effect of P nutrition on the concentration of P in stems of VAM-infected and noninfected 'Corona' cucumber plants. Data has been averaged for three VAM species (*G. mosseae*, *G. dimorphicum* and *G. intraradices*) and three harvest dates (24 to 38 DAP). Linear regression coefficients and coefficients of determination appear in the inset. F-values for the main effect of $[P]_{linear}$ and the interaction of VAM x $[P]_{linear}$ were both significant at the 0.01 level. (B) Change in P concentration of roots of VAM-infected and noninfected plants from 24 to 38 DAP. Data has been averaged for the three VAM species (as above) and three levels of P nutrition (4 to 20 ppm P). Linear regression coefficients and coefficients of determination appear in the inset.. F-values for the main effects of VAM, DAP_{linear}, $DAP_{deviations}$, and the interaction of VAM x DAP_{linear}, were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively.


A
Stem P (mg/g dry wt)
12
10
8
6
4
2
6
10
14
18
22
Phosphorus (ppm)
VAM mg/g/[P] r 2
- 0.37 0.94
+ 0.22 0.99
B
Root P (mg/g dry wt)
9
8
7
6
5
23
27
31
35
39
DAP
VAM mg/g/d r 2
- -0.133 0.75
+ -0.190 0.92

affected the rates of decline (Table II-5). For plants grown with 4 ppm, 12 ppm and 20 ppm P, leaf nitrate-N concentration decreased at rates of 238, 419 and 345 μg g^{-1} dry wt d^{-1}, respectively. On average, leaf nitrate-N concentration also fell by 60 μg g^{-1} dry wt for every ppm increase in available P. The concentration of root nitrate-N also declined as available P increased (by 33 μg N g^{-1} dry wt for every ppm increase in P); however, unlike leaf nitrate-N, the rates of decline were not significantly affected by P level and averaged 134 μg N g^{-1} dry wt loss per day. In addition, the rates at which leaf (Fig. II-12A) and root (Fig. II-13A) nitrate-N concentrations declined with time were altered by VAM infection. Despite the fact that control (nonmycorrhizal) plants had 27% (leaf) and 101% (root) more nitrate-N than VAM-infected plants at 24 DAP, a 32% and 130% faster rate of decline in nitrate-N concentration in leaves and roots of control plants, respectively, resulted in concentrations equal to that of the VAM-infected plants by 38 DAP.

Similar to nitrate-N, amino-N concentrations of leaves and roots were inversely related to the level of P nutrition (Table II-5). For every ppm increase in available P, the concentration of amino-N in leaves and roots decreased by 43 and 19 μg g^{-1} dry wt, respectively. Leaf amino-N also decreased with time and the rate depended on level of P nutrition. The rates of decrease were 61, 119 and 92 μg amino-N g^{-1} dry wt d^{-1} in leaves of plants grown with 4, 12 and 20 ppm P, respectively. In addition, VAM influenced the rate of decline in leaf amino-N (Fig. II-12B). At 24 DAP, the concentration of leaf amino-N was 19% greater in control plants compared with VAM-infected plants; however, this difference in concentration had disappeared by 38 DAP, due to a 50% greater rate of decline in leaves of control plants. Amino-N concentration of roots remained relatively constant over time for plants grown with 4 ppm P, but decreased by 13 and 28 μg g^{-1} dry wt d^{-1} in plants grown with 12 and 20 ppm P, respectively (Table II-5). Thus, for every ppm increase in P nutrition, root amino-N concentration declined at a rate of 1.8 μg g^{-1} dry wt d^{-1} ($P<0.01$). The effect of VAM on the concentration of root amino-N depended on the level of P nutrition (Fig. II-13B). Relative to VAM-infected plants, a greater reduction in amino-N concentration in roots of control plants was evident as the level of P nutrition increased from 4

Table II-5. Effect of P nutrition on the concentrations of soluble nitrogen in leaf and root tissue during growth of young cucumber plants. Data is the average for nonmycorrhizal and VAM-infected plants.

Treatment		Leaf			Root		
[P]	DAP	Nitrate- N	Amino-N	Protein-N	Nitrate- N	Amino-N	Protein-N
		mg g^{-1} dry wt			mg g^{-1} dry wt		
4 ppm P	24	5.81	2.86	16.11	2.40	2.04	7.93
	31	3.73	2.29	10.80	1.56	2.17	7.76
	38	2.48	2.01	5.75	0.80	2.16	7.00
12 ppm P	24	7.17	2.78	17.96	2.27	1.98	8.82
	31	3.04	1.65	11.16	1.05	1.86	8.32
	38	1.30	1.11	4.97	0.34	1.80	6.92
20 ppm P	24	5.79	2.41	17.82	2.31	1.95	8.99
	31	2.40	1.56	11.16	0.65	1.92	8.59
	38	0.96	1.12	5.47	0.22	1.56	6.51
$[P]_{lin}$[a]		0.01[b]	0.01	ns	0.05	0.05	ns
$[P]_{dev}$		ns	0.01	ns	ns	ns	ns
DAP_{lin}		0.01	0.01	0.01	0.01	0.01	0.01
DAP_{dev}		0.01	0.01	ns	ns	ns	0.05
$[P]_{lin}$ x DAP_{lin}		0.05	0.05	0.05	ns	0.01	0.05
$[P]_{lin}$ x DAP_{dev}		0.01	0.01	0.05	ns	ns	ns
$[P]_{dev}$ x DAP_{lin}		ns	ns	ns	ns	ns	ns
$[P]_{dev}$ x DAP_{dev}		ns	ns	ns	ns	ns	ns

[a] sources of variation.

[b] significance levels for indicated sources of variation (lin, linear trend; dev, deviations from linearity; ns, not significant)

Figure II-12. Change in leaf nitrate (A), free amino (B) and soluble protein (C) nitrogen concentrations of VAM-infected and noninfected 'Corona' cucumber plants from 24 to 38 DAP. Data was averaged for three VAM species (*G. mosseae*, *G. dimorphicum*, and *G. intraradices*) and three levels of P nutrition (4 to 20 ppm P). Linear regression coefficients and coefficients of determination appear in the insets. For nitrate-N, F-values for the main effects of VAM, DAP_{linear}, $DAP_{deviations}$, and the interaction of VAM x DAP_{linear}, were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively. For amino-N, F-values for the main effects of VAM, DAP_{linear}, $DAP_{deviations}$, and the interaction of VAM x DAP_{linear}, were all significant at the 0.01 level. For protein-N, F-value for the main effect of DAP_{linear} was significant at the 0.01 level.

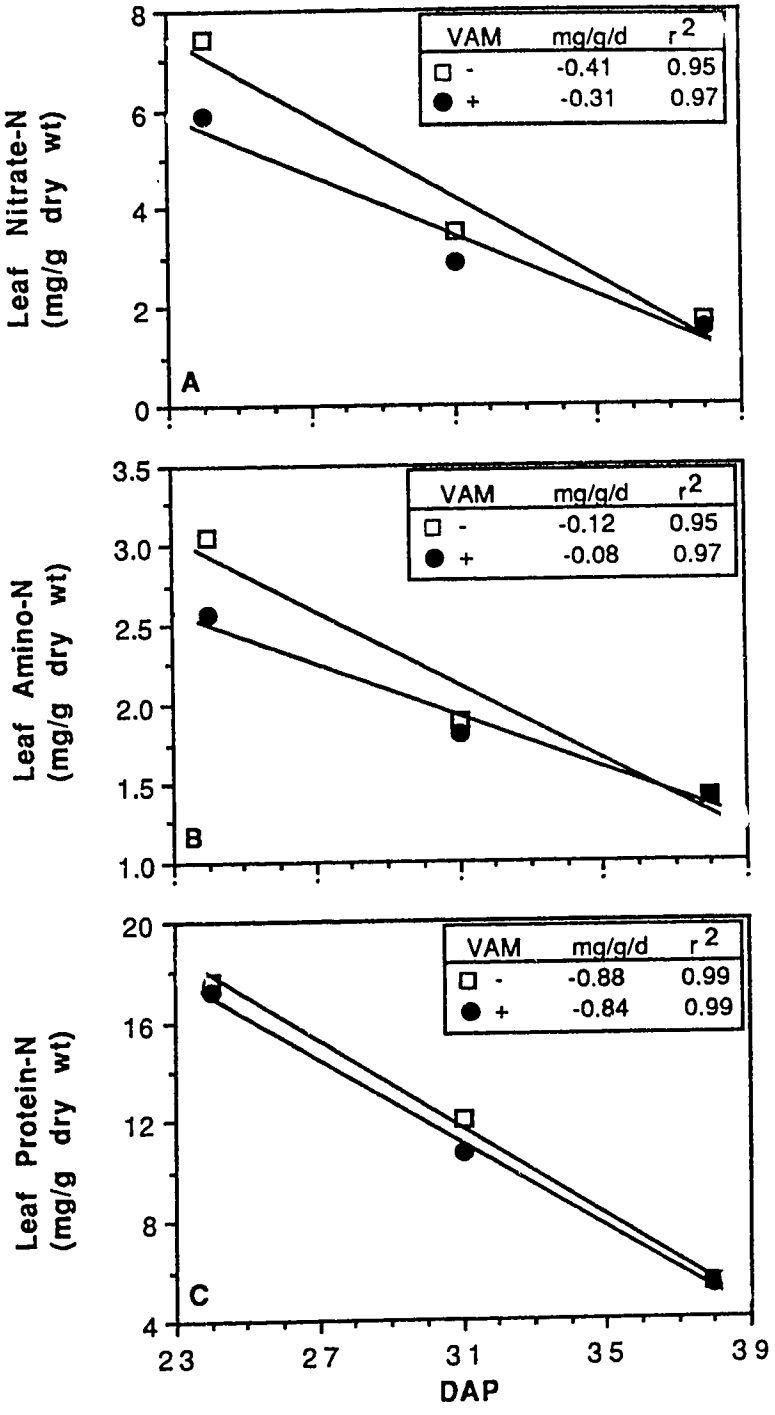
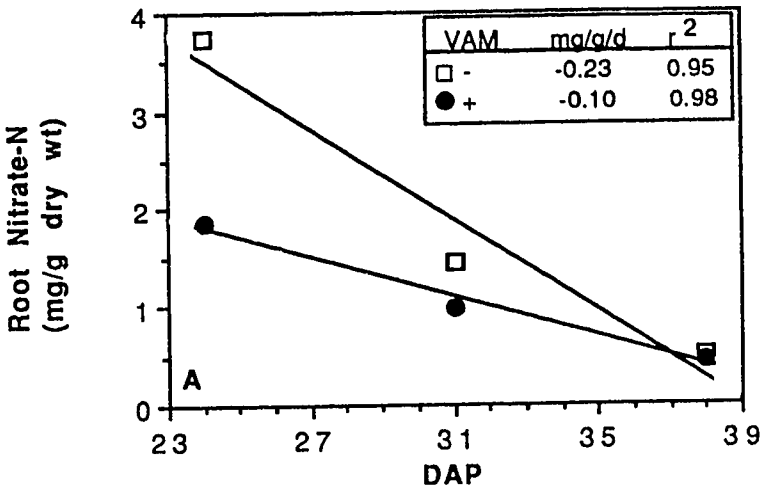
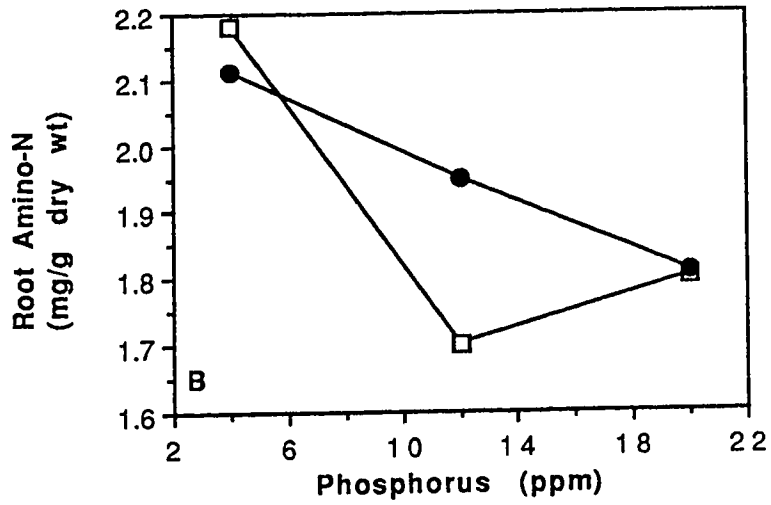

Leaf Nitrate-N (mg/g dry wt)
VAM mg/g/d r2
- -0.41 0.95
+ -0.31 0.97
A
Leaf Amino-N (mg/g dry wt)
VAM mg/g/d r2
- -0.12 0.95
+ -0.08 0.97
B
Leaf Protein-N (mg/g dry wt)
VAM mg/g/d r2
- -0.88 0.99
+ -0.84 0.99
C
DAP

Figure II-13. (A) Change in free-amino nitrogen concentration of roots of VAM-infected and noninfected 'Corona' cucumber plants from 24 to 38 DAP. Data was averaged for three VAM species (*G.mosseae*, *G. dimorphicum* and *G. intraradices*) and three P fertilization levels (4 to 20 ppm P). Linear regression coefficients and coefficients of determination appear in the inset. F-values for the main effects of VAM and DAP_{linear}, and the interaction of VAM x DAP_{linear}, were all significant at the 0.01 level. (B) Effect of P nutrition on the concentration of amino nitrogen in roots of VAM-infected (●) and noninfected (□) plants. Data was averaged for the three VAM species (as above) and three harvest dates (24 to 38 DAP). F-values for the main effect of $[P]_{linear}$, and the interaction of VAM x $[P]_{deviations}$, were significant at the 0.01 and 0.05 levels, respectively.


VAM
mg/g/d
r 2
- -0.23 0.95
+ -0.10 0.98
Root Nitrate-N (mg/g dry wt)
A
DAP


Root Amino-N (mg/g dry wt)
B
Phosphorus (ppm)

ppm to 12 ppm. Roots of control and VAM-infected plants grown with 20 ppm P had equal concentrations of amino-N.

Unlike the other N pools, level of P nutrition did not significantly change the concentration of soluble protein-N in leaves, and root protein concentration increased by only 6% as P nutrition increased from 4 ppm to 20 ppm (Table II-5). However, leaf and root soluble protein-N concentrations decreased substantially with time, and rates of decline depended on the level of P-nutrition. The concentration of leaf protein-N in plants grown with 4 ppm P decreased at a rate of 740 μg g^{-1} dry wt d^{-1}, as compared with 928 and 882 μg g^{-1} dry wt d^{-1} for those grown with 12 ppm and 20 ppm P, respectively. In roots, protein-N concentration declined by 6.9 μg g^{-1} dry wt for every ppm increase in available P ($P<0.05$). VAM did not alter the rates at which leaf (Fig. II-12C) and root (data not shown) protein-N concentrations decreased with time; however, roots of VAM-infected plants averaged a 10% higher concentration of protein-N (8.06 mg g^{-1} dry wt) than those of control (nonmycorrhizal) plants (7.30 mg g^{-1} dry wt) throughout the study. In contrast to roots, leaf protein-N was equal for control and VAM-infected plants throughout the study (Fig. II-12C).

Plant soluble carbohydrates. Changes in total soluble carbohydrate (TSC) and reducing sugar (RS) concentrations in leaves and roots of VAM-infected and non-infected (control) plants are characterized by the data in Table II-6.

Leaves of control (nonmycorrhizal) plants contained a slightly higher (4%) concentration of TSC (48.0 mg g^{-1} dry wt) than those of VAM-infected plants (46.3 mg g^{-1} dry wt) when averaged over harvest date (Table II-6). More importantly, leaf TSC concentration increased with time, and the rate depended on VAM infection. The rate of increase in leaf TSC concentration of VAM-infected plants (432 μg g^{-1} dry wt d^{-1}) was 38% lower than that of control plants (696 μg g^{-1} dry wt d^{-1}). Leaf RS concentration also increased from 24 to 38 DAP, but the rate of increase (589 μg g^{-1} dry wt d^{-1}) and average concentration (17.6 mg g^{-1} dry wt) were not altered by VAM infection. For every ppm increase in P nutrition, leaf TSC and RS concentrations decreased by 118 μg g^{-1} dry wt ($r^2 = 0.85$, $P<0.05$) and 96 μg g^{-1} dry wt ($r^2 = 0.95$, $P<0.05$), respectively, in

Table II-6. Effects of VAM infection and time on the concentrations of total soluble carbohydrates (TSC) and reducing sugars (RS) in leaves and roots of cucumber plants during establishment. Values have been averaged over three levels of P nutrition.

Treatment		Leaf			Root		
		TSC	RS	RS/TSC	TSC	RS	RS/TSC
VAM[a]	DAP	mg g^{-1} dry wt		%	mg g^{-1} dry wt		%
-	24	43.9	13.8	31.5	32.8	12.2	37.1
	31	46.4	16.4	35.4	32.9	17.4	52.9
	38	53.7	22.8	42.5	47.4	35.0	73.8
+	24	44.4	13.7	30.9	36.1	13.5	37.4
	31	44.0	17.7	40.2	41.5	21.2	51.1
	38	50.4	21.1	42.0	45.8	28.9	63.1
VAM[b]		0.05[c]	ns	ns	0.01	ns	ns
DAP_{lin}		0.01	0.01	0.01	0.01	0.01	0.01
DAP_{dev}		0.01	ns	0.05	ns	ns	ns
VAM x DAP_{lin}		0.05	ns	ns	ns	0.05	0.05
VAM x DAP_{dev}		ns	ns	0.05	0.01	0.05	ns

[a] VAM data is the average for plants infected with *Glomus mosseae*, *G. dimorphicum*, and *G. intraradices*.

[b] sources of variation

[c] significance levels for indicated sources of variation (lin, linear trend; dev, deviations from linearity; ns, not significant)

both VAM-infected and non-infected plants (data not shown).

Phosphorus nutrition did not affect the concentration of TSC or RS in roots. The average concentration of TSC in roots of VAM plants (41.2 mg g^{-1} dry wt) was 9% greater than that for control plants (37.8 mg g^{-1} dry wt) over the course of the study (Table II-6). Similar to the trend in leaf TSC, root TSC concentration increased with time, and the rate was 50% greater in control plants (1,041 µg g^{-1} dry wt d^{-1}) than in those infected with VAM (692 µg g^{-1} dry wt d^{-1}). From 24 to 38 DAP, the average RS concentration of roots (21.4 mg g^{-1} dry wt) was equal for VAM-infected and control (nonmycorrhizal) plants; however, in contrast to leaf tissue, RS concentration of roots of control plants increased 48% faster (1,631 µg g^{-1} dry wt d^{-1}) than in those of VAM-infected plants (1,099 µg g^{-1} dry wt d^{-1}) during growth.

On average, RS accounted for about 37% and 53% of TSC in leaves and roots, respectively. Moreover, as a proportion of TSC, leaf RS concentration increased 35% from 24 to 38 DAP, versus an 84% increase in roots. The TSC concentration of roots was 16% lower than that of leaves; however, RS concentration of roots was 21% higher than that of leaves.

DISCUSSION

Establishment and early growth of cucumber plants were very dependent on P nutrition. Control (nonmycorrhizal) and VAM-infected plants grown under low-P (4 ppm P) conditions showed restricted leaf area expansion (Fig. II-9) and slowed dry matter accumulation in roots, stems and leaves (Fig. II-4). Plants with rapid growth rates, such as cucumbers, have a high demand for P (Hayman, 1983; Koide, 1991). The nature of cucumber plant growth is that once the plant has established a root system and a certain degree of leaf area, it then enters an exponential shoot growth phase which is characterized by rapid stem elongation. Low rates of P fertilization restricted plant growth rates during establishment and, if maintained, would most likely delay, or even inhibit, the normal exponential growth phase. This would potentially limit the number of nodes and hence fruit yield per plant.

Changes in dry weight ratios of the various plant yield components over time indicated that foliar growth benefited the most by increasing P level, which is consistent with results reported for other crops (Rufty *et al.*, 1990; Fredeen *et al.*, 1989; McArthur and Knowles, 1993). Increases in Sw/W with improved P nutrition were mainly offset by decreases in Lw/W (Fig. II-6). These changes occurred with a concomitant increase in the proportion of total plant P allocated to stems, and a decrease in that allocated to leaves (Fig. II-10), which reflects the large increase in stem weight relative to the other plant yield components with advancing plant age . Leaf area and leaf growth indices (LAR, LA/LF, SLA) increased substantially as P nutrition increased, characterizing the dependency of cucumber leaf growth and expansion on P nutrition. Similar effects of P nutrition were reported for soybean (Fredeen *et al.*, 1989). The decrease in LAR (Fig. II-9C) and SLA with time probably reflect the greater allocation of plant dry matter to stems. High P promoted leaf area development which, when compared with that from low P plants, would render the plant more efficient at photosynthesis on a whole-plant basis.

'Corona' cucumbers demonstrated a high compatability to colonization by VAM fungi, as shown by an average infection level of 49% at 24 DAP. VAM fungal species may differ in their ability to enhance P uptake and/or growth of a host plant, even at similar levels of root infection. This variation may be due to many factors, including differences in external hyphae of the VAM species, or differences in compatability with the host (Graham *et al.*, 1982). The fungal species used in our study differed with respect to infection level, and high levels of infection did not necessarily correlate with the greatest growth enhancement or the most P uptake by plants. This was not surprising since previous studies have also demonstrated little correlation between internal VAM infection level and plant growth enhancement (Hayman, 1983). When averaged over the three harvest dates, plants inoculated with *G. mosseae* had a significantly higher level of infection than those inoculated with *G. dimorphicum*, yet shoot growth and leaf areas of these VAM-infected plants were quite similar. *G. intraradices* was the least effective species of VAM for stimulating plant growth, despite the fact that plants inoculated with this species developed a level of infection intermediate to that induced by the other two species (Table II-1, Fig. II-1).

The general result of VAM infection was increased plant growth relative to nonmycorrhizal plants. Plant growth rate (dry weight basis) was 13% higher in VAM-infected plants than in control plants (Table II-1). Leaf area was also influenced by VAM infection, with leaves of VAM-infected plants expanding 15% faster than those of control plants (Table II-1). Partitioning of dry matter to stems of control plants was inhibited relative to that of VAM-infected plants (Tables II-1 and II-2), and thus stem elongation appeared inhibited (Fig. II-7). The effect of VAM on the rate of leaf production from 24 to 38 DAP depended on the level of P nutrition (Fig. II-8). When grown at low levels of P (4 ppm P), control plants produced leaves faster than VAM-infected plants. However, the rate of leaf production by VAM-infected plants increased with P nutrition, and was significantly greater than that of control plants when both were grown with 20 ppm P. Leaves of VAM-infected plants were also significantly larger than those of nonmycorrhizal plants (Table II-1). With few exceptions, VAM-induced increases in the various growth parameters were similar to those induced by increases in P fertilization level. For many of the growth parameters, lack of interactions between VAM infection and level of P nutrition indicated that VAM stimulated growth by enhancing plant P nutritional status.

Phosphorus fertilization level had a significant effect on the content and allocation of P within the plant (Fig. II-10). High-P nutrition (20 ppm P) resulted in a large increase in total plant P, much of which was allocated to the stem, reflecting enhanced stem growth relative to plants grown with low-P (4 ppm P). VAM-infected plants had an overall greater P content (20%) than control plants, emphasizing the P uptake advantage of the VAM infection. In contrast, no difference was evident in the concentration of shoot P between control and VAM-infected plants. Since VAM greatly stimulated dry matter accumulation of shoots relative to control plants (Table II-1), it is evident that VAM plants increased their P uptake to match (support) the higher shoot growth rate. Therefore, VAM-induced differences in growth rate were due to the additional P provided by the symbiosis, underscoring the increased efficiency with which VAM-infected plants were able to acquire P. When compared with control plants, the higher root P concentration of VAM-infected

plants was most likely due to the additional P content of the VAM fungi (Gianinazzi-Pearson and Gianinazzi, 1983).

Concentrations of mineral elements such as P and N in plants decrease with time because the rate of carbon-based dry matter accumulation in structural tissues greatly exceeds the rate of mineral nutrient assimilation (Hocking and Steer, 1983). The ratio of structural tissues to more actively metabolizing nonstructural tissues thus increases during plant growth, and this was readily apparent for cucumber by the increase in stem and decrease in leaf weights with time, as a proportion of total plant weight (Fig. II-6). The rates at which plant P concentration declined with time in low-P (Table II-4) and nonmycorrhizal plants (Fig. II-11B) were significantly lower than those of high-P or VAM-infected plants. Because of a slower growth rate, low-P and nonmycorrhizal plants were simply in an earlier phase of growth relative to high-P and VAM-infected plants.

Phosphorus nutrition can affect the efficiency of N assimilation and metabolism. Oliver *et al.* (1983) found that, under P limiting conditions, mycorrhizal plants had a greater ability to assimilate nitrate via nitrate reductase than nonmycorrhizal plants. Moreover, the enhanced activity of nitrate reductase was attributed to improved P nutrition provided by the VAM infection. Thus, P deficiency would be expected to inhibit the assimilation of N. Nitrate, free amino and soluble protein nitrogen pools of cucumber leaf and root tissues declined from 24 to 38 DAP (Table II-5). Plants grown on a low-P (4 ppm P) regime had higher leaf and root nitrate-N and amino-N concentrations, but lower protein-N concentrations, than high-P (20 ppm P) plants. These responses were similar to those reported for tobacco (Rufty *et al.*, 1990), soybean (Fredeen *et al.*, 1989) and potato (McArthur and Knowles, 1993). Under P deficient conditions, absorbed nitrate may accumulate due to reduced nitrate reductase activity, and free amino acid concentration may increase because of enhanced protein degradation (Rufty *et al.*, 1990). A high concentration of nitrate-N in leaves of low-P potato plants was associated with low leaf nitrate reductase activity, as compared to that found in high-P plants (McArthur and Knowles, 1993). Moreover, P deficiency in tobacco resulted in a buildup of nitrate within roots, apparently caused by a restricted ability to translocate nitrate to leaves (Rufty *et al.*, 1990). Rufty *et al.* (1990) also demonstrated that P

deficiency leads to the accumulation of amino acids in shoot tissues. Early in our study, VAM-infected plants had significantly lower concentrations of leaf nitrate-N and amino-N compared to nonmycorrhizal plants (Fig. II-12). Roots of VAM-infected plants also had a lower nitrate-N concentration than nonmycorrhizal plants early in the study (Fig. II-13A), but amino-N and protein-N concentrations of roots were not altered by VAM infection. Since the effects of VAM and P nutrition on plant soluble N content were similar, the VAM effect on plant soluble N was most likely manifested through improved P nutrition provided by the symbiosis. Moreover, the enhanced growth effects induced by VAM fungi can be partially attributed to effects of VAM-enhanced P nutrition on the efficiency of N utilization by the plant.

Phosphorus nutrition did not greatly influence the concentration of soluble carbohydrates in leaf and root tissues. In leaves, TSC and RS concentrations decreased with improved P nutrition, while root TSC and RS concentrations were not altered by P nutrition. P deficiency limits carbon export from leaves (Rao *et al.*, 1990), and this may account for the higher level of soluble carbohydrates in leaves of low-P cucumber plants. On average, TSC concentration in leaves of VAM-infected plants was only slightly lower (4%) than that of nonmycorrhizal plants over the study interval; however, TSC concentration in leaves of control plants increased 61% faster than that in leaves of VAM-infected plants from 24 to 38 DAP. This faster rate of buildup in TSC's may reflect the higher degree of P deficiency stress in leaves of control plants, relative to those of VAM-infected plants.

When averaged over the study interval, VAM-infected plants had a higher concentration of TSC in roots than control plants, while control plants had a higher concentration of TSC in leaves than VAM-infected plants (Table II-6). This suggests that the VAM infection increased the sink strength of roots for carbohydrates, greater quantities of which would be needed to support the symbiosis. Relative to leaves, RS comprised a greater percentage of TSC in roots, which no doubt reflects the hydrolysis of sucrose (the translocated carbohydrate) to glucose and fructose to support intermediary metabolism and thus growth of the roots.

This study characterized the early growth response of greenhouse cucumber plants to P nutrition and VAM infection. The level of P nutrition was shown to be very important to the establishment of vigorous cucumber plants. Since VAM enhanced the growth of cucumber plants at all levels of P nutrition, use of VAM during transplant production may be advantageous to the greenhouse cucumber industry. If the accelerated early development of VAM-infected plants translates into earlier fruit production, or greater overall yield, then use of VAM may be of even further benefit to growers. The next chapter characterizes the effects of VAM and P nutrition on yield response and growth of fruiting plants.

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Chapter III

EFFECTS OF PHOSPHORUS NUTRITION AND MYCORRHIZAE ON GROWTH AND YIELD OF FRUIT-BEARING PLANTS

INTRODUCTION

European seedless cucumbers (*Cucumis sativus* L.) are a semi-tropical vegetable with an indeterminate growth habit. Grown under greenhouse conditions, these plants require high levels of light, heat, relative humidity, moisture, and fertilizer (Wittwer and Honma, 1979). Thus, the precise control of plant nutrition and the growing environment is extremely important to achieving a vigorous and marketable cucumber crop (Mirza, 1990). In commercial production, cucumber seedlings are established in small pots and are transplanted four to five weeks after seeding to the growing medium of choice, where they remain for the duration of the cropping season (Wittwer and Honma, 1979; Mirza, 1990). Cucumbers can be grown on a wide variety of media and, under optimum nutritional and environmental conditions, will produce marketable fruit fifty to sixty days after planting (DAP) (Adamson and Maas, 1981).

Phosphorus (P) is an essential element required in the plant's growth processes. Since P is involved in plant energy transformations, P nutrition can have a great influence upon plant growth in general (Brady, 1984). Phosphorus deficiency is very detrimental to plants, causing reduced growth rates, death of older leaves, delayed plant maturity, restricted root development and poor crop quality (Brady, 1984; Salisbury and Ross, 1985). Hence, the intensive production practices needed to sustain high growth rates of greenhouse cucumbers target optimum nutrition, as the plants have a low tolerance for nutrient deficiencies. Phosphorus deficiency in cucumbers can result in plant stunting and desiccation of older leaves (Roorda van Eysinga and Smilde, 1981; Mirza, 1990), and therefore, has severe effects upon transplant quality, plant growth rates and fruit production.

The relationship between vesicular-arbuscular mycorrhizal (VAM) fungi and the plant host is considered to be mutualistic. In exchange for carbohydrate nutrition from the host plant, VAM fungi benefit the plant primariiy through enhanced uptake of immobile soil nutrients such as phosphorus, zinc and copper (Miller, 1986). This enhanced mineral uptake is facilitated by the external hyphae of VAM fungi, which can exploit a greater volume of soil than can roots, thus sequestering nutrients (especially P) not normally accessible to the plant's root system (Brady, 1984; Hayman, 1983).

The VAM-induced improvement in plant growth and P nutrition is well documented for a variety of crops, but there is little evidence of the value of VAM to greenhouse crops such as cucumbers. The intent of this study was to characterize the effects of VAM fungi (specifically, *Glomus intraradices* Schenck and Smith) and P nutrition on growth and yield dynamics of fruit-bearing cucumber plants. Previous studies in our laboratory characterized the benefits of P nutrition and VAM infection to the nutritional status and growth of cucumber plants during establishment. VAM enhanced the P status and increased the growth rates of young cucumber plants at all levels of P nutrition. This study seeks to determine the extent to which the VAM-stimulated early growth influences subsequent growth and yield of mature, fruit-producing plants.

MATERIALS AND METHODS

Growing Conditions. For all studies, *Glomus intraradices* Schenck and Smith spores were used to inoculate the cucumber plants at seeding. Spores were supplied (Nutri-link research grade spores, NPI, Salt Lake City, Utah) on an inert carrier at a concentration of one million spores per carrier disc (6.47 g). The spores (38,640 spores/pot) were placed 1 cm below cucumber seeds, which were sown into an autoclaved mixture of coarse sand, fine sand, and loam (2:2:1 v/v/v). Phosphorus was undetectable in a 2:1 (v/v) water extract of the medium. Control (nonmycorrhizal) plants were not inoculated with spores.

In the first study, the effects of three levels of P nutrition (see below) and VAM infection on growth and yield of the cucumber cultivar Corona were characterized. Seeds were sown 2 cm

deep in VAM inoculated or control media (see above) in 15 L pots (3 seeds/pot). At 10 DAP (first to second true leaf stage), plants were thinned to one plant per pot, blocked for size, and P-level and VAM treatments were randomized within each block. The study was conducted from May 3 to July 19, 1991 under natural light in a University of Alberta research greenhouse. The greenhouse temperature was maintained between 21 and 25°C. In the Edmonton area, daylength ranged between 15.2 h and 16.4 h between these dates. The plants were pruned and trained to maintain a single leader according to guidelines for a sequence cropping production system (Mirza, 1990; Adamson and Maas, 1981). Insects were controlled by chemical and biological methods. Thrips (*Frankliniella occidentalis*) were controlled by spraying the soil with Diazinon (2 mL/L, 12.5% a.i.) at 12, 20, and 28 DAP, coupled with release of thrips predator (*Amblesius cucumeris*) at two-week intervals throughout the study. Whiteflies (*Trialeurodes vaporariorum*) were controlled by the whitefly predator, *Encarsia formosa*.

The experiment was set out in a randomized complete block design comprised of five replications of all combinations of three P fertilization levels and two VAM treatments (one VAM species and a nonmycorrhizal control). Depending on the treatment, plants were hand fertilized with 250 mL of 120, 240 or 480 ppm P (KH_2PO_4), three times per week, starting 26 DAP. Thus, P treatments consisted of 90, 180 or 360 mg of elemental P per plant per week. Potassium levels were held constant by adding KCl to the 90 and 180 mg P/week treatments. All other nutrients were applied uniformly (pH 6.0) to each pot in the experiment by an automated fertigation system (Harrow Fertigation Manager, Labbate Control Systems, Leamington, ON), according to levels recommended for cucumber plant growth and fruit production (Mirza, 1990; Adamson and Maas, 1981; Chérif and Bélanger, 1992). Fertigation began 26 DAP. Each plant initially received 1 L/day of nutrient solution containing 50 ppm NO_3-N, 5 ppm NH_4-N, 75 ppm K, 40 ppm Ca, 40 ppm Mg, and 400 ppm (max) S. The volume of fertilizer and concentration of nutrients were gradually increased with plant growth, so that by 64 DAP each plant received 5.4 L/day of solution containing 200 ppm NO_3^--N, 20 ppm NH_4^+-N, 275 ppm K, 120 ppm Ca, 40 ppm Mg and 400 ppm (max) S. Micronutrients, also included in the fertigation solution, were maintained at constant

levels (0.840 ppm Fe, 0.547 ppm Mn, 0.164 ppm Zn, 0.200 ppm B, 0.102 ppm Cu, and 0.072 ppm Mo) throughout the study.

Fruit was harvested after reaching a minimum of 30 cm length and 42 mm diameter (Canada #1 Grade, Mirza, 1990), which corresponded to at least 300 grams fresh weight. Foliar prunings were oven-dried (72 ^{o}C) and the dry weights recorded. Fruit fresh weight and fruit number were also recorded. Fruit harvest started at 53 DAP and the experiment was terminated at 77 DAP. At crop termination, the stem length and number of nodes were recorded, and leaf (including petioles) and stem dry weights determined. The dry weight of prunings was added to the total leaf dry weight. The harvest index (proportion of economic yield to biological yield) was determined by dividing total fruit dry weight per plant by the sum of shoot and fruit dry weight. Fruit dry weight was determined to be 4.55% of the fresh weight, and this percentage was not altered by treatment. Hence, fruit dry weights were calculated in order to determine the effect of the various treatments on harvest index.

Root infection was determined on roots isolated from a longitudinal 'pie-slice' of the medium from each pot, which represented approximately one eighth of the root system. The roots were washed to remove soil and debris and were stored in FAA (5% formalin, 5% a acid, 45% ethanol) until clearing and staining (Phillips and Hayman, 1970). Infection counts were made on four slides per root sample, using the gridline intersect method of Giovanetti and Mosse (1980). Growth and yield data were subjected to ANOVA and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Based on the ANOVA results, regression analysis was used to determine growth and yield response to increasing P nutrition. Only significant results are reported in this document.

A second study tested the growth and yield response of 'Carmen' cucumbers to P fertilization level and VAM infection. Seeds were inoculated at planting in 1.3 L plastic pots (2 seeds/pot) containing the growing medium described for the first study. At 12 DAP, plants were thinned to one plant per pot, blocked for size, and the treatments were randomized within each

block. At 23 DAP, the seedlings were transplanted to 15 L plastic pots containing the growth medium described previously.

The experiment consisted of five replications of all combinations of four P fertilization levels and two VAM treatments (one VAM species and a nonmycorrhizal control). The four P fertilization levels were 90, 180, 360 and 720 mg elemental P/plant/week from KH_2PO_4. Potassium chloride was again used to balance the K levels of the lower P treatments to match that present in the highest P treatment. Phosphorus was applied three times per week by hand (250 mL/pot/fertilization) starting 25 DAP. Other nutrients were supplied uniformly to all pots by the Harrow Fertigation Manager starting 25 DAP and increasing until 61 DAP, at volumes and concentrations equivalent to those outlined previously. Plants were grown from July 4 to October 4, 1991 under long-day conditions (ranging from 16.9 h to 11.4 h natural light) in a University of Alberta greenhouse at 21 to 25°C. Insects were controlled biologically, as previously described. The use of biological methods to control insects on 'Carmen' can be seen in figure III-1. Figure III-2 shows the cucumber plants at various stages of growth, and depicts the training technique and fertigation system used in all of the greenhouse studies.

Fruit harvest began 54 DAP, and the experiment was terminated at 92 DAP. Data was collected as described previously, with the exception of low-P (90 mg P/week) and high-P (720 mg P/week) plants from three of the replicates. For plants from these three replicates, individual leaves and petioles were harvested separately from alternate nodes, starting at the apex and ending 26 nodes down the mainstem. The leaves and petioles from these nodes were lyophilized. The remaining plant material was oven-dried. Dry weights of the lyophilized components were determined and included with the oven-dried weights from the other replicates for analysis of treatment effects on yield components.

Lyophilized leaf tissue was ground through a 40 mesh screen with a Wiley mill, and 50 to 100 mg of ground tissue was ashed overnight in a muffle furnace (550°C). The ash was digested in 1 mL of HCl for 20 minutes, followed by the addition of 9 mL of 0.72 N H_2SO_4. The mixture was

Figure III-1. Photograph shows how biological predators were applied to greenhouse cucumbers ('Carmen') to control thrips (*Frankliniella occidentalis*) and whitefly (*Trialeurodes vaporariorum*). The thrips predatory mite, *Amblesius cucumeris,* was shipped in a bran-like material which was shaken directly onto the leaves. The white card attached to the leaf petiole contains eggs of the whitefly predator, *Encarsia formosa*. Blue (or yellow) sticky cards were used to trap insects and thus monitor pest populations. Note the drippers placed in the pots to facilitate automatic fertigation.

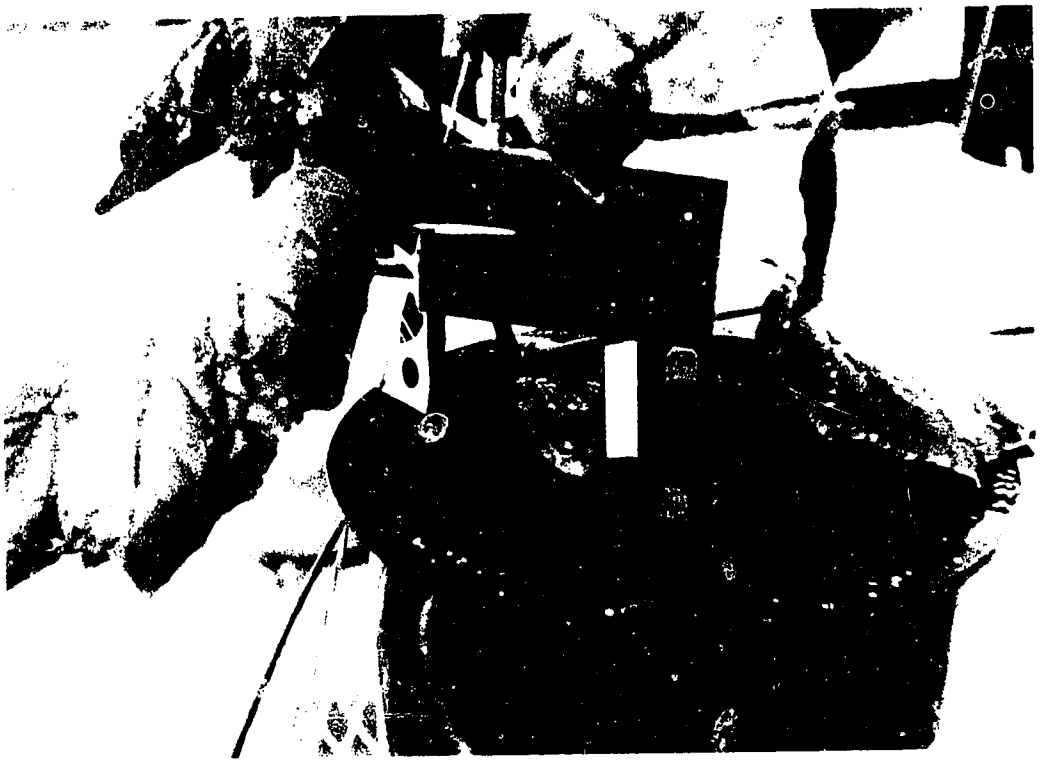


Figure III-2. Different stages of growth of 'Carmen' cucumbers in the greenhouse. (A) Young cucumber plants, approximatedly 0.76 m tall, growing under supplemental light in 15 L plastic pots containing an autoclaved sand/soil medium. (B) A main fertigation line was used to deliver nutrients (except P) to each plant in the study, through the two drippers (4 L h^{-1}) placed in each pot. Black plastic clips were used to fasten the plants to training lines during growth. The lower leaves have been pruned. The lush foliar growth produced by the mature, fruit-bearing plants later in the experiment is shown in (C) and (D).

centrifuged (1640 g, 20°C for 30 minutes) to settle any undigested matter. An aliquot of the supernatant (200 µL) was then analyzed for P by the methods of Serrano *et al.* (1976), using a sodium phosphate standard. All data from this study were statistically analyzed as previously described.

A final experiment was constructed so that the growth and yield responses of 'Corona' and 'Carmen' cucumbers to P nutrition and VAM infection could be directly compared. Seeds (3 seeds/pot) were sown into inoculated or control growing medium as described in the first study. At 19 DAP, plants were thinned to one plant per pot, blocked for size and randomized within each block. The experiment consisted of four replications of all combinations of three P fertilization levels, two VAM treatments (one VAM species and a nonmycorrhizal control), and two cucumber cultivars ('Carmen' and 'Corona'). The three P fertilization levels (90, 180, 360 mg elemental P/plant/week) were applied three times per week (250 mL/pot/fertilization) starting 27 DAP. Other nutrients (including micronutrients) were provided at the same concentrations as in the first study, starting at 27 DAP and increasing until 57 DAP. However, in contrast to the previous studies, the volume of nutrient solution was initially 1 L/plant/day at 27 DAP, and was increased to 4 L/plant/day by 57 DAP. The experiment was conducted in a University of Alberta greenhouse from November 21, 1991 to February 24, 1992, under 16 h supplemental light (450 µE m^{-2} sec^{-1} at mid canopy) provided by HID lamps with high pressure sodium bulbs (Sylvania 400 watt). The temperature ranged from 21 to 25°C. Insect control was facilitated by the biological agents previously described. Fruit harvest started at 54 DAP, and the experiment was terminated at 96 DAP. Data were collected and statistically analyzed as previously described.

Microscopy. Cucumber roots ('Corona'), infected with *Glomus intraradices* Schenck and Smith, were harvested approximately 3.5 months after planting. Roots were carefully washed in water to remove soil and debris, cut into 1 cm segments, and fixed overnight in a sodium phosphate (0.01 M, pH 7.4) buffer containing 5% (v/v) glutaraldehyde. The root tissue was then rinsed in sodium phosphate buffer (0.01 M, pH 7.4) three times, and fixed for 1.5 hours in a sodium phosphate (0.01 M, pH 7.4) buffer containing 4% (v/v) osmium tetroxide. The tissue was

again rinsed in sodium phosphate buffer three times, and then dehydrated in a graded alcohol series ranging from 10% to 100% ethanol. The roots were then resin embedded or prepared for scanning electron microscopy (SEM). Tissue to be resin-embedded was transferred from 100% ethanol to propylene oxide and then embedded with Spurr's resin (Spurr, 1969). Ultramicrotome sections (2 µm thick) were made using a Reichert OMU2 ultramicrotome. The sections were stained with Toluidine blue O (0.5% w/v TBO in 0.1% sodium carbonate solution, pH 11.1) for 5 minutes at 60°C and observed using brightfield microscopy. Roots for SEM were removed from 100% ethanol, frozen in liquid nitrogen, and fractured with a scalpel. The fractured roots were rinsed three times with 100% ethanol and critical-point dried. Roots were mounted on stubs with double-sided sticky tape, sputter-coated with gold, and viewed using a Cambridge Stereoscan 150 SEM or a Hitachi S-2500 SEM.

RESULTS

Root Infection. Since the absolute levels of root infection by *Glomus intraradices* (and the trend in root infection with increasing P nutrition) were similar for all three studies, percent infection data were averaged for each of the cultivars and plotted against level of P nutrition (Fig. III-3). Infection of roots from low-P (90 mg/plant/week) 'Carmen' and 'Corona' cucumber plants was well established, averaging 55% and 71%, respectively, at crop termination (average 88 DAP). Percent infection declined linearly with increasing P nutrition in all experiments ($P < 0.01$). When grown with 360 mg supplemental P/week, infection was less than 10% in both cultivars. 'Corona' plants supported a 54% higher level of internal VAM infection than 'Carmen' plants over all P fertilization levels. However, the rate of decline in percent infection with increasing P nutrition was similar for the two cultivars. On average, percent infection dropped by 10% for every 50 mg increase in supplemental P per plant per week. No infection was evident when plants were grown with 720 mg P/week (data not shown). Freshly harvested, VAM-infected roots had a characteristic yellow pigmentation that clearly distinguished them from nonmycorrhizal roots (Fig. III-4). This

Figure III-3. Effect of P nutrition on root infection of mature cucumber plants by *Glomus intraradices*. 'Carmen' (▲) and 'Corona' (○) cucumbers were inoculated with VAM spores at seeding and were grown in a greenhouse at three levels of P nutrition for 88 days (average of three studies). F-value for the main effect of $[P]_{linear}$ was significant at the 0.01 level in all three studies.

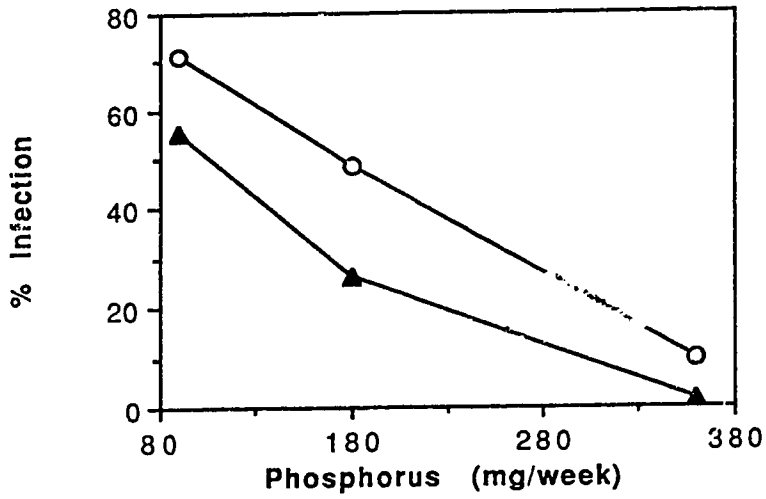

80
60
40
20
0
% Infection
80
180
280
380
Phosphorus (mg/week)

Figure III-4. Characteristic yellow pigmentation of mycorrhizal (M) roots compared to the white nonmycorrhizal (C) roots of cucumber plants. The VAM-infected (*Glomus intraradices*) roots on the left are from 'Corona' plants grown in the greenhouse for approximately 3 months (fully mature plants), while those on the right are from relatively immature plants harvested 38 DAP. Nonmycorrhizal roots are from plants harvested at 38 DAP. The plants were grown with 90 mg supplemental P/plant/week.


dM
C
M

characteristic is not unique to cucumber roots, and has been reported for a number of other plant species infected with VAM (Carling and Brown, 1982).

VAM infection consists of two phases, one external and the other internal to the plant root. *Glomus* spp. form clusters of chlamydospores (Morton and Benny, 1990) formed singly on subtending hyphae (Fig. III-5AB). An external hypha penetrates the root epidermis by forming a swollen appressorium (Fig. III-5C) at the infection point. VAM infection can be quite extensive in cucumber roots under low-P (90 mg P/week) conditions. Figure III-5D illustrates the invasive nature of the fungus as it fills most of the parenchyma cells of the root cortex with fungal structures such as arbuscules. Both arbuscules (Fig. III-5E) and vesicles (Fig. III-5F) appear to fill most of the plant cell lumen, thus having a major spatial impact on the infected root cells. The VAM infection spreads throughout the root by growth of internal hyphae, both intracellularly (Fig. III-6AB) and intercellularly (Fig. III-6CD, Fig. III-7AB). The arbuscules form in the root cells by repeated dichotomous branching of a central intracellular hyphal 'trunk', ending in short, bifurcate branches (Fig. III-7AB). Infection does not extend past the endodermis into the vascular cylinder (Fig. III-5DF, Fig.III-6C, Fig. III-7AB). VAM infection can also be easily observed using low-power brightfield microscopy (Fig. III-7C), which can be useful in noting internal VAM structure such as lipid droplets in vesicles.

Growth and yield response of 'Corona'. Phosporus nutrition and VAM infection had a significant effect on the foliar yield components of 'Corona' cucumber plants at 77 DAP (crop termination). On average, the number of leaves per plant (equivalent to nodes/plant) increased by about 9% as P nutrition increased from 90 to 360 mg P/plant/week ($P < 0.01$) (Fig. III-8A). However, nonmycorrhizal plants were more responsive to additional P in the low-P to medium-P (90 to 180 mg P/week) range, while VAM-infected plants were more responsive in the medium-P to high-P (180 to 360 mg P/week) range, with regard to leaf production. Although statistically significant ($P < 0.01$), the difference between the number of leaves produced by VAM-infected and control plants was only 2%.

Figure III-5. Scanning electron micrographs of 'Corona' cucumber roots infected with the VAM fungus *Glomus intraradices*. (A) External hyphae (h) and clusters of chlamydospores (s) (bar = 40 μm). (B) Single spore (s) with subtending external hypha (h) (bar = 10 μm). (C) External hypha forming an appressorium (ap) to facilitate infection entry through the epidermis (ep) (bar = 4 μm). (D) Transverse fracture of a root showing the invasive nature of the arbuscules (a), that fill most of the cortical cells between the epidermis (ep) and the endodermis (e) (bar = 60 μm). (E) Arbuscules (a) formed from a main hyphal trunk (h) within adjacent cortical cells (cw = cell wall, bar = 20 μm). (F) Fractured vesicle (v) contained within a cortical cell next to the endodermis (e). Note the intercellular hypha (h) below the vesicle (cw = cell wall, bar = 20 μm).

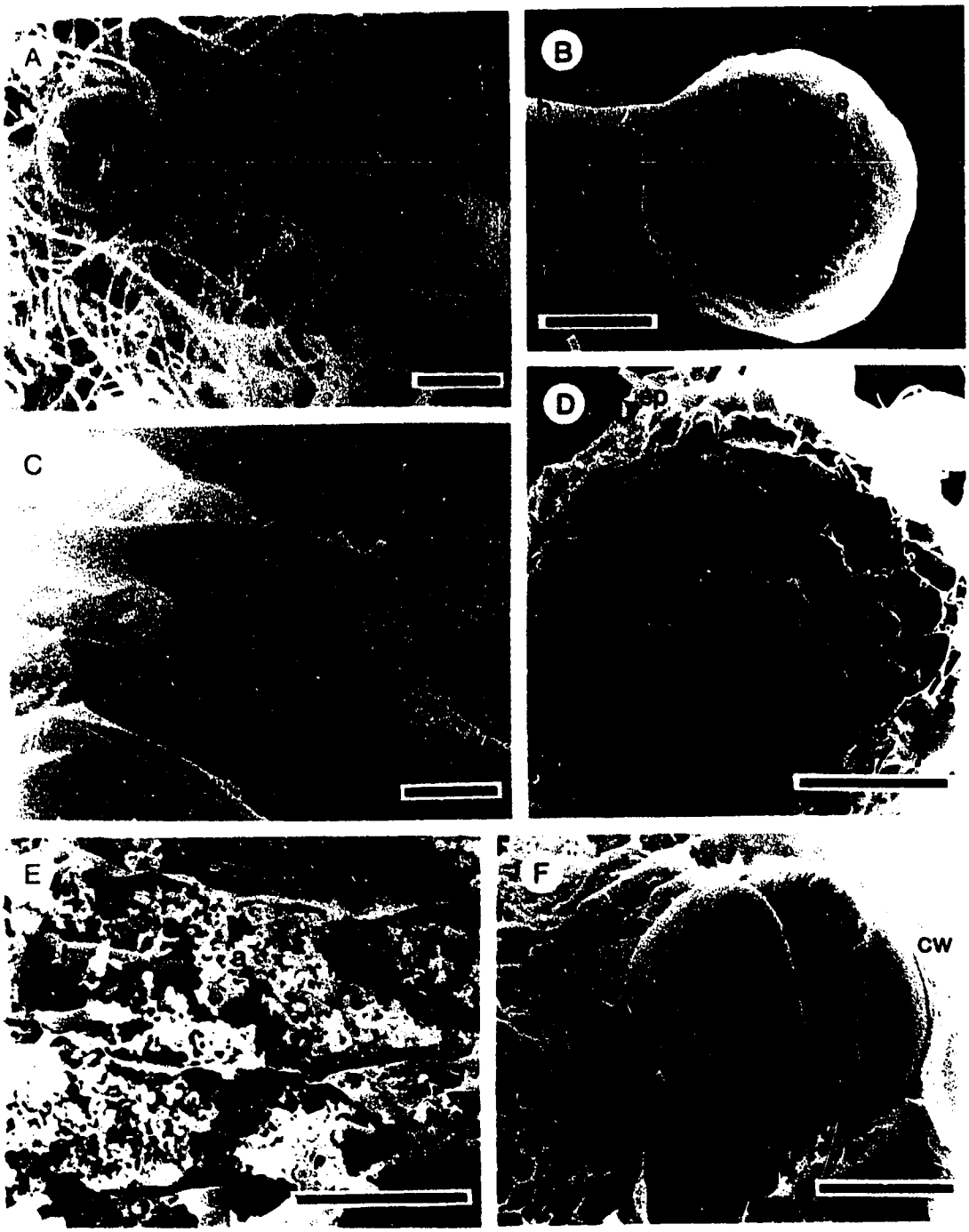

A
B
C
D
E
F
CW

Figure III-6. Scanning electron micrographs of 'Corona' cucumber roots infected with the VAM fungus, *Glomus intraradices*. (A) Epidermis (ep) has been peeled off to reveal the arbuscules (a) and hyphae (h) contained within cells of the root cortex (bar = 40 μm). (B) Close-up view of A revealing the intracellular hypha (h) and arbuscule (a) contained within cortical cells (cw = cell wall, bar = 10 μm). (C) Transverse section of cucumber root showing the arbuscules (a) and intercellular hyphae (h) in the cortex region of the root. VAM infection does not extend past the endodermis (e) into the vascular portion of the root (bar = 38 μm). (D) Close-up view of C showing intercellular hyphae (h) between adjacent cortical cells (cw = cell wall, bar = 10 μm).

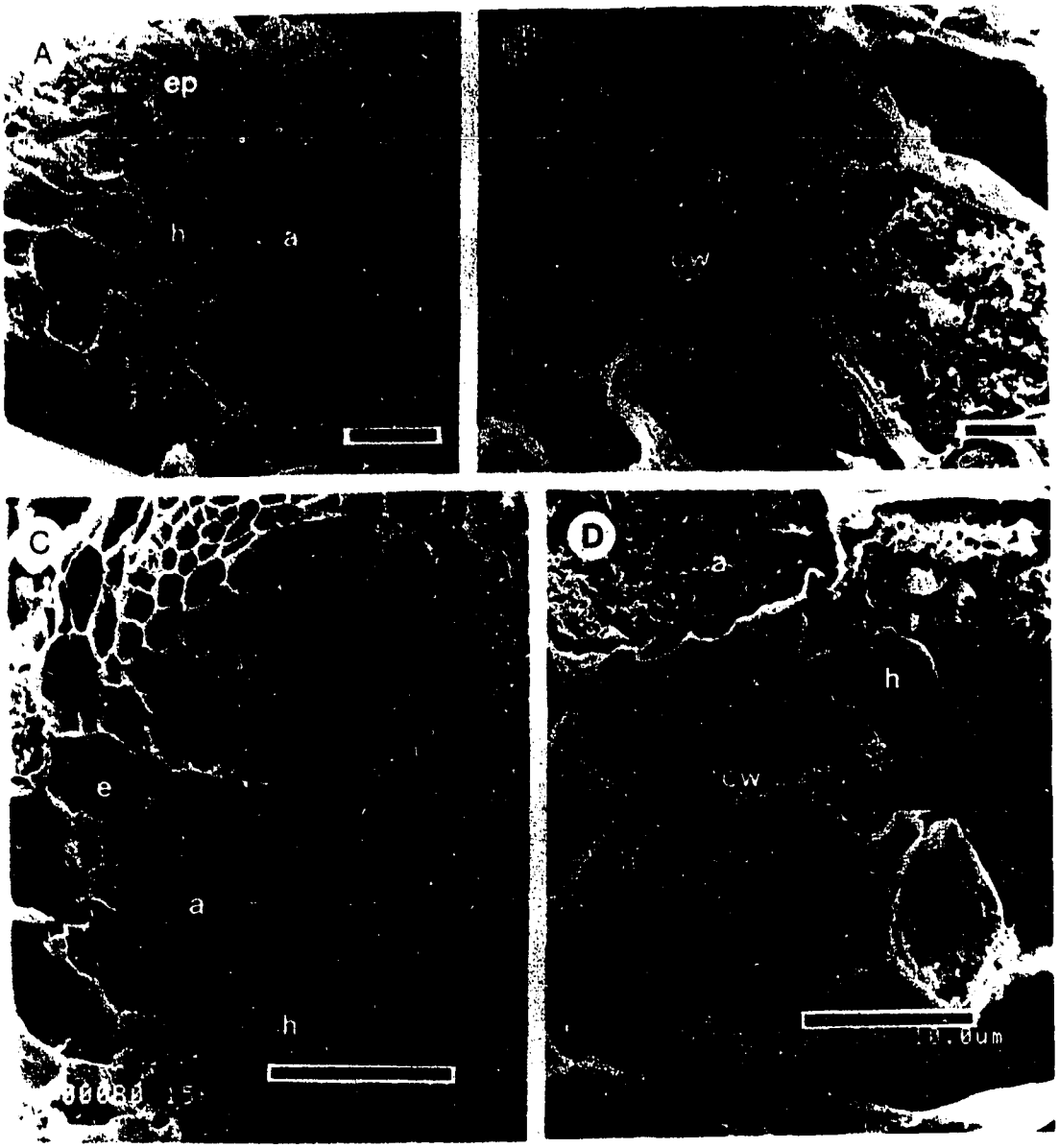
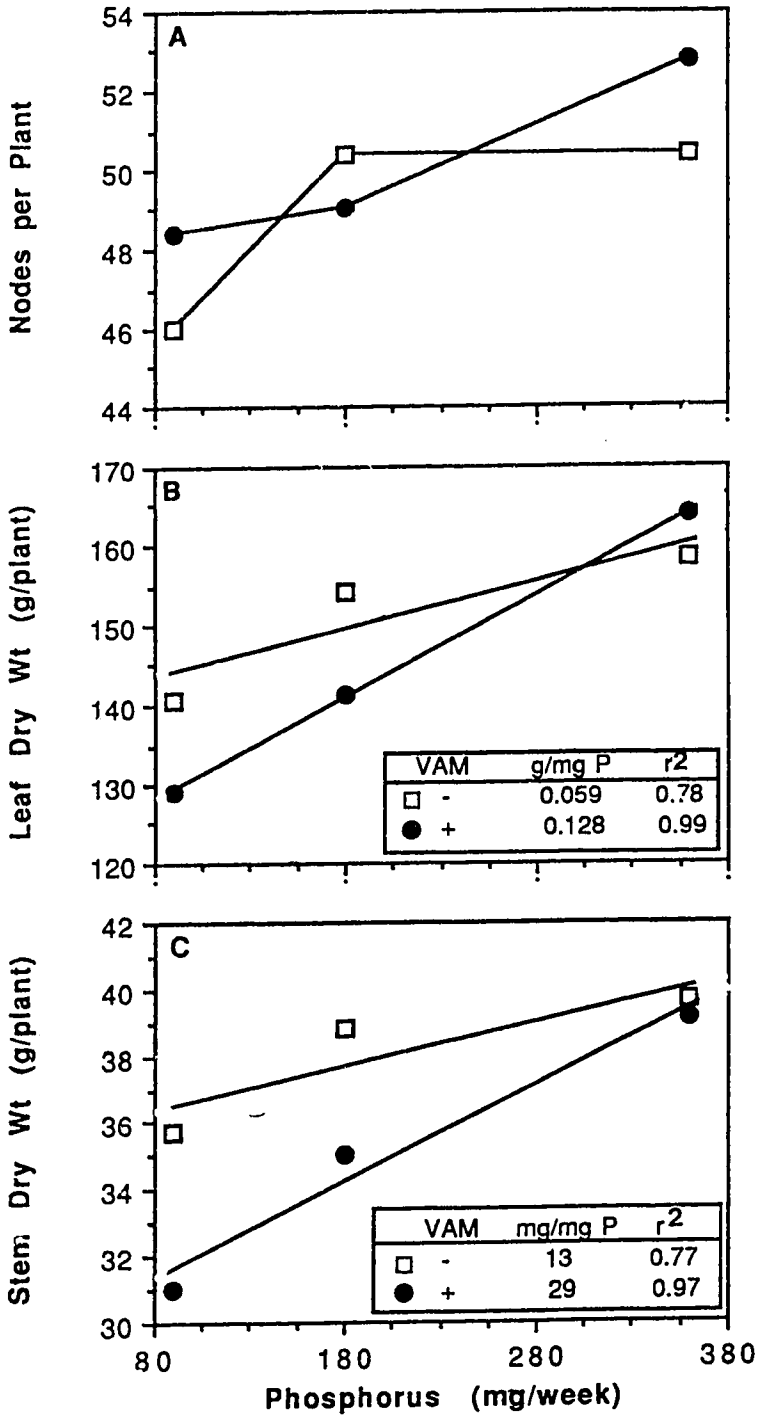

A
ep
h
a
cw
C
e
a
h
D
a
h
cw

Figure III-7. Bright-field micrographs of 'Corona' cucumber roots infected with the VAM fungus, *Glomus intraradices*. (A) Thin-section (2 μm thick) mounts of a transverse section of VAM-infected root tissue showing the highly branched arbuscule (a) contained within a cortical cell (cw = cell wall), intercellular hyphae (h), and enlarged cortical cell nucleus (n). Note that the VAM infection does not extend into the endodermis (e) (bar = 60 μm). (B) Thin section as in A showing arbuscules (a) and intercellular hyphae (h) in relation to the endodermis (e) (bar = 45 μm). (C) Squash mount of VAM-infected root showing a vesicle (v) containing lipid droplets, and arbuscules (a) forming from intercellular hyphae (h) (bar = 45 μm).


A
e
a
n
cw
B
e
a
h
C
v
h
a

Figure III-8. Effect of infection by *Glomus intraradices* on the foliar growth response of 'Corona' cucumbers to increased weekly P fertilization level. Plants were harvested at 77 DAP. (A) The number of main stem nodes produced by control (□) and VAM-infected (●) plants. F-values for the main effects of VAM, $[P]_{linear}$, and $[P]_{deviations}$, and the interaction of VAM x $[P]_{deviations}$, were significant at the 0.01, 0.01, 0.05 and 0.01 levels, respectively. (B) Total leaf dry matter (including petioles) produced by VAM-infected and control plants. F-values for the main effect of $[P]_{linear}$ and the interaction of VAM x $[P]_{linear}$ were significant at the 0.01 and 0.10 levels, respectively. (C) Total stem dry matter produced by VAM-infected and control plants. F-values for the main effects of VAM and $[P]_{linear}$ and the interaction of VAM x $[P]_{linear}$ were significant at the 0.01, 0.01 and 0.10 levels, respectively. Linear regression coefficients and coefficients of determination for VAM-infected and control plants are depicted in the insets of (B) and (C).

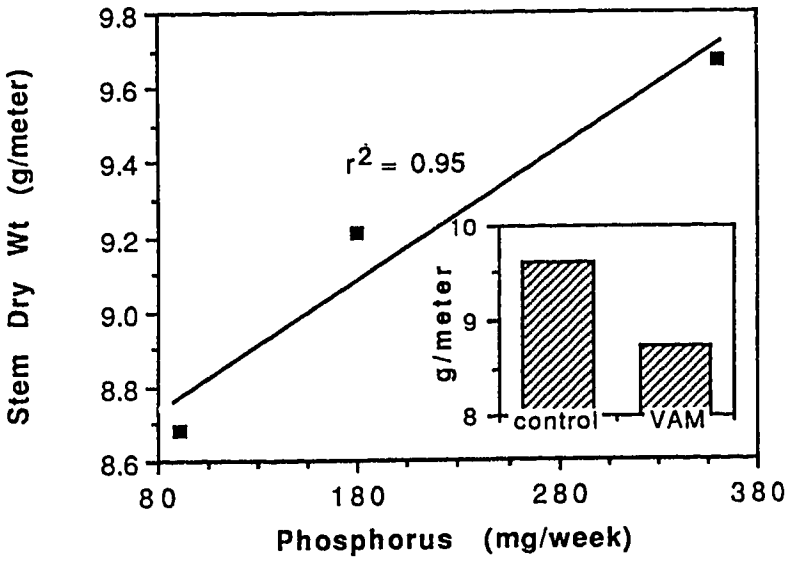

A
Nodes per Plant
54
52
50
48
46
44
B
Leaf Dry Wt (g/plant)
170
160
150
140
130
120
VAM g/mg P r2
- 0.059 0.78
+ 0.128 0.99
C
Stem Dry Wt (g/plant)
42
40
38
36
34
32
30
VAM mg/mg P r2
- 13 0.77
+ 29 0.97
80
180
280
380
Phosphorus (mg/week)

When averaged over VAM treatments, total leaf dry weight increased linearly ($P < 0.01$) with increasing P fertilization level. Medium-P and high-P plants had 10% and 19% more leaf dry weight, respectively, than low-P plants (Fig. III-8B). Of greater relevance, the increase in leaf dry matter of VAM-infected plants with increasing P nutrition was 98% greater than that of nonmycorrhizal plants, when calculated over the range of P treatments. Hence, leaf growth of VAM-infected plants was more responsive to P. This was due to the fact that control plants had 9% more leaf dry matter than VAM-infected plants at the lowest level of P nutrition (90 mg P/plant/week), whereas at the highest P level (360 mg P/week), VAM-infected plants had 3% more leaf dry weight than control plants. The leaf dry weight of VAM-infected plants increased by 6.4 g for every 50 mg increase in P/plant/week. In contrast, leaf dry weight of control plants increased by only 2.95 g for every 50 mg increase in P/plant/week.

When averaged over VAM treatments, stem dry weight also increased linearly ($P < 0.01$) with increasing P nutrition (Fig. III-8C). Medium-P and high-P plants had incorporated 10% and 18% more dry weight into stems, respectively, than low-P plants by the end of the study (77 DAP). However, the magnitude of the P-dependent increase in stem dry weight was 107% greater for the VAM-infected plants, relative to controls. When grown with 90 mg P/week, stem weight of control plants was 15% greater than that of VAM-infected plants, but this difference disappeared as P nutrition increased to 360 mg P/plant/week. The stem dry weight response of VAM-infected plants to additional weekly P was 123% greater than that of control plants, with VAM-infected plants increasing stem dry matter by 1.45 g for every 50 mg increase in P/week, compared with only 0.65 g increase from control plants.

Stem dry weight per meter fresh length (g m^{-1}) also increased linearly ($P < 0.05$) with improved P nutrition (Fig. III-9). Hence, the length-based densities of stems of medium-P and high-P plants were 6% and 11% greater, respectively, than that of low-P plants. Stem density increased by 175 mg m^{-1} for every 50 mg increase in weekly P fertilization level. When averaged over the levels of P nutrition, stems of control plants contained 10% more dry weight per meter than stems of VAM-infected plants (Fig. III-9 inset).

Figure III-9. Effect of P nutrition and VAM infection (inset) on the dry weight accumulated per meter of stem from greenhouse-grown 'Corona' cucumber plants. Plants were inoculated at seeding with *Glomus intraradices* and were harvested 77 DAP. Stem length was determined at harvest (fresh basis). F-values for the main effects of $[P]_{linear}$ and VAM were significant at the 0.05 level.


9.8
9.6
9.4
9.2
9.0
8.8
8.6
Stem Dry Wt (g/meter)
r2 = 0.95
80
180
280
380
Phosphorus (mg/week)
10
9
8
g/meter
control
VAM

The rates of fruit production, and the total number of fruits produced per plant, were significantly affected by P nutrition (Fig. III-10). As P nutrition increased from 90 to 360 mg P/week, fruit production rate increased from 0.41 fruit/plant/d (r^2 = 0.94) to 0.63 fruit/plant/d (r^2 = 0.99), respectively, over the 25 day production period (a 53% increase based on the linear functions). The result was 1.6 more fruit per plant for every 100 mg increase in weekly P fertilization. Hence, by 77 DAP, high-P plants had produced 46% more fruit than low-P plants (10.3 fruits/plant versus 15.0 fruits/plant) ($P< 0.01$). Moreover, a slight but significant shift in the onset of fruit production, that favored earlier fruit development, was clearly evident with increasing P level (Fig. III-10 and insets). By 59 DAP, VAM plants produced an average 23% more fruit than noninfected plants (4.8 fruit versus 3.9 fruit) ($P< 0.10$). From 53 to 59 DAP, VAM plants grown with 90 mg P/week produced fruit 62% faster than control plants (0.77 versus 0.47 fruit/day, based on linear models) (Fig. III-10 insets). Differences in the rates of early fruit production were still apparent when plants were grown with 180 and 360 mg P/week, with VAM plants producing fruit 49% (1.06 versus 0.71 fruits/day) and 30% (0.92 versus 0.71 fruits/day) faster, respectively, than control plants. However, over the entire growing period, control plants made up this early fruit production deficit and, by 77 DAP, the VAM-infected and control plants had produced an equal number of fruits at all P levels (Fig. III-10).

When averaged over P nutrition levels, VAM-infected plants produced fruits that were 6% heavier than control plants ($P< 0.01$) (data not shown). More importantly, P nutrition and VAM infection interacted ($P< 0.01$) to affect fruit fresh weight, such that VAM fruit (326.8 g) were 11% heavier than control fruit (295.5 g) at low-P and medium-P levels, but were about equal in weight to control fruit at high-P levels (average 319 g). The increase in harvest index (dry weight basis), with increasing P nutrition (from 90 to 180 mg P/week), indicated an improved efficiency of fruit production (Fig. III-11). When averaged over VAM treatments, plants grown with low-P and medium-P had partitioned 48.7% and 53.3% of their dry weight, respectively, into fruit production by 77 DAP (a 9.4% difference, $P< 0.05$). No further improvement in harvest index was apparent as P level increased from 180 to 360 mg P/week. VAM infection and P nutrition interacted ($P<$

Figure III-10. Effect of VAM infection and P fertilization level on cumulative fruit production from 'Corona' cucumber plants. Plants were inoculated at seeding with *Glomus intraradices*, and were grown with 90, 180 or 360 mg supplemental P per week in a greenhouse. The F-value for the main effect of [P] at 77 DAP was significant at the 0.01 level. Insets depict the early fruit production (53 to 59 DAP) from VAM-infected (●) and control (□) plants at each level of P fertilization. F-values for the main effects of VAM, and [P] at 59 DAP were significant at the 0.10 level.

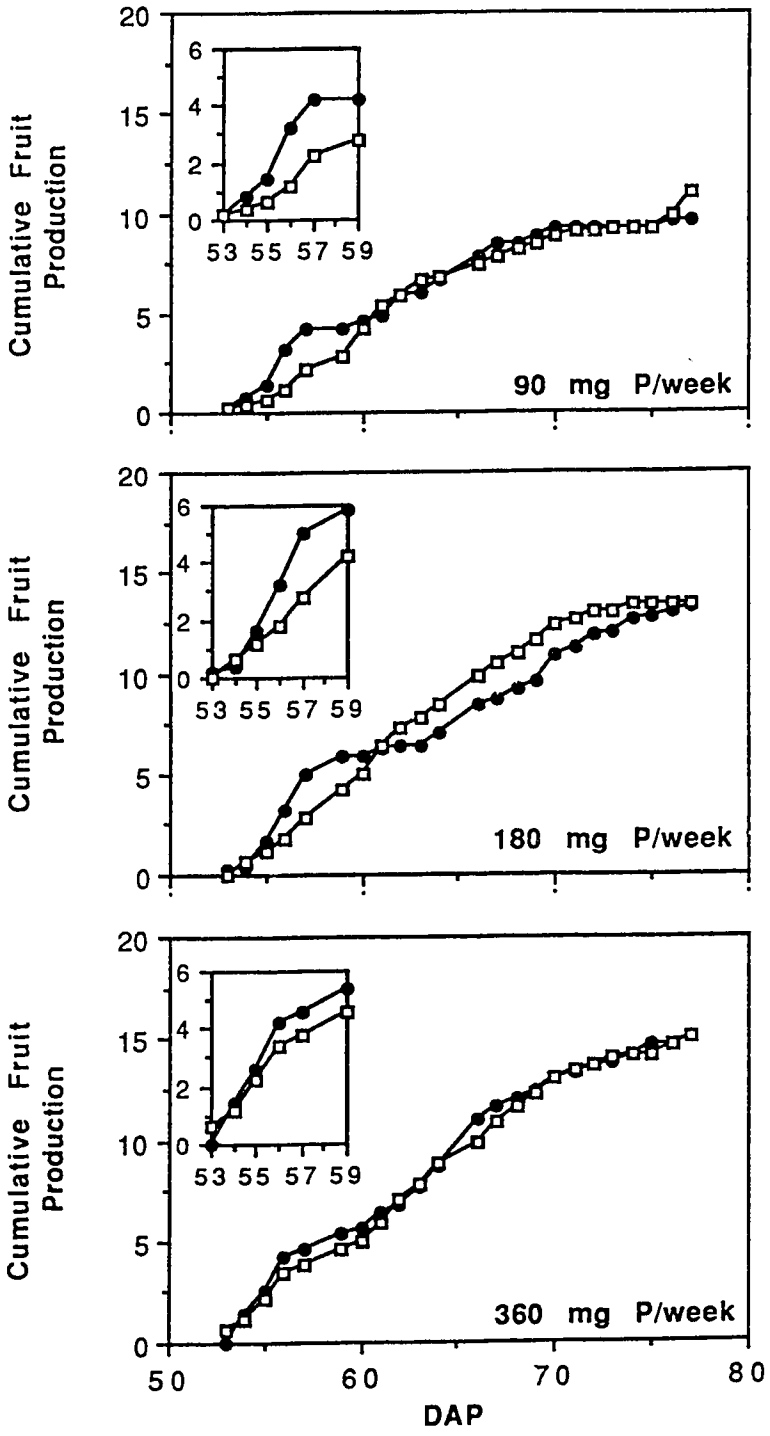
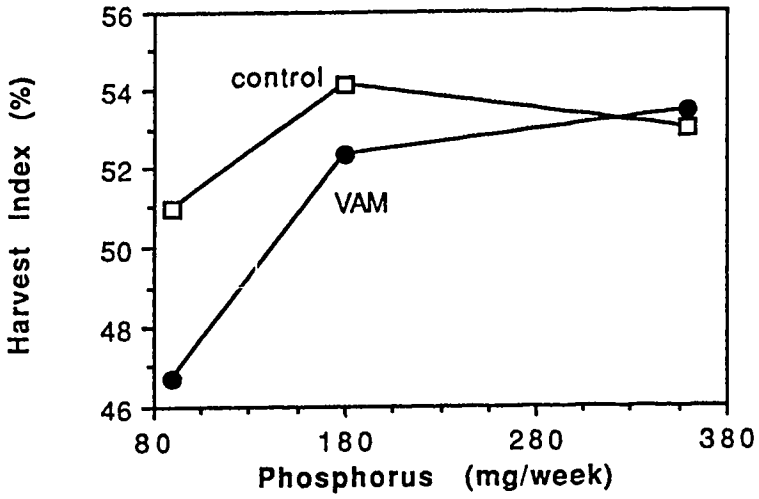

Cumulative Fruit Production
90 mg P/week
180 mg P/week
360 mg P/week
DAP

Figure III-11. Effects of VAM infection and P nutrition on the harvest index of 'Corona' cucumbers at crop termination (77 DAP). Plants were inoculated with *Glomus intraradices* at seeding. Harvest index was determined on a dry weight basis. F-values for the main effects of VAM, $[P]_{linear}$, $[P]_{deviations}$, and the interaction of VAM x $[P]_{linear}$, were significant at the 0.10, 0.01, 0.05 and 0.10 levels, respectively.


56
54
52
50
48
46
Harvest Index (%)
control
VAM
80
180
280
380
Phosphorus (mg/week)

0.10) to affect harvest index, such that at low-P levels, control plants partitioned 4.3% more of their total dry matter to fruits than VAM-infected plants, but at high-P levels, the harvest index was equal for control and VAM-infected plants.

Growth and yield response of 'Carmen'. Similar to the response of 'Corona', P nutrition significantly affected the foliar yield components of 'Carmen' cucumber plants by 92 DAP (crop termination). However, in contrast to the response of 'Corona', there were no significant growth effects due to VAM infection, nor any interactions between P nutrition and VAM infection. The foliar yield component data were thus averaged for VAM-infected and control plants (Table III-1). The number of nodes (leaf number) increased linearly with increasing P fertilization level, such that high-P (720 mg P/week) plants had 16% more nodes than low-P (90 mg P/week) plants. On average, plants had produced an additional leaf for every 100 mg increase in weekly P fertilization level by 92 DAP ($r^2 = 0.85$, $P < 0.01$). Leaf dry weight also increased in response to increasing P nutrition. Plants grown with 180, 360 and 720 mg supplemental P/week produced 17%, 16% and 19% more leaf dry matter than those grown with 90 mg P/week. Stem growth was particularly sensitive to P nutrition, as seen by a 30% increase in stem dry weight as the level of P increased from 90 to 720 mg/week. As with leaf dry weight, the growth response of stems to increasing P level was greatest between the two lowest P levels, and was much reduced between the higher levels of P nutrition. In contrast to the P-induced increase in stem density per meter characterized for 'Corona' plants in the previous study, stem density of 'Carmen' plants was not affected by P nutrition. The overall effect of P on foliar growth is reflected in the total shoot dry weight, which increased by 21% as P nutrition increased from 90 to 720 mg/week. Intermediate P levels stimulated shoot growth by an average of 18% over that from low-P plants. Hence, the greatest shoot growth response to P fertilization occurred between the 90 and 180 mg P/week treatments.

Phosphorus fertilization level greatly affected the concentration of P (mg/g dry wt) in leaf tissue by 92 DAP. The average leaf P concentration of high-P (720 mg P/week) plants (9.4 mg/g dry wt) was 213% greater than that of low-P (90 mg P/week) plants (3.0 mg/g dry wt). In fact, the

Table III-1. Effect of P fertilization level on foliar yield components of 'Carmen' cucumbers at crop termination (92 DAP). Data has been averaged for VAM-infected (*Glomus intraradices*) and control (nonmycorrhizal) treatments.

		Dry Weight			
Treatment	Nodes	Leaf	Stem		Shoot
mg P week^{-1}	(no. plant^{-1})	(g plant^{-1})	(g plant^{-1})	(g meter^{-1})	(g plant^{-1})
90	43.4	133.2	32.7	7.8	165.9
180	45.7	156.4	40.6	9.6	197.1
360	48.9	153.9	40.7	8.3	194.6
720	50.2	158.7	42.5	8.6	201.0
Linear [a]	0.01 [b]	0.05	0.01	ns	0.05
Quadratic	ns	ns	0.01	ns	0.10

[a] sources of variation

[b] significance levels for indicated sources of variation (ns, not significant)

leaf P concentration of low-P plants was well below the range considered sufficient (7 to 10 mg/g dry wt) for cucumber leaf tissue (Roorda van Eysinga and Smilde, 1981; Adams, 1978). Leaf P concentration was highest for younger leaves (those toward the shoot apex) and declined with increasing distance from the plant apex (Fig. III-12). The amount of decline in leaf P depended on the level of P nutrition. For plants grown with 720 mg P/week, leaf P concentration declined linearly by 1.00 mg/g dry wt every six leaves from the apex. For plants grown with 90 mg P/week, leaf P concentration fell from 6.00 to 2.97 mg/g dry wt over the first ten leaves, and then remained constant at about 2.3 mg/g dry wt for the remaining leaves. Moreover, low-P plants were under such a high level of P stress that their youngest leaf contained about the same concentration of P as that of the older, fully expanded base leaves of high-P plants. Phosphorus is a mobile element in plants and, upon mobilization from older leaves, is transported to support new growth (Brady, 1984). This results in the appearance of various P deficiency symptoms in older leaves, eventually contributing to their senescence. Symptoms of P deficiency were readily apparent on older leaves of the low-P cucumber plants (Fig. III-13).

Fruit harvest from 'Carmen' cucumber plants began 54 DAP and significant effects of P nutrition on yield were clearly evident by 70 DAP. The cumulative number of fruit produced per plant increased linearly with increasing P nutrition, such that plants grown with 720 mg P/week produced 36% more fruit than those grown with 90 mg P/week by 70 DAP (Table III-2). The linear yield response to increasing P nutrition was even more apparent by 92 DAP, with high-P (720 mg P/week) plants producing 41% more fruit than low-P (90 mg P/week) plants. Moreover, from 70 to 92 DAP, fruit yield from low-P plants increased by 55% (4.8 fruit/plant), compared with a 62% (7.3 fruit/plant) increase from high-P plants. When averaged over all levels of P nutrition, VAM-infected plants had produced 10% more fruit (11 fruit) than control plants (10 fruit) by 70 DAP ($P < 0.05$); however, VAM infection had no effect on final fruit production (92 DAP). It appeared that the slight stimulation of early yield by VAM was lost due to a longer fruit abortion period (flattened area of production curve) in VAM-infected plants relative to control plants (Fig III-14). There were no significant effects of P nutrition or VAM infection on average fruit weight. At 92 DAP, the

Figure III-12. The concentration of P (mg g^{-1} dry wt) in leaves of 92-day-old 'Carmen' cucumber plants grown with 90 (□) or 720 mgP/week (●). Phosphorus content was determined for leaves harvested from alternate nodes starting at the shoot apex (youngest leaf) and continuing through node twenty six (alternate leaf number thirteen). F-values for the main effect of [P] and the interactions of [P] x $Leaf_{linear}$ and [P] x $Leaf_{quadratic}$ were significant at the 0.01 level.

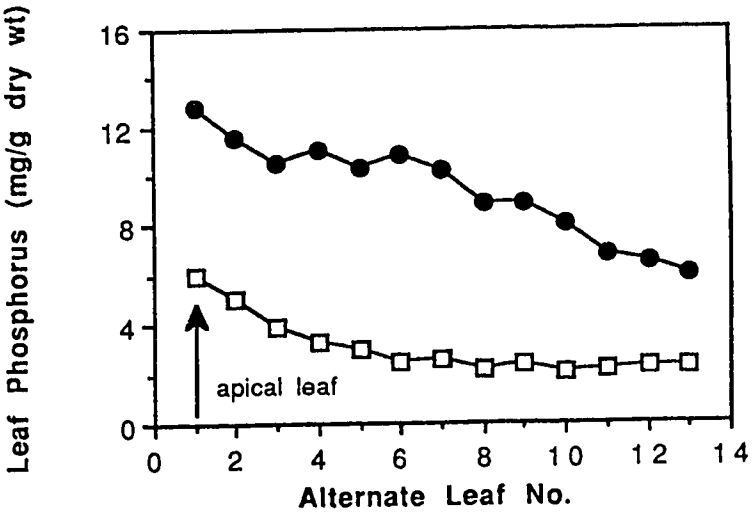

Leaf Phosphorus (mg/g dry wt)
16
12
8
4
0
0
2
4
6
8
10
12
14
apical leaf
Alternate Leaf No.

Figure III-13. Advanced symptom of P deficiency in older, fully expanded leaves of 'Carmen' cucumber plants grown with 90 mg P/week. Phosphorus deficiency characteristically resulted in localized, interveinal lesions of desiccated tissue (B) that rapidly spread (3 to 5 days) across the entire leaf (A), leaving a dry and brittle remnant.

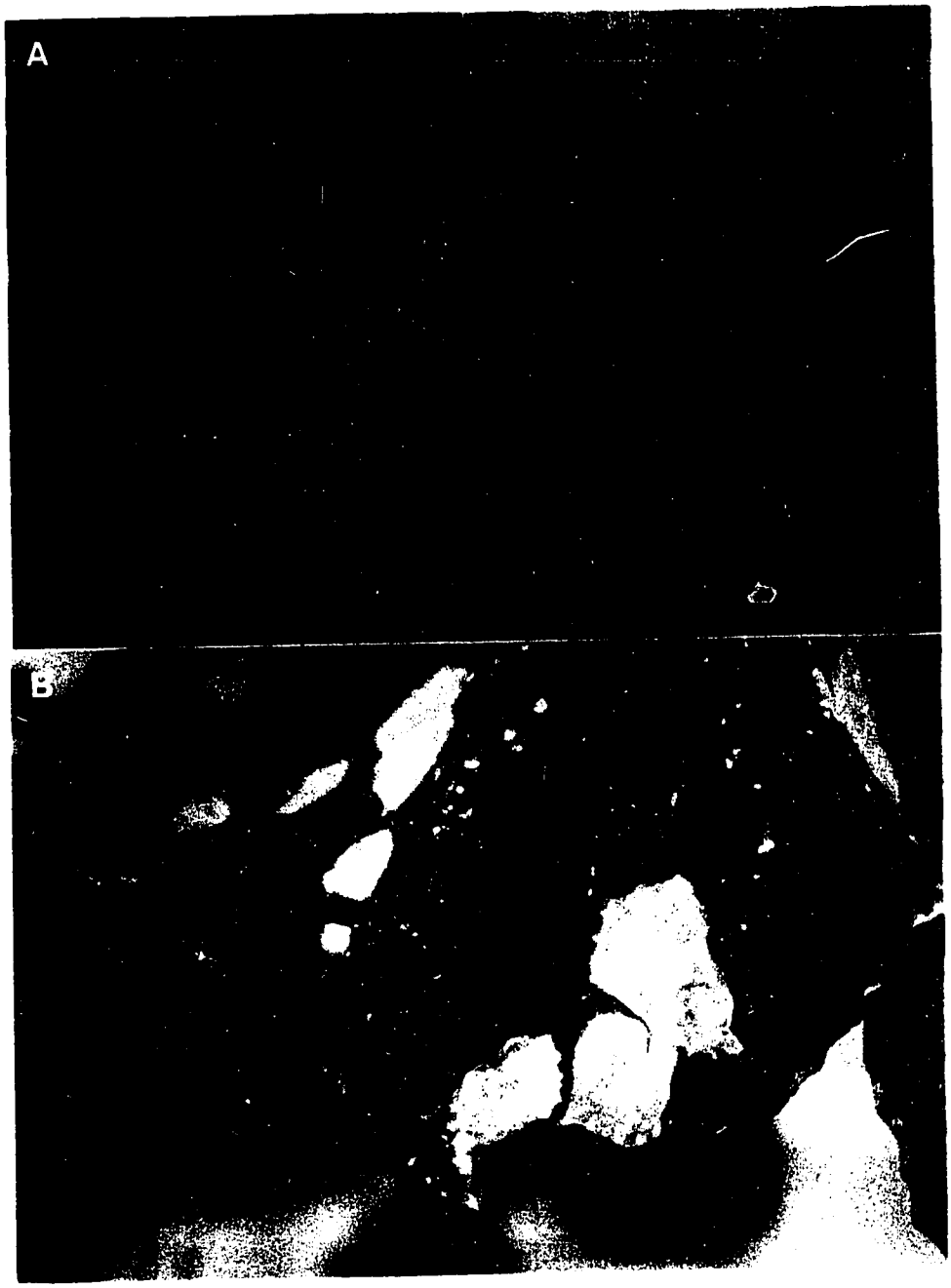

A
B

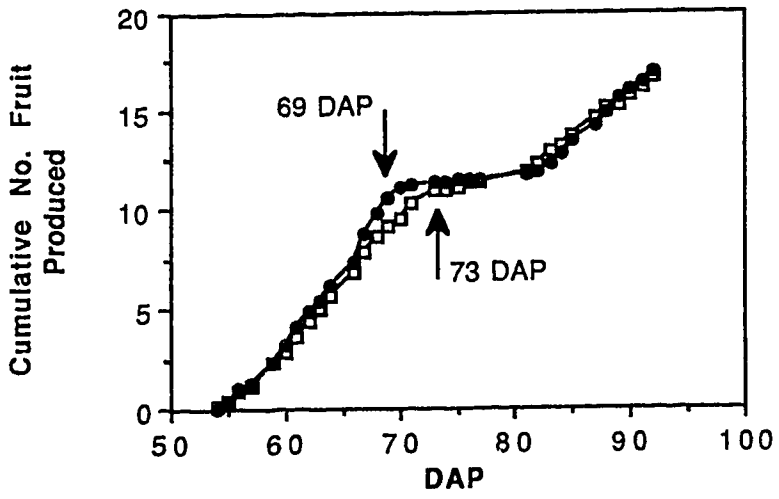
Table III-2. Cumulative number of marketable fruit produced by 'Carmen' cucumber plants at 70 and 92 DAP as affected by P nutrition. Phosphorus nutrition had no effect on average fruit size. The harvest index was determined on a dry weight basis (see Materials and Methods). Data has been averaged for VAM-infected (*Glomus intraradices*) and control (nonmycorrhizal) treatments.

Treatment	Number of Marketable Fruit		Harvest Index
(mg P week^{-1})	70 DAP	92 DAP	(%)
90	8.7	13.5	55.7
180	10.7	17.4	57.6
360	11.2	17.0	57.7
720	11.8	19.1	59.5
Linear [a]	0.01 [b]	0.01	0.01
Quadratic	ns	ns	ns

[a] sources of variation

[b] significance levels for indicated sources of variation (ns, not significant)

Figure III-14. Cumulative production of marketable fruits from control (□) and VAM-infected (●) 'Carmen' cucumber plants, averaged over four levels of P nutrition. Arrows indicate the time at which fruit production rate decreased due to fruit abortion.


Cumulative No. Fruit Produced
20
15
10
5
0
69 DAP
73 DAP
50
60
70
80
90
100
DAP

harvest index (HI) increased linearly with increasing P fertilization level, such that for every 150 mg increase in weekly P, plants partitioned 1% more of their dry matter into fruit production (Table III-2). Hence, increasing P nutrition improved the efficiency of dry matter partitioning to fruits.

Growth and yield response of 'Corona' and 'Carmen'. By 96 DAP, P fertilization level and VAM infection (*Glomus intraradices*) had significantly influenced most of the foliar growth parameters of 'Carmen' and 'Corona' cucumber plants (Tables III-3 and 4). The average number of nodes (leaves) increased linearly (r^2 = 0.96, P<0.01) with increasing P nutrition (Table III-3). Medium-P (180 mg P/week) and high-P (360 mg P/week) plants produced 7% and 13% more leaves (nodes), respectively, than low-P (90 mg P/week) plants. Moreover, when averaged over all levels of P nutrition, 'Corona' plants produced significantly (P< 0.01) more nodes (5%) than 'Carmen' plants. The P-induced increase in leaf number was equal for both cultivars by 96 DAP, and averaged 2.4 leaves for every 100 mg/week increase in P. The effect of VAM on the number of leaves (nodes) per plant was independent of P nutrition and differed depending on the cultivar (Table III-4). Relative to their respective noninfected controls, VAM-infected 'Carmen' plants produced 3% fewer leaves per plant, while VAM-infected 'Corona' plants produced 3% more leaves per plant.

When averaged over P and VAM treatments, the total leaf dry matter produced by 'Corona' plants was 24% greater than that from 'Carmen' plants after 96 days of growth (Table III-3). However, leaf dry weight per plant increased with increasing P nutrition (P< 0.01), and the trend was different for each cultivar. Leaf dry weight of 'Corona' plants increased linearly (r^2 = 0.93, P< 0.01) in response to increasing P fertilization level, with medium-P and high-P plants producing 19% and 34% more leaf dry weight, respectively, than low-P plants. This translated into a leaf dry matter increase of 151 mg for every mg increase in weekly P fertilization level. In contrast, the medium-P level stimulated the greatest increase in leaf dry weight of 'Carmen' plants; 18% greater than the low-P level and 12% greater than the high-P level. When averaged for the two cultivars, VAM infection did not influence leaf dry weight, and there was no VAM by cultivar interaction. However, when the response of 'Carmen' plants to VAM infection was analyzed separately (i.e.

Table III-3. A comparison of the effects of P nutrition on foliar yield components of 96-day-old 'Corona' and 'Carmen' greenhouse cucumber plants. Data has been averaged for control and VAM-infected treatments.

Treatment		Nodes	Dry Weight			
			Leaf	Stem		Shoot
Cultivar	[P] week^{-1}	(# plant^{-1})	(g plant^{-1})	(g plant^{-1})	(g metre^{-1})	(g plant^{-1})
Corona	90	52.5	127.6	30.3	6.77	157.9
	180	54.5	152.1	33.8	6.82	186.0
	360	59.6	170.4	37.5	7.02	207.8
Carmen	90	49.1	112.5	26.3	6.52	138.8
	180	54.3	132.7	29.2	6.46	161.9
	360	55.6	118.0	26.1	5.72	144.1
Cultivar [a]		0.01 [b]	0.01	0.01	0.01	0.01
[P]		0.01	0.01	0.05	ns	0.01
Cultivar x [P]		ns	0.01	0.05	0.10	0.01
$[P]_{lin}$ (Corona)		0.01	0.01	0.01	ns	0.01
$[P]_{dev}$ (Corona)		ns	ns	ns	ns	ns
$[P]_{lin}$ (Carmen)		0.01	ns	ns	0.05	ns
$[P]_{dev}$ (Carmen)		0.05	0.05	0.10	ns	0.05

[a] Sources of variation. The experiment was analyzed as a two-factor (cultivar and P-level) factorial and, in addition, the effect of P was analyzed separately for each cultivar (single factor ANOVA) (lin, linear trend; dev, deviations from the linear trend)

[b] significance levels for indicated sources of variation (ns, not significant)

Table III-4. Effect of VAM infection (*Glomus intraradices*) on foliar yield components of 96-day-old 'Corona' and 'Carmen' greenhouse cucumber plants. Data has been averaged over three levels of P nutrition.

Treatment		Nodes	Dry Weight			
			Leaf	Stem		Shoot
Cultivar	VAM	(# plant^{-1})	(g plant^{-1})	(g plant^{-1})	(g metre^{-1})	(g plant^{-1})
Corona	-	54.8	150.0	33.5	6.86	183.4
	+	56.3	150.1	34.3	6.59	184.4
Carmen	-	53.9	128.5	29.1	6.87	157.6
	+	52.1	113.7	25.3	5.88	138.9
Cultivar [a]		0.01 [b]	0.01	0.01	0.01	0.01
VAM		ns	ns	ns	ns	ns
Cultivar x VAM		0.05	ns	0.05	0.10	0.10
VAM (Corona)		ns	ns	ns	ns	ns
VAM (Carmen)		ns	0.05	0.05	0.05	0.05

[a] Sources of variation. The experiment was analyzed as a two-factor (cultivar and VAM) factorial and, in addition, the effect of VAM was analyzed separately for each cultivar (single factor ANOVA) (lin, linear trend; dev, deviations from the linear trend)

[b] significance levels for indicated sources of variation (ns, not significant)

using a single factor ANOVA), nonmycorrhizal plants had produced 13% more leaf dry weight than VAM-infected plants by 96 DAP (Table III-4).

On average, 'Corona' plants produced 25% more stem dry matter than 'Carmen' plants, but cultivar interacted with P nutrition to affect this foliar yield component (Table III-3). Stem dry matter of 'Corona' plants increased linearly with increasing P nutrition (2.6 mg per 100 mg P/week increase, $r^2 = 0.97$), such that the medium-P and high-P treatments resulted in 12% and 24% more stem dry weight, respectively, than the low-P treatment. For 'Carmen' plants, the medium-P fertilization level resulted in the greatest stem dry weight. The effect of VAM on stem dry weight depended on cultivar (Table III-4). Stem dry matter of 'Corona' plants was not affected by VAM infection; however, VAM-infected 'Carmen' plants had 13% less stem dry weight than control plants.

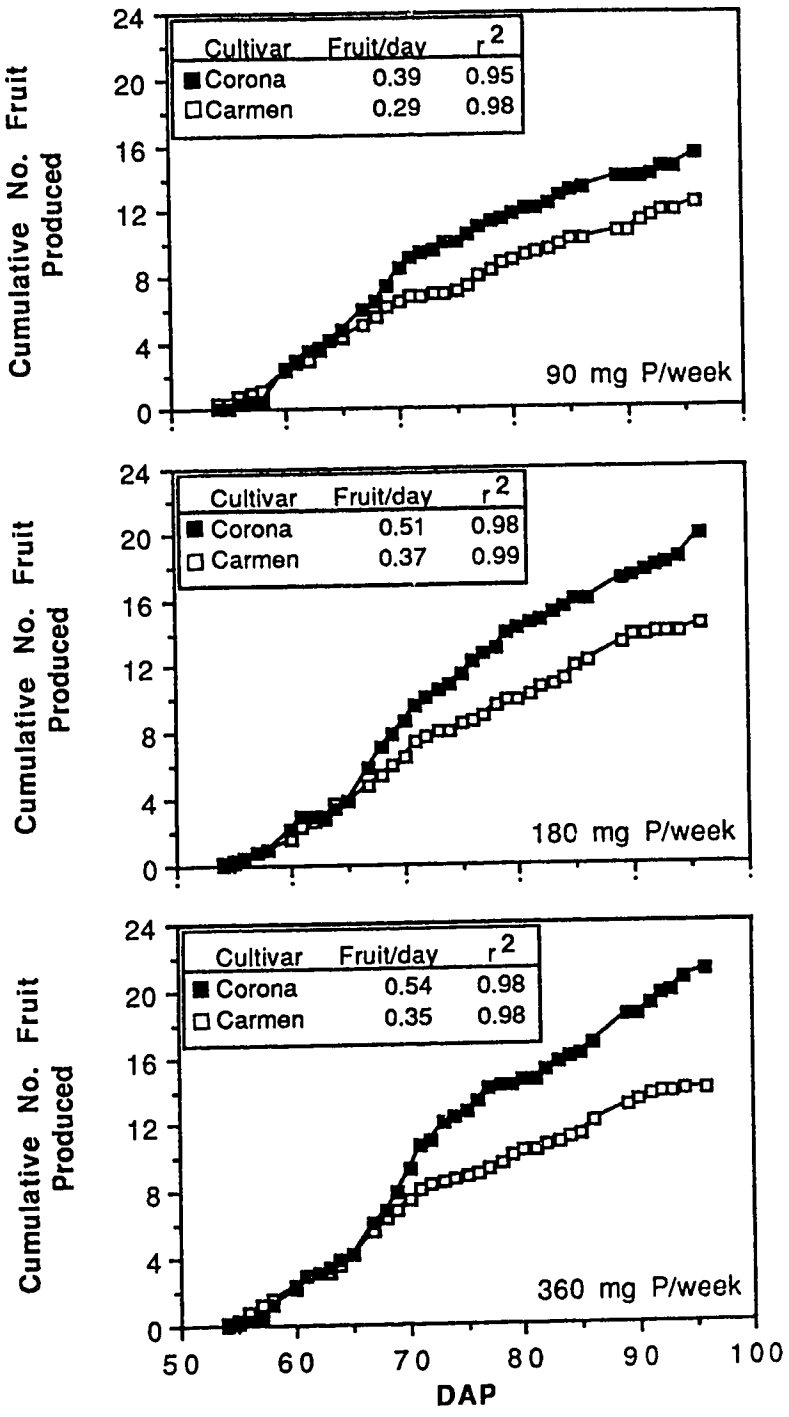
On a per meter fresh length basis, stems of 'Corona' plants were 10% heavier than stems of 'Carmen' plants (Table III-3). Moreover, P nutrition interacted ($P< 0.10$) with cultivar to affect the length-based stem density. Unit density of stems of 'Corona' plants remained relatively constant as P nutrition increased, whereas that of 'Carmen' plants decreased linearly with increasing P nutrition. The effect of VAM on stem unit-length density depended on cultivar. 'Carmen' control plants had 17% heavier stems per meter than VAM-infected plants (Table III-4). In contrast, stem unit weights of 'Corona' control and VAM-infected plants were equal. Furthermore, VAM infection interacted with the level of P nutrition to affect stem unit weights of 'Carmen' plants (data not shown). When grown for 96 days with 90 and 180 mg P/week, 'Carmen' plants produced stems that were 12% and 29% heavier per meter, respectively, than those of VAM-infected plants. Conversely, with 360 mg P/week, stems of VAM-infected 'Carmen' plants were 4% heavier per meter than those of nonmycorrhizal plants after 96 days of growth (VAM x [P] deviations, $P< 0.10$).

When averaged over all treatments, 'Corona' plants produced 24% more shoot dry matter than 'Carmen' plants by 96 DAP (Table III-3). More importantly, the shoot dry weight response to P nutrition differed between the two cultivars. Shoot dry weights of 'Corona' plants grown with 180

and 360 mg P/week were 18% and 32% greater, respectively, than that of plants grown with 90 mg P/week. In contrast, 'Carmen' plants grown with 180 mg P/week produced 17% more shoot dry matter than those grown with 90 mg P/week, and 12% more than those grown with 360 mg P/week. VAM infection did not alter final shoot dry weight of 'Corona' plants relative to controls; however, nonmycorrhizal 'Carmen' plants produced 13% more shoot dry matter than those infected with VAM (Table III-4).

Total fruit production by 'Corona' and 'Carmen' plants at crop termination (96 DAP) was significantly influenced by P fertilization level. When averaged over cultivar and VAM treatments, plants grown with 180 and 360 mg P/week had produced 21% and 24% more fruit by 96 DAP, respectively, than those grown with 90 mg P/week ($P < 0.01$). However, the yield response of the two cultivars to increasing P nutrition differed. (Fig. III-15). At the lowest level of P nutrition, the final yield of 'Corona' plants (15.5 fruit/plant) was 23% higher than that of 'Carmen' plants (12.6 fruit/plant). The difference in final yield between the two cultivars increased substantially with increasing P level and, at the highest level of P nutrition, 'Corona' plants had produced 50% more fruit than 'Carmen' plants (21.0 versus 14.0 fruit/plant). The yield response of 'Corona' plants was thus much more sensitive to increases in P nutrition than that of 'Carmen' plants. These differences in final yield were due to treatment effects on the rates of fruit production (Fig. III-15 insets). For example, over the 43 day production period, 'Corona plants produced fruit 34% faster than 'Carmen' plants when both were grown with 90 mg P/week. Moreover, the rate of fruit production from "Corona' plants increased by 38% as P nutrition increased from 90 to 360 mg/week, while that from 'Carmen' plants increased by only 21%. At the highest level of P nutrition, 'Corona' plants produced fruit 54% faster than 'Carmen' plants. It should be noted that cultivar differences in the overall rates of fruit production were manifested later in the study. From 54 to 66 DAP, yield and rates of fruit production were equal for the two cultivars at all levels of P nutrition. At about 67 DAP, the rate of fruit production by 'Corona' plants increased relative to that from 'Carmen' plants (Fig. III-15).

Figure III-15. Effect of P nutrition and cultivar on cumulative fruit production from 'Corona' and 'Carmen' cucumber plants. Plants were inoculated at seeding with *Glomus intraradices* and were grown with 90, 180 or 360 mg supplemental P per week in a greenhouse. Data for nonmycorrhizal and VAM-infected plants has been averaged. Insets depict the significant ($P<0.01$) linear regression coefficients and coefficients of determination for each cultivar at each level of P nutrition. The main effects of cultivar, [P] and time, and the interactions of cultivar x [P] and cultivar x [P] x time, were significant at the 0.01 level.


Cumulative No. Fruit Produced
Cultivar Fruit/day r 2
Corona 0.39 0.95
Carmen 0.29 0.98
90 mg P/week
Cumulative No. Fruit Produced
Cultivar Fruit/day r 2
Corona 0.51 0.98
Carmen 0.37 0.99
180 mg P/week
Cumulative No. Fruit Produced
Cultivar Fruit/day r 2
Corona 0.54 0.98
Carmen 0.35 0.98
360 mg P/week
0 4 8 12 16 20 24
50 60 70 80 90 100
DAP

When averaged over cultivar and level of P nutrition, VAM-infected (*Glomus intraradices*) plants produced 8% more fruit than control plants by 96 DAP (16.8 fruit versus 15.6 fruit) ($P< 0.05$). This average increase in overall yield was due entirely to an 10% lower yield from VAM-infected 'Corona' plants, relative to controls ($P< 0.05$). Total fruit production from 'Carmen' plants was not influenced by VAM infection.

The average fresh weight of individual fruits produced by 'Carmen' plants was not influenced by P nutrition or VAM infection. However, for 'Corona' plants, average fruit weight was dictated by an interaction between VAM infection and P nutrition (VAM x P_{linear}, $P< 0.10$). When grown with 90 mg P/week, nonmycorrhizal plants produced fruit (366.5 g) that were 2% heavier than those from VAM-infected plants (358.1 g); however, when grown with 360 mg P/week, fruits from VAM-infected plants(386.9 g) were 7% heavier than those from noninfected plants(361.9 g). The effects of VAM on fruit yield and weight resulted in small differences in harvest index (dry weight basis) between the two cultivars at 96 DAP ($P< 0.10$). The HI of nonmycorrhizal 'Corona' plants was 5% higher than that for VAM-infected plants (64.1% HI versus 61.6% HI), whereas the HI of VAM-infected 'Carmen' plants was 3% higher than that for noninfected plants (61.6% HI versus 60.6% HI) (VAM x cultivar, $P< 0.10$).

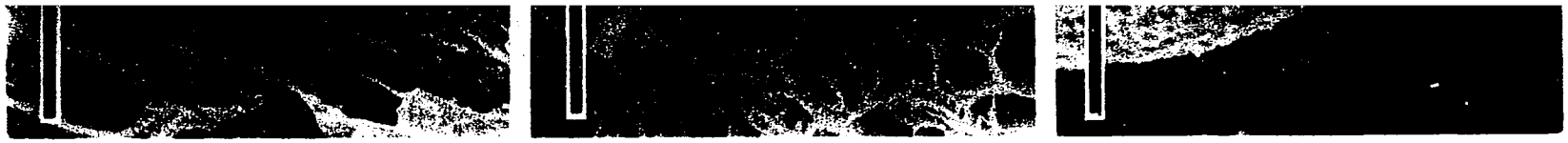
DISCUSSION

The large quantity of foliage and fruit produced by mature cucumber plants (Fig. III-2) necessitates large amounts of water (up to 5L/plant/day) and nutrients to support this growth. Figure III-16 illustrates the massive vessel members of the root xylem tissue which facilitate water and nutrient transfer to the foliar and fruit tissues. Cucumber plants have rapid growth rates and thus require high quantities of P to maintain growth and fruit production (Hayman, 1983). Low rates of P fertilization (90 mg P/week) were shown to restrict shoot development and yield when cucumber plants were in the fruit-bearing stage of growth.

Foliar development was enhanced by increasing P fertilization level in each of the three studies, which is consistent with results reported for potatoes (McArthur and Knowles, 1993),

Figure III-16. (A) Transverse section of a cucumber root showing the large quantity of xylem tissue (x) necessary to meet the extensive nutrient and water requirements of the mature plant. Note that the cortex has been sloughed off leaving the endodermis (e) surrounding the vascular system (bar = 200 μm). (B) and (C) Close-up view of root xylem consisting of large vessel members (vm), tracheary elements (te) and pits (arrow). Bar = 10 μm in both (B) and (C).

A
B
C



onions (Smith *et al.*, 1986), pepper (Sreenivasa *et al.*, 1993) and soybean (Fredeen *et al.*, 1989). Shoot dry weights increased substantially as P fertilization increased in all of our studies. More importantly, the number of main stem nodes produced in each of the three studies (Fig. III-8, Tables III-1 and 3) increased significantly with increased P fertilization. Fruit is produced in the axils of the leaves at stem nodes. Thus, more nodes equate to more sites available for fruit production. Increased P fertilization level significantly increased the total number of fruit produced per plant (Figs. III-10 and 15, Table III-2). The harvest index indicates the proportion of biological yield that is of economic importance. In our studies, the harvest index ranged from 48% to 60%, depending upon the level of P fertilization. The significant increase in harvest index with increased P nutrition (Fig. III-11, Table III-2) underscores the importance of P to shoot growth and fruit production, and indicates an increased efficiency for fruit production at high-P levels. Low-P nutrition (90 mg P/week) resulted in P deficiency symptoms (Fig. III-13). The concentration of P within leaves of these plants (Fig. III-12) was well below that recommended for cucumber leaf tissue (Roorda van Eysinga and Smilde, 1981; Adams, 1978). Higher P levels (720 mg P/week) resulted in leaf P concentrations that are considered nonlimiting to growth, and when leaf P concentrations are compared with growth data (Table III-1), a P fertilization level of about 360 mg P/week appeared to be the most beneficial to the plant. The P fertilization level recommended for greenhouse cucumber production in soilless culture systems in Alberta is approximately equivalent to 360 mg P/week (Mirza, 1990).

Mature 'Corona' (Figs. III-5, 6, 7) and 'Carmen' cucumber plants demonstrated a high compatability to colonization by *Glomus intraradices* at low-P levels. The level of infection of both cultivars was greatly reduced as the level of P fertilization increased; however, 'Corona' plants tolerated a higher level of VAM infection at all P levels (Fig. III-3). On average, VAM infection of 'Corona' plants decreased foliar growth relative to that from nonmycorrhizal plants. On the other hand, foliar growth of 'Carmen' plants was either not affected, or was slightly depressed by VAM infection, depending on the study. This is contrary to results obtained in potato, strawberry, pepper, and onion where VAM infection increased shoot growth (McArthur and Knowles, 1993;

Vestberg, 1992; Sreenivasa *et al.*, 1993; Smith *et al.*, 1986, respectively). Considerable variability has been found among cultivars in their response to the same VAM species under comparable treatments (Miller, 1986). The variability between 'Carmen' and 'Corona' cucumbers in foliar growth response to VAM infection may be based upon differences in root colonization level, since the VAM carbon requirements are met by the host plant (Miller, 1986). This may also indicate why VAM-infected plants produced less shoot dry matter under low-P conditions than control plants. The high VAM infection level of plants grown with low-P, when coupled with the carbohydrate demand of fruit production, may have constituted such a large nonphotosynthetic sink for carbon that the plant became more stressed and produced less foliar dry weight than nonmycorrhizal plants grown with low levels of P. At high-P levels, when VAM infection percentage was low (<10%), shoot dry weight of VAM-infected plants was comparable to that produced by control plants. VAM infection may increase the number of fruit produced in the first one to two weeks of the production period; however, by crop termination, VAM infection had no effect on the total number of fruit produced per plant (Fig. III-10). The early effect of VAM on fruit production may have been due to the fact that VAM substantially enhanced growth of young cucumber plants during establishment (see Chapter II), resulting in larger, more vigorous plants at the onset of fruit production. However, by crop termination, the carbon requirements needed to support the VAM infection may have reduced plant vigour, such that any initial increase in fruit production was lost. The extended fruit abortion period evident in VAM-infected plants, relative to nonmycorrhizal plants (Fig. III-14), may reflect the greater carbon cost of the symbiosis. Hence, fruit-bearing, VAM-infected plants may be under a greater stress than nonmycorrhizal plants, resulting in an attenuation of the potential early benefits of VAM on total fruit yield.

'Corona' plants produced significantly more fruit and had greater shoot growth than 'Carmen' plants for all P and VAM treatments. Thus, it would appear that 'Corona' is a more efficient cultivar in terms of dry matter produced per unit P required. The proportion of total plant dry matter distributed to fruits was equal for the two cultivars, resulting in equal harvest indices, even though 'Corona' plants produced more shoot and fruit dry matter than 'Carmen' plants at a

given P fertilization level. Hence, on a total dry matter basis, the two cultivars are equally efficient at allocating dry matter to fruit production.

This study characterizes the growth responses of mature, fruit-bearing cucumber plants to P nutrition and VAM infection. The level of P nutrition was very important in dictating the number of marketable fruit produced per plant. Previous studies showed that VAM accelerated the early development of young cucumber plants. VAM stimulated early fruit production and, depending on the cultivar, fruits from VAM-infected plants were slightly larger than those from noninfected plants. However, since VAM infection either slightly decreased or did not affect the total number of fruit produced per plant, the yield from cucumber crops grown over an extended season such as produced in Alberta (Mirza, 1990) may not benefit from VAM infection. Multiple, short-season cucumber crops (Adamson and Maas, 1981) that require vigorous and fast-growing transplants would probably benefit the most from a VAM infection.

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Chapter IV

POSTHARVEST FRUIT RESPIRATION AS AFFECTED BY P STATUS AND MYCORRHIZAL INFECTION

INTRODUCTION

Phosphorus (P) is an essential nutrient required by plants for numerous metabolic processes. Plants benefit from P in many ways, including flowering and fruiting, improved crop quality (especially of vegetables), and crop maturation (Brady, 1984). Phosphorus deficiency symptoms in cucumbers include plant stunting and desiccation of older leaves (Roorda van Eysinga and Smilde, 1981; Mirza, 1990), resulting in lower plant vigor and reduced fruit production (Chapter III). Phosphorus deficiency may also result in an increase (up to three-fold) of poorly developed fruit (Adams, 1978).

The mutualistic relationship between vesicular-arbuscular mycorrhizal (VAM) fungi and plants is facilitated by nutrient exchange in the root cortex. The VAM fungus benefits plant growth primarily through enhanced uptake of relatively immobile soil nutrients such as P, zinc and copper, in exchange for plant carbohydrate, which the fungus uses to support its metabolism (Miller, 1986; Gianinazzi-Pearson and Gianinazzi, 1983; Hayman, 1983). However, depending on their stage of development, VAM-infected plants may exhibit growth depression when compared with nonmycorrhizal plants, and this may be caused by increased fungal competition for carbohydrate (Gianinazzi-Pearson and Gianinazzi, 1983). When the additional nutrient demand of fruit production is considered, the carbohydrate expense of supporting both fruit production and VAM infection will be great. Fruit growth is sustained by a combination of newly formed carbohydrate from photosynthesis, mobilization and translocation of existing carbohydrate and other nutrient stores from vegetative to reproductive tissues, and uptake and translocation of mineral nutrients from roots to the fruit (Koide, 1991). Thus, even though VAM infection enhances the uptake of certain nutrients, the increased demand for carbohydrates resulting from

simultaneous fruiting and VAM infection may be too much for cucumber plants to maintain. Probable symptoms of such a source-sink imbalance would be a greater amount of fruit abortion and slower vegetative growth in VAM-infected plants, relative to that occuring in nutritionally equivalent nonmycorrhizal plants (Chapter III).

The European seedless cucumber (*Cucumis sativus* L.) plant requires high nutrient levels, high light intensity, a warm growing environment and a large amount of water for maximum fruit production (Wittwer and Honma, 1979; Mirza, 1990). Under optimal conditions, plants should produce marketable fruit fifty to sixty days after planting (DAP) (Adamson and Maas, 1981); however, marketable fruit may not necessarily be of the highest quality. In this regard, the physiological age of a cucumber fruit appears to have a strong negative influence on its shelf-life, i.e. the longer it takes a fruit to mature from the onset of flowering, the shorter the shelf-life (Lin and Ehret, 1991). Cucumbers produced on young, vigorous plants quickly reach commercial maturity and, therefore, will be of higher marketable quality (Adamson and Maas, 1981). Also, since an actively growing fruit exerts an inhibitory effect on subsequent fruit set and growth, the faster a fruit reaches harvest maturity, the less time subsequent fruit growth will be inhibited (Kanellis *et al.*, 1986). Phosphorus nutrition had a major effect on cucumber plant growth and rate of fruit production (see Chapter III). This study was conducted to determine how P nutrition and VAM infection affect fruit P concentration, and to determine if a relationship exists between P content and respiration rate of harvested cucumber fruit. High postharvest respiration rates may limit the shelf-life of fruit, thus revealing a lower quality fruit.

MATERIALS AND METHODS

Growing Conditions. Cucumber plants (cultivar Carmen) were inoculated at the time of seeding with *Glomus intraradices* Schenck and Smith spores as previously described (see Chapter 3). Seeds were sown 1.5 cm deep in VAM inoculated or control media (Chapter 3, first study) in 15 L pots (3 seeds/pot). At 12 DAP (first to second true leaf stage), plants were thinned to one plant per pot, replicates were blocked for size, and three P fertilization levels and two VAM

treatments (one VAM species and a nonmycorrhizal control) were randomized within each replicate (4 replicates total). The study was conducted in a University of Alberta research greenhouse from September 4 to November 24, 1992 under 16 h supplemental light (450 μE m^{-2} sec^{-1} at mature mid canopy) provided by HID lamps with high pressure sodium bulbs (Sylvania 400 watt). The greenhouse temperature was maintained between 21 and 25 ^{o}C. Plants were pruned and trained to maintain a single leader according to guidelines for a sequence cropping production system (Mirza, 1990; Adamson and Maas, 1981). Insects were controlled by a combination of chemical and biological methods. Thrips (*Frankliniella occidentalis*) were controlled by spraying the soil with Diazinon (2 mL/L, 12.5% a.i.) at 28 DAP, coupled with the release of a thrips predator, *Amblesius cucumeris*, at two-week intervals throughout the study. Whiteflies (*Trialeurodes vaporariorum*) were controlled by the whitefly predator, *Encarsia formosa*. Mites were controlled by weekly applications of a Vendex 50W (1g/L, fenbutatin oxide 50%) and Safer's insecticidal soap (7.5 mL/L) mixture to the apical meristem.

Depending on treatment, plants were fertilized with 250 mL of 0, 120, or 480 ppm P (KH_2PO_4), three times per week, starting at 26 DAP. Thus, P treatments consisted of 0, 90 or 360 mg of elemental P per plant per week. Potassium levels were held constant by adding KCl to the 0 and 90 mg P/week treatments. All other nutrients were applied uniformly to each pot in the experiment by an automated fertigation system (Harrow Fertigation Manager, Labbate Control Systems, Leamington, ON) at concentrations equivalent to those described in the methods section of Chapter III (third study). Each plant initially received 1L/day of nutrient solution starting 20 DAP. The volume of solution and concentration of nutrients were gradually increased with plant growth, so that by 63 DAP each plant received 4 L/day. Micronutrients were also included in the fertigation solution at concentrations previously described (Chapter III).

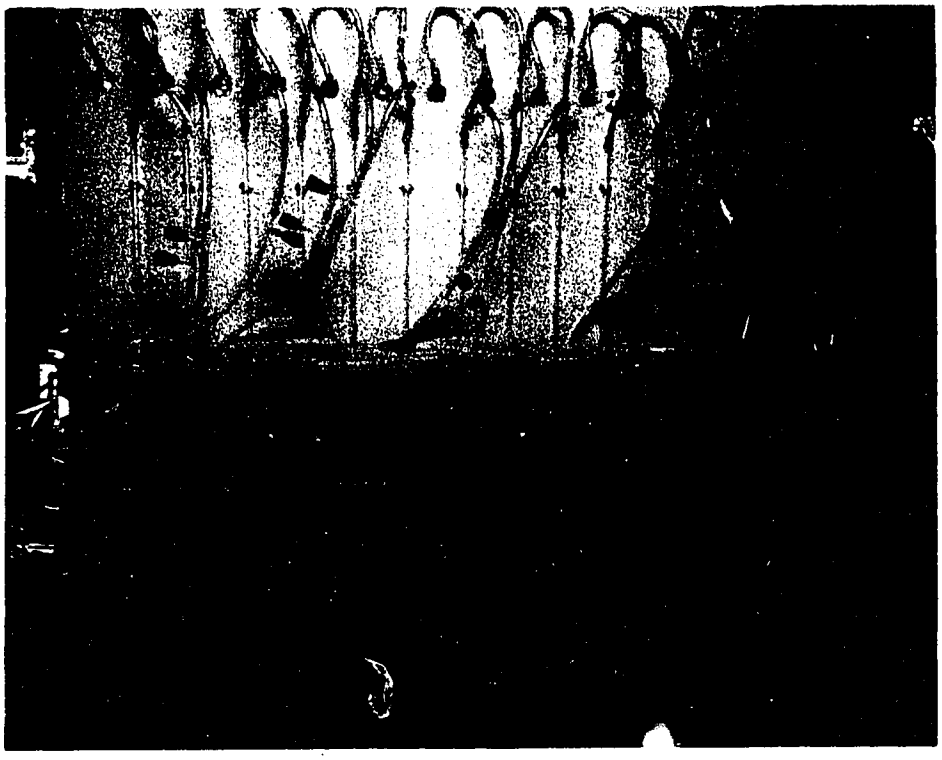
Fruit was harvested after reaching a minimum of 30 cm length and 42 mm diameter (Canada #1 Grade; Mirza, 1990), which corresponded to at least 300 grams fresh weight. Fruit fresh weight and fruit number were recorded. Fruit harvest started 48 DAP and the experiment was terminated 82 DAP.

Fruit Respiration. Fruit were harvested from three replicates of low-P (90 mg P/week) and high-P (360 mg P/week) control and VAM-infected plants at approximately 54 and 77 DAP. To profile respiration rates, single fruit were placed in plastic chambers (5.7 L) that had inlet and outlet ports. The chambers were attached to a flow-board (Fig.IV-1) which distributed a constant flow of humidified air (120 mL min^{-1}) to each chamber. Fruit respiration was determined daily by calculating the difference in CO_2 concentration between inlet and outlet ports of each chamber through 10 or 16 days for fruit harvested at 54 or 77 DAP, respectively. Carbon dioxide was analyzed by injecting 1 mL gas samples from the ports of each chamber into a Hewlett-Packard 5890A gas chromatograph (GC). The GC had a 2.4 m stainless steel column (3.2 mm o.d.) packed with HayeSep T (Hewlett-Packard) and a thermal conductivity detector. The carrier gas (He) flow rate was 30 mL min^{-1}, and the column was isothermal at 100 ^{o}C. Injector and detector port temperatures were 140 ^{o}C. Cucmber respiration rates were expressed as µL CO_2 min^{-1} kg^{-1} fresh weight.

Fruit Phosphorus Analysis. At termination of respiration monitoring, the fruit was sliced longitudinally, frozen, lyophilized and stored for further analysis. The dried fruit tissue was ground with a Wiley mill through a 40 mesh screen and 100 mg of the ground tissue was ashed overnight in a muffle furnace (550 ^{o}C). The ash was acid digested in 1 mL of HCl for 20 minutes, diluted with 9 mL of 0.72 N H_2SO_4 and centrifuged (1640 g, 30 minutes) to settle any undigested matter. Total P concentration was determined using the methods of Serrano *et al.* (1976) with a sodium phosphate standard and 200 µL of sample supernatant.

Statistical Analysis. All data collected from this study were subjected to ANOVA and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions.

Figure IV-1. Open air-flow system for monitoring respiration rates of cucumber fruit. Fruit were sealed in plastic chambers connected to a flow-board/manifold system. Humidified air (120 mL min^{-1}) was continuously passed through the chambers. Respiration was calculated as the difference in CO_2 concentration between inlet and outlet ports from each chamber.



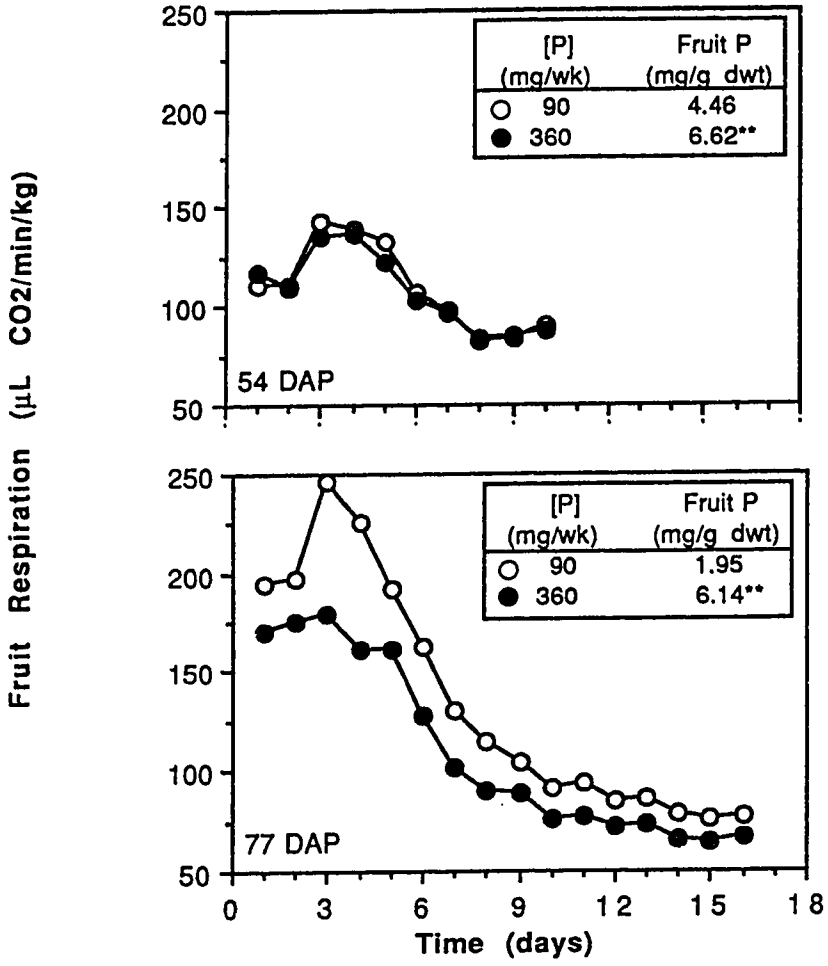
RESULTS

Fruit Production. By 82 DAP (crop termination), the total number of fruit produced was greatly enhanced by increasing P fertilization level. High-P plants (360 mg P/week; 11.5 fruit) and low-P plants (90 mg P/week; 10.6 fruit) produced 67% and 55% more total fruit , respectively, than did plants receiving no supplemental P (0 mg P/week; 6.9 fruit) ($P< 0.01$). This translates to approximately 0.20, 0.30 and 0.33 fruit produced per day from no-P, low-P and high-P plants, respectively, over the 35-day fruit harvest period. These fruit production rates are comparable to those characterized for 'Carmen' cucumbers in our previous studies (see Chapter 3). VAM infection did not influence the total number of fruit produced by 82 DAP.

Postharvest fruit respiration and P concentration. Since VAM treatment had no main effect on fruit respiration, and did not interact with P level or time to affect respiration rates, data for VAM and control plants were averaged and plotted against time. The respiration rates of fruit harvested at 54 DAP from low-P (90 mg P/week) and high-P (360 mg P/week) plants were equal over a 10-day postharvest period (Fig. 2). Fruit respiration increased 22% over the first three days from harvest, and then declined significantly through the next five days, such that the respiration rate at day 3 was 68% higher than at day 8. Phosphorus nutrition significantly ($P< 0.01$) influenced P concentration of fruit harvested at 54 DAP. High-P plants produced fruit containing 48% more P (mg/g fruit dry wt) than fruit produced by low-P plants (Fig. IV-2, inset). This substantial difference in fruit P concentration was evidently not enough to influence postharvest respiratory metabolism.

By 77 DAP, fruit harvested from low-P plants had significantly higher (23%; $P< 0.01$) average (over time) respiration rates (134.5 $\mu L\ CO_2 min^{-1}\ kg^{-1}$) than fruit from high-P plants (109.2 $\mu L\ CO_2\ min^{-1}\ kg^{-1}$). Respiration rates significantly declined ($P<0.01$) over the 16-day postharvest period, such that the average respiration rate at day 1 was 153% higher than that at day 16, regardless of P fertilization level (Fig. IV-2). However, low-P plants produced fruit which exhibited a greater initial increase in respiration (from harvest through day 3) than high-P plants. In low-P plant fruit, maximum respiration at day 3 was 27% and 221% higher than at day 1 and day

Figure IV-2. Effect of harvest date (54 or 77 DAP) and P fertilization level on the postharvest respiration rates and P concentrations of cucumber fruit. For fruit respiration at 54 DAP, F-values for the main effect of time was significant at the 0.01 level. For fruit respiration at 77 DAP, F-values for the main effects of time and P fertilization level were significant at the 0.01 level. Comparison of data from the two harvest dates yield significant F-values at the 0.01 level for the main effects of harvest date, time and P fertilization level and for the interactions of harvest date x time and harvest date x P level. Fruit P concentrations at each level of P nutrition are shown in the insets. F-values (**) for the main effects of P fertilization level and harvest date and the interaction of P x harvest date were significant at the 0.01 level.


Fruit Respiration (µL CO2/min/kg)
250
200
150
100
50
[P] (mg/wk)
Fruit P (mg/g dwt)
90
4.46
360
6.62**
54 DAP
250
200
150
100
50
[P] (mg/wk)
Fruit P (mg/g dwt)
90
1.95
360
6.14**
77 DAP
0
3
6
9
12
15
18
Time (days)

16, respectively; whereas in high-P fruit, the maximum respiration was only 5% and 165% higher than at day 1 and day 16, respectively. Average respiration of fruit from VAM-infected plants (129.23 μL CO^2/min/kg) was 13% greater ($P< 0.01$) than that of fruit from control plants (114.53 μL CO^2/min/kg). Fruit respiration was not affected by interactions between P level, VAM infection and incubation time. Phosphorus nutrition significantly influenced ($P< 0.01$) fruit P concentration at 77 DAP; high-P plants produced fruit with 215% more P (mg/g dry wt) than low-P plants (Fig. IV-2, inset).

Respiration rates were significantly ($P< 0.01$) affected by harvest date such that respiration of fruit harvested 77 DAP was 41% higher than that of fruit harvested 54 DAP. More importantly, P nutrition and time of harvest interacted to affect fruit respiration rate. Low-P and high-P plants produced fruit at 77 DAP that averaged (over time) 57% and 25% higher rates of respiration, respectively, than comparable fruit produced at 54 DAP ($P< 0.01$; Fig. IV-2).

Interestingly, P concentration of fruit produced by high-P plants at 54 DAP was 8% greater than that of fruit from high-P plants at 77 DAP, while fruit produced by low-P plants at 54 DAP had 128% greater P concentration than fruit from low-P plants harvested at 77 DAP (P nutrition by DAP $P< 0.01$). Thus, high-P plants were able to maintain relatively constant fruit P concentrations over the entire harvest period, whereas low-P plants were not (Fig. IV-2, insets). According to Roorda van Eysinga and Smilde (1981), the range of P concentrations found in healthy cucumber fruit is 4.96 to 10.85 mg/g fruit dry wt. Thus, it is apparent that the low-P fertilization level (90 mg P/week) resulted in P deficient fruit even at the early production date of 54 DAP. High-P plants (360 mg P/week), although showing a slight decrease in fruit P concentration from 54 to 77 DAP, produced fruit with P concentrations well within the healthy range as outlined above.

DISCUSSION

As with previous studies (Chapter III), P nutrition greatly influenced the total number of cucumber fruit produced per plant. Although deficient P fertilization levels (0 and 90 mg P/week)

resulted in P deficiency symptoms in mature leaves (localized desiccated areas), fruit produced by these plants appeared healthy (Fig. IV-3). Adams (1978) indicated that P deficiency may result in up to a three-fold increase of poorly developed fruit. However, this was not measured in our experiment as misshapen fruit were pruned at an early developmental stage.

The postharvest respiration rate of a fruit can be a good indicator of storage life, since respiration rate and shelf-life are often inversely related (Salunkhe and Desai, 1984; Kader, 1987). Cucumber respiration rates determined in this experiment depended upon plant age, VAM infection and the corresponding P fertilization level. Fruit produced from a young and vigorous plant will be of the highest quality (Adamson and Maas, 1981); thus one would expect 'early' fruit to have lower respiration rates than fruit produced from older plants. Kanellis *et al.* (1986), demonstrated that the longer a fruit requires to reach commercial maturity, the shorter its subsequent shelf-life. Since developing fruit impose an inhibitory effect on the growth of later set fruit (Kanellis *et al.*, 1986), it is reasonable to expect fruit produced later in the harvest period to have a shorter shelf-life. Thus, any factors that hasten and promote rapid fruit growth early in a cropping season (for example, high nutrient concentrations and fruit thinning) should result in physiologically younger fruit at commercial maturity and, therefore, increased shelf-life (Lin and Ehret, 1991).

Fruit harvested at 54 DAP (7 days into the harvest period) from low-P (90 mg P/week) and high-P (360 mg P/week) plants did not have significantly different postharvest respiration rates (Fig. IV-2). Fruit harvested at approximately 77 DAP (30 days into the 35-day harvest period) showed significant differences in respiration rate due to plant P fertilization level. Moreover, below a critical P concentration, differences in fruit respiration corresponded with differences in P concentration. Although the P concentration of high-P and low-P plant fruit at 54 DAP were significantly different (Fig. IV-2, inset), the concentrations were within, or very close to within, the sufficient range as determined by Roorda van Eysinga and Smilde (1981). Thus, apparently the P concentration of fruit produced by low-P plants had not reached a level low enough to significantly affect postharvest respiration rate. However, by 77 DAP, fruit produced by low-P plants were

Figure IV-3 Fruit developing on a 'Carmen' cucumber plant growing under P-deficient conditions (90 mg P/week). Although the cucumber plant leaves showed advance symptoms of P deficiency (desiccated areas), the plants were still capable of producing high quality fruit in terms of appearance. Fruit production is thus a priority for the plant, and will occur at the expense of vegetative growth.

extremely P deficient (Fig. IV-2, inset), whereas those from high-P plants were still well within the P sufficient range. Hence, two main conclusions may be drawn from this study. First, P deficient plants are able to maintain almost adequate fruit P levels early in the harvest period, most likely through mobilization and translocation of P from mature leaves (Fig. IV-3). However, as more and more fruit are harvested over time, fruit from these these low-P plants gradually become P-deficient, since P is being permanently lost from the vegetative system and availability and uptake of P are insufficient to adequately support further vegetative growth and fruit production. Thus, subsequent harvestable fruit are of lower quality. Secondly, P deficiency in a fruit that appears in all other respects to be normal and of high quality results in a higher respiration rate and, most likely, a shorter shelf-life. Low-P plants produced fruit at 77 DAP that had significantly higher respiration rates than all other treatments (Fig. IV-2). However, since high-P plants produced fruit at 77 DAP with significantly higher respiration rates than fruit from low-P and high-P treatments at 54 DAP, P deficiency apparently magnifies the effect of advancing harvest date on postharvest fruit respiration.

Fruit from VAM-infected plants had a 13% higher respiration rate at 77 DAP than those from control plants, regardless of P fertilization level. There was no effect of VAM on the total number of fruit produced or on fruit P concentration. Thus, the increase in fruit respiration attributable to VAM infection may be related to slower fruit maturation due to an altered source:sink ratio caused by the additional carbohydrate requirements of the VAM fungal infection. This effect of VAM may only be manifested later in development when the plant growth rate has slowed (sigmoidal growth curve) and there is less active leaf area to provide photoassimilates to sustain fruit production and the VAM infection. Indeed, evidence of the inability of VAM-infected plants to sustain fruit growth later in development was demonstrated by increased fruit abortion and slowed fruit production in the latter portion of the harvest period (see Chapter III).

This study demonstrates that plant P nutrition level, although important in terms of growth and fruit production, is also very important in dictating fruit P concentration and postharvest respiration rates. Postharvest respiration in cucumbers has been demonstrated to increase with

increased physiological age at harvest, and the 'older' a fruit is at harvest, the shorter the shelf-life (Kanellis *et al.*, 1986). High nutrient concentrations (150% of recommended values) were shown to prolong shelf-life (Lin and Ehret, 1991). Our study indicates that insufficient P nutrition increases postharvest fruit respiration and decreases fruit P concentration, thus it is reasonable to conclude than low-P conditions will result in a lower quality fruit as defined by a shorter shelf-life. Although previous studies (Chapter II) showed an advantage of VAM infection on enhanced early growth, it is obvious that the fungus can also compete with developing fruit for photoassimilates.

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Chapter V

GENERAL DISCUSSION AND CONCLUSIONS

The purpose of this research was to characterize the effects of phosphorus (P) nutrition and vesicular-arbuscular mycorrhizal (VAM) infection on growth and development of cucumber plants. Studies on plant establishment and early growth were undertaken since, in commercial production systems, it is important to have a strong and healthy seedling which will survive the stress of transplanting. Faster plant establishment should result in earlier and more vigorous fruit production. Studies on fruit-bearing plants were therefore undertaken to see if the initial beneficial effects of P and VAM persisted and improved mature shoot biomass and fruit production. A mature plant must be able to support the carbohydrate demand of fruiting in order to produce an acceptable commercial product and overall yield. Since cucumber plants responded greatly to increasing P fertilization and, to a lesser extent, VAM infection, a study was conducted to determine if P nutrition influenced fruit respiratory metabloism after harvest.

Phosphorus nutrition was important to the establishment and growth of cucumbers. Although young plants did not show P deficiency symptoms in their leaves (desiccated areas) as did mature plants, low rates of P fertilization in young plants restricted growth rates of the shoot and, to a lesser extent, the root (Fig. II-4). Low P fertilization levels in young plants resulted in restricted stem growth which, if maintained, could reduce fruit yield per plant by limiting nodal sites for fruit production. This early conclusion was confirmed by mature plant studies where the number of nodes and the number of fruit produced per plant increased significantly with increasing P fertilization level (see Chapter III).

As with younger plants during establishment, foliar development of mature plants was enhanced by increasing P fertilization level, as reported for tobacco (Rufty *et al.*, 1990), soybean (Fredeen *et al.*, 1989) and potato (McArthur and Knowles, 1993). At low P fertilization levels, young plants exhibited restricted leaf growth and reduced leaf area expansion. This effect of low

P was most likely the cause of reduced foliar dry weight in mature plants. The harvest index of mature plants at final harvest was also influenced by P nutrition (see Chapter III). Harvest index increased with increasing P fertilization level, underscoring the importance of P to shoot growth and fruit production, and indicating an increased efficiency for fruit production at high-P levels.

High P nutrition in young plants greatly increased total plant P relative to low-P plants, and much of this P was allocated to the stem, enhancing stem growth (Fig. II-10). In mature plants, P concentration decreased from apical to basal leaves (Figs. III-12 and IV-2), reflecting mobilization and translocation of P from older to younger leaves. Low-P, fruit-bearing plants were unable to maintain leaf or fruit P concentrations within a healthy range (Roorda van Eysinga and Smilde, 1981).

Part of the deleterious effects of low P fertilization on plants may be manifested indirectly, through reduced ability to assimilate nitrogen (N), as was shown in early plant growth studies (see Chapter II). In young plants, nitrate, free amino, and soluble protein N concentrations of leaf and root tissues declined with time. Plants grown on a low-P (4 ppm P) regime had higher leaf and root nitrate-N and amino-N concentration, but lower protein-N concentrations, than high-P (20 ppm P) plants. These responses were similar to those reported for tobacco (Rufty *et al.*, 1990), soybean (Fredeen *et al.*, 1989), and potato (McArthur and Knowles, 1993). Under P deficient conditions, absorbed nitrate-N may accumulate due to reduced nitrate reductase activity, and free amino acid concentration may increase because of enhanced protein degradation (Rufty *et al.*, 1990). Thus, effects of low P nutrition on the assimilation of N may play an important part in the mature plant system and be a factor in the detrimental effects of low-P on fruit production. Phosphorus deficiency also limits carbon export from leaves (Rao *et al* ., 1990) and this may account for a higher level of soluble carbohydrates in the leaves of young, low-P cucumber plants. This limited carbon export from leaves may have detrimental effects on fruit production.

In young "Corona' plants, the average percent infection at 24 DAP was 49%. In mature plants (average 88 DAP), percent infection was 55% for 'Carmen' and 71% for 'Corona'. However, unlike young plants where high-P fertilization levels gave an infection level of 38%, in mature plants,

infection at high-P (360) levels was less than 10%, regardless of cultivar. Thus, it appears that under high (adequate) P conditions, the extent of the VAM infection is reduced over time (DAP) since the plant is not receiving a benefit in return for supporting the fungus. Cucumber roots exhibited a yellow pigmentation when infected with VAM. The fungus appeared to be very compatable with the plant, and fungal structures such as arbuscules and vesicles were present in a large number of root cortical cells, Indeed, many arbuscules and vesicles appeared to fill entire cells (Figs. III-5, 6, 7).

VAM infection strongly influenced plants in the establishment and early growth stages. VAM plants were significantly larger than non-infected plants, regardless of P fertilization level. Leaf area in VAM-infected plants expanded 15% faster than controls. In general, VAM-induced increases in growth of young plants were similar to those induced by increases in P fertilization level (see Chapter II). A lack of P by VAM interactions indicated that a majority of the growth effects attributable to VAM were the result of increased P nutrition afforded by the VAM infection. However, by the time plants reached maturity and began to produce fruit, the benefits of VAM diminished. Early fruit production (first one to two weeks of the harvest period) was increased by VAM infection at all P fertilization levels (see Chapter III). However, by crop termination, fruit yield of VAM-infected plants was equal to that produced by noninfected plants. This reduction in early fruit production rate may be due to an extended fruit abortion period in VAM-infected plants, perhaps as a result of the plant's inability to support both the VAM infection and fruit production. Indeed, the carbohydrate demand on a plant due to VAM infection could be substantially higher, considering that fungal biomass within the roots was reported to be 10% to 17% of total root dry weight (Hayman, 1983; Abbott and Robson, 1984). VAM infection in mature cucumber plants resulted in less foliar growth relative to that from nonmycorrhizal plants. This is contrary to results obtained in other crops such as strawberry (Vestberg, 1992), chilli pepper (Sreenivasa *et al.*, 1993) and onion (Smith *et al.*, 1986). Maintenance of a high level of VAM infection in low-P plants, combined with the increased nutritional requirements needed to sustain fruit production, probably resulted in less foliar growth.

Phosphorus nutrition and VAM infection also greatly influenced postharvest fruit respiration (see Chapter IV). Respiration of fruit picked early in the harvest period was not influenced by P fertilization level, even though there was a significant difference in total fruit P concentration. However, respiration of fruit harvested at a later date was significantly influenced by P, such that low-P plants produced fruit that respired at a much higher rate than high-P plants. In addition, fruit from VAM-infected plants respired at a rate higher than fruit from noninfected plants at the later harvest date. Thus, low P nutrition and VAM infection, in addition to reducing foliar growth and fruit production, also affect postharvest behavior of the fruit. The higher fruit respiration rates associated with low P and VAM infection may translate into a shorter shelf-life, since postharvest respiration and subsequent shelf-life are inversely related (Lin and Ehret, 1991).

The effects of inadequate P nutrition were found in all aspects of cucumber production. Phosphorus is extremely important in enabling the young plant to grow vigorously. Leaf production and expansion are restricted under low P conditions and this will result in later fruit production and a plant that has less reserves and photosynthetic area to support fruit growth. Although not measured in these studies,increased fruit abortion may result from this. Many of the growth depressions attributed to P deficiency may also be indirectly affected by the effects of P on N assimilation, as was found in the early growth studies. Further examination of plant N concentrations may be useful in determining the long-term effects (to crop termination) of P deficiency on N assimilation in a cucumber crop.

VAM infection of cucumbers appears to be beneficial during establishment and early growth, but becomes less so as the plant ages. The longer a fruit-bearing cucumber plant is sustained, the less value VAM is to the plant. In fact, VAM may actually limit vegetative growth and fruit production in older plants, possibly as a result of the added sink demand of the fungus. At later harvest dates, VAM-infected plants yielded fruit with higher respiration rates, and these fruit may thus have a reduced shelf-life. It appears that VAM infection of greenhouse-grown cucumbers may only be advantageous if used to produce fast growing, vigorous young plants to facilitate multiple, short-season crops.

In summary, P nutrition of the cucumber plant is a very important aspect of growth and fruit production. VAM infection, although readily established in cucumber, does not enhance growth and yield of mature plants under adequate levels of nutrition. Further studies to characterize the effects of P nutrition and VAM infection on plant N assimilation and source-sink relationships in mature plants are warranted. In addition, studies on fruit respiration should be expanded to investigate the effects of VAM and P on the actual shelf-life of fruit. Results from these studies may indicate whether a short-season crop would be more advantageous in terms of quality of fruit produced than a long-season crop.

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