

ENVIRONMENT, ETIOLOGICAL FACTORS, AND VIRUS DISEASES IN  
RELATION TO BACTERIAL STEM ROT OF PELARGONIUMS

By

ALEKSANDER KIVILAAN

AN ABSTRACT

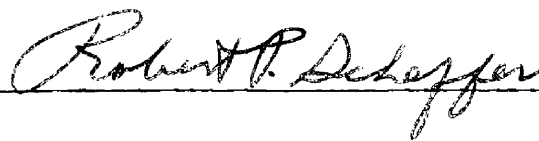
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Department of Botany and Plant Pathology  
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Approved

  
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Stem rot development in Pelargonium hortorum Bailey varieties inoculated with Xanthomonas pelargonii (Brown) Starr et Burkh., was found to be significantly affected by temperature, nutritional factors, and certain previously undescribed virus diseases. Lack of knowledge about many aspects of disease development made certain exploratory experiments necessary before the above factors could be studied.

Facts about etiology and disease development were established. X. pelargonii was found to have a narrow host range in the Geranium family. Systemic invasion of the host occurred through roots and stems, but not through leaves, where localized leafspots were formed. The organism can be present in vessels without causing visible effects; it probably causes stem rot only when breaks allow invasion of parenchyma. The pH of healthy plant sap was well below the range which allowed growth of the pathogen in culture, but infection resulted in a substantial decrease in acidity. Strains of the pathogen were found which differed in their ability to cause disease in host plants.

Stem inoculated rooted cuttings (var. Better Times) from stem-rot-free stock plants were kept at 10°, 16°, 21°, and 27° C night temperatures. Disease development increased linearly with increasing night temperatures

during the first five weeks following inoculation. Symptoms were almost absent at 10°C night temperature, developed slowly at 16°C and at 21°C, and rapidly killed plants at 27°C. During the latter part of experiments, plants at 10°C also showed severe disease development, probably because of a deleterious effect of low temperature on host plants.

Mineral nutrient concentrations below and above optimum for host growth enhanced disease development. Rooted cuttings, var. Ricard, were grown in sand cultures with concentrations 0.1, 0.5, 1, 2, and 3 times that of the basal (optimum) salt solution. Plants were inoculated after 30 days' growth in their respective nutrient solutions. Disease development was significantly higher in solutions 0.1, 0.5, and 3, than in 1 and 2. Comparison of disease indices in inoculated plants with average weights of control plants in nutrient solutions showed an inverse relationship.

Rooted cuttings inoculated with X. pelargonii were grown in sand and watered with nutrient solutions with low, optimum, and high levels of N, P, and K. Very low, low and optimum levels of Ca were used in another experiment. Disease developed more rapidly in plants in low K and in high P and N than in their high or low counterparts. As Ca was increased in nutrient solutions, disease decreased. In unbalanced Ca and K solutions, disease development decreased as growth of plants increased, while disease increased with

increasing plant growth in unbalanced N and P solutions. The N and P effects are unique in that all other factors which favor pelargonium growth also retard disease. Possible explanations are that X. pelargonii is favored by high organic N, and in high P the plants mature more quickly and soon lose resistance.

Several graft-transmissible conditions of pelargonium were found. These reactions apparently were of virus origin and included necrosis, ring-spot, necrotic ringspot, crinkle and mottle. Attempts to transmit the virus(es) mechanically and by dodder were not successful. Development of stem rot was slowest in virus-free plants, fastest in those expressing mottle, crinkle, and necrotic ringspot symptoms, and intermediate in plants with ringspot.

Commercial stocks of six common pelargonium varieties (Ricard, Radio Red, Olympic Red, Irvington Beauty, Enchantress Supreme, and Fiat) were found to carry X. pelargonii and virus diseases in latent forms. Twenty-six per cent of 600 plants carried X. pelargonii and 62 per cent carried some form of virus disease. Night temperature of 27° C for 2 weeks was used to force stem rot symptoms in latently infected pelargonium stock plants. Veneer grafting to pelargonium seedlings and to virus-free seedling clones was used to detect latent viruses and to index stock plants for virus diseases. Virus assays together with high temperature treatment to eliminate latent bacterial infections has made possible the development of disease-free propagating stock.

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## INTRODUCTION

Pelargoniums were the leading potted plant in the United States in 1949, when more than 25 million unfinished plants and cuttings were produced (6). Pelargoniums once were comparatively free of destructive pests and diseases, but in recent years serious losses have resulted from bacterial leaf spot and stem rot, caused by Xanthomonas pelargonii (Brown) Starr et Burkh. These diseases have been known for many years and in recent years have become a limiting factor in production. This situation is true in Michigan as well as in other parts of the world.

Although stem rot and leaf spot are the only diseases of epidemic proportions, other diseases are well known. The Index of Plant Diseases in U. S. A. (21) lists two nematode diseases, four bacterial diseases, sixteen diseases caused by fungi, one physiological disorder, and four diseases of virus origin, on Pelargonium domesticum Bailey, P. graveolens L'Her., P. hortorum Bailey, and P. peltatum Ait. The knowledge of virus diseases of pelargonium is scant, and the economic importance of this group is unknown. Crown-gall and leafy-gall are of minor importance among bacterial diseases. Fungi, causing root-rots, leafspots, and other diseases, have

been of local importance. Leaf nematodes and root nematodes have been reported occasionally. Stem rot has been considered the limiting factor in pelargonium production, therefore the present work started as an attempt to study the cause and evaluate the role of some factors affecting disease development. Our own observations, as well as those of others, have indicated that certain environmental and genetic factors also may affect the occurrence and development of stem rot in pelargonium plants. At first the study was concerned primarily with bacterial stem rot, but as the program developed it became apparent that this could not be separated entirely from virus diseases.

The natural habitat of pelargonium is in the Great Karoo region in South Africa. Silurian soil, rich in calcium and potassium, moderately high altitude with cool nights and hot days, and low rainfall are the main characteristics of the environment in which the genus originated. Such factors should be taken into account in a consideration of environmental effects on diseases. Cultivated pelargoniums (P. hortorum, P. zonale Ait., P. peltatum, and P. domesticum) are undoubtedly the result of long hybridization and mutation. There is a large number of cultivated varieties of world-wide distribution, but all prefer an environment resembling that of the original habitat.

## REVIEW OF LITERATURE

### Distribution of the Disease

Bacterial pathogens have been reported repeatedly on cultivated pelargoniums as causing stem rot, leaf spot, or both. Lequet (26) reported in 1888 the occurrence of bacterial stem rot of pelargoniums in France; this report was later verified by Prillieux and Delacroix (43). Galloway (15) noticed the disease in the United States two years later. Stone and Smith (52) observed leaf spot on leaves of several pelargonium varieties growing out of doors during the rainy summer of 1897 in Massachusetts. They assumed that the disease was caused by a bacterium, although they could not isolate bacteria from leaf spots. Further reports from New Zealand by Kirk in 1907 (24), from Switzerland by Tuinmann in 1927 (55), from Holland by van Poeteren in 1932 (41), from Italy by Passalacqua in 1933 (36), from the U. S. S. R. by Vergovsky and Vodolaghin in 1938 (56), from Hungary by Krenner in 1941 (25), from Brazil by Robbs in 1946 (44), and from Denmark by Hellmers in 1952 (19), indicate the wide geographical distribution of the disease.

## Symptoms

X. pelargonii attacks leaves and stems on P. hortorum varieties.

Leaf spot is commonly reported on greenhouse plants, especially during the winter months, and on certain varieties. Often the appearance of bacterial leaf spot can be correlated with occurrence of edema (19). This may be due to increased ease of infection through edema pustules or because the same environmental conditions cause pustules and favor bacterial infection.

In an early stage of bacterial infection spots on upper surfaces of leaves are darker green than uninvaded tissue. The deep green infected area has a water-soaked appearance. After three to five days the spots become brownish, necrotic, circular in shape, with depressed epidermis, surrounded by pale yellow zones (19, 28). The size of leaf spots may vary greatly, but is most commonly five tenths to five mm in diameter. Hellmers (19) and Munnecke (28) have observed that in advanced stages of the disease the leaf area between spots may also turn brown and die. In younger leaves the disease may cause some wrinkling of leaf blades, resembling somewhat the "leaf curl" caused by virus infection (19). However, the spots of leaf curl differ from bacterial spots

in that the former are mostly irregular and indistinct in outline, lack the yellowish rims and are never necrotic (19). Both workers cited above observed that spots rarely coalesce, but the whole attacked leaf in extreme cases may become withered and flaccid. A controversy exists concerning bacterial penetration from invaded lamina into the stem through the petiole. Hellmers (19) believes that such penetration rarely takes place, but Munnecke (28) thinks the bacteria can spread through the vascular system of the petiole downward to the stem and become systemic from the original leaf spot.

Considerably less attention has been paid to stem rot than to leaf spot, despite the much greater damage caused by the former. As the first symptom of stem rot or "black rot", the leaves of affected plants turn dull green, indicating improper functioning of vessels. Appearance of angular or v-shaped spots on the leaves, wilting of some leaves, and occasional necrosis of growing points alone or all combined are the next external signs of stem rot (19, 28). These symptoms are later accompanied by discoloration of the stem; brown color followed by blackening is the most positive characteristic of stem rot. Cross sections of discolored stems show that vascular and fibrous tissues and epidermis are the only undamaged parts, with medulla and cortex parenchyma becoming a dark nonviscous mass (19). In still later stages the diseased stem becomes a dry hollow cylinder.

## The Pathogen

The causal organism can be isolated easily from diseased plants.

Stem isolations usually yield pure cultures. Smith in volume 2 of "Bacteria in Relation to Plant Diseases" (48) stated that a yellow organism with a polar flagellum was isolated from pelargonium leaf spots and the disease was reproduced on inoculation. Lewis (27) probably was the first to discover the causal organism in leaf spots of Erodium and Pelargonium; he named it Bacterium erodii. Later Brown (5) studied the leaf spot organism and made inoculation tests. Since her isolate behaved differently in biochemical and inoculation tests from those described by Lewis, she suggested the name Bacterium pelargonii for the causal organism.

Dodge and Swift (10) in 1932 inoculated pelargonium plants with bacteria isolated from leaf spots and produced stem rot. Although they did not conclude that the same organism was causing both leaf spot and stem rot, their work suggests such a conclusion. On the basis of comprehensive bacteriological and plant pathological studies, Hellmers (19) showed that leaf spot and stem rot were two phenomena of a single disease caused by Xanthomonas pelargonii (Brown) Starr et Burkh. Munnecke (28) in 1954, working with leading American pelargonium varieties, confirmed Hellmers'

findings. Starr et al (51) made comparative biochemical and cultural determinations and cross inoculation tests with authentic cultures of X. pelargonii and X. geranii and showed that these two species were identical and suggested that they should be combined as Xanthomonas pelargonii (Brown) Starr et Burkh.

Lewis (27), Brown (5), Hellmers (19), and Starr et al (51) have described the organism in detail, along with results of biochemical and inoculation tests. The organism is a rod-shaped, gram negative bacterium with rounded ends, measuring 1.0 to 1.5 by 0.5 to 0.7  $\mu$ . No spores are formed. It is motile by means of one polar flagellum. In culture it is usually borne singly, in pairs, or occasionally in chains of 2-16 cells. Brown found that cells from agar cultures were slightly smaller than those from diseased plant materials. The organism was found to be aerobic by Lewis (27) and Brown (5). Hellmers (19) found that the bacteria survived three months at minus 7°C. Optimal temperature was about 27°C, and thermal death point was 51-52°C. Cells resisted drying at room temperature for six days (5, 27) and did not grow in nutrient cultures below pH 5.7 and above pH 8.7 (5).

X. pelargonii grows on a wide variety of nutrient media, making especially good growth on two per cent potato dextrose agar and on autoclaved potato plugs (19). At first the dirty-white colonies are circular, convex,



shiny, with entire margins. On autoclaved potato plugs creamy to yellow colonies are formed. Gelatin is liquefied slowly (19, 51).

Most characteristic biochemical properties of the organism are the formation of acid from a wide variety of carbon compounds without gas production (Table I), utilization of many organic acids, some lipolytic and pectinolytic activity, breakdown of peptones and other complex nitrogenous compounds producing ammonia, production of hydrogen sulfide, and inability to reduce nitrates. Since all workers do not agree entirely as to biochemical reactions, comparative data are given in Table I.

### The Disease

Brown states that pelargonium leaf spot in eastern United States, is a greenhouse disease occurring less frequently out of doors. She lists high temperature, high humidity, and all other factors which weaken the host as factors which promote disease development. Unless the physical condition of the plant is weakened, the disease is not serious. She also makes a general statement "from all the evidence gathered the organism seems to be one harbored in the soil". Brown's opinion was that insects did not play an important part in the transmission of the disease, and therefore splashing, contaminated water drops were the main means of dissemination.

TABLE I  
COMPARISON OF BIOCHEMICAL REACTIONS OF X. PELARGONII

Substrate or Reaction	Products Formed According To	
	Hellmers (19)	Starr et al (51)
<u>Carbon Compounds:</u>		
xylose		acid <sup>a</sup> , no gas <sup>b</sup>
dextrose	acid, no gas	" "
galactose	"	"
mannose	"	"
sucrose		"
lactose		"
maltose	no acid, no gas	no acid, no gas
starch	no hydrolysis	no hydrolysis
dextrin		no acid, no gas
glycerol	acid, no gas	acid, no gas
mannitol		some acid, no gas
sorbitol		no acid, no gas
salicin	no acid, no gas	" "
<u>Organic Acids:</u>		
acetic		alkaline reaction
citric		"
formic		"
lactic		"
malic		"
malonic		"
pyruvic		"
hippuric		not utilized
tartaric		"
<u>Reduction of NO<sub>3</sub></u>	no reduction	no reduction
<u>Formation of NH<sub>3</sub></u>	ammonia formed	
<u>Formation of H<sub>2</sub>S</u>	hydrogen sulfide formed	hydrogen sulfide formed
<u>Action on litmus milk</u>	peptonized	peptonized
<u>Formation of indole</u>	none	
<u>Voges-Proskauer re-</u> <u>action</u>		negative
<u>Lipolytic activity</u>		slightly positive
<u>Pectinol. activity</u>		slow

<sup>a</sup>Organic acids formed from carbon compounds.

<sup>b</sup>Positive for gas indicates production of CO<sub>2</sub> from carbon compounds.

Hellmers found that leaves were attacked first, but in the presence of moisture the bacteria could attack wounded stems and petioles as well. He did not find evidence to indicate that the organism invaded stems from leaf spots via petioles. Therefore, he was convinced that spread of bacteria took place from leaf spots by means of contaminated water drops or by insects. In this way leaf spots were a source of primary inoculum from which soil was infested and the stem rot stage initiated. Infection took place either through the stomata and hydathodes of the leaf, or through wounds and leaf scars on the stem. Hellmers also considered spread of disease through cuttings as an important factor.

Brown's and Hellmers' statements were based mostly on observations rather than on controlled experiments. Munnecke (28) has presented experimental evidence on some of the problems. His experiments included determination of host range, varietal susceptibility, survival of the organism in soil, carry over of the bacteria in cuttings, and dissemination. He demonstrated the susceptibility of P. domesticum, P. hortorum, and P. peltatum, thus confirming the results of Hellmers (19). Starr, Volcani and Munnecke (51) found that Geranium maculatum L., G. sanguineum L., G. pratense L., G. sylvaticum L., and G. yedoense Franch. et Sav. were susceptible to leaf inoculations by the organism. However, the degree of symptom-expression by different species varied.

In a field trial by Munnecke (29) heavy loam soil was infested by plowing under pelargonium plants previously inoculated with pure culture of X. pelargonii. A portion of the field was planted immediately with Radio Red rooted cuttings and other portions were planted at intervals of one, two and 12 months thereafter. The number of diseased plants decreased rapidly as the interval after plowing increased. The percentage of infected plants planted immediately and at intervals of one, two, and 12 months was found to be 100, 20, 11, and 0 respectively. From these data Munnecke concluded that survival of X. pelargonii in soil depended upon the rate of decay of infected plant material and was presumably nil after six months.

Carry-over of bacteria in cuttings was tested by taking cuttings from stem inoculated plants of five varieties before inoculation, and at intervals of one, three, nine, 23 and 35 days after inoculation (28). Disease was found to be transmitted from symptomless stock plants even in early stages after inoculation. Approximately 20 per cent of all the cuttings were infected regardless of the time interval after inoculation.

In dissemination tests Munnecke (28) proved that causal bacteria were spread from diseased cuttings to adjacent healthy cuttings through the rooting medium, by contaminated cutting-knives, and by rain or overhead watering.

### Environmental Factors

No reports are known dealing with the effect of environmental factors on development of bacterial blight in pelargoniums, except general statements from Brown (5) and Post (42). Brown stated that "the disease is not a serious one unless the physical condition of the plant is weakened by too rapid growth and too moist or too warm an atmosphere". Post gives high temperature as the sole reason for failure of pelargonium culture in the southern states.

### Virus Diseases

Some information is available concerning pelargonium virus diseases. Seeliger (46) in 1926, and Pape (33, 34) in 1927 and 1928, reported and described a virus disease spreading rapidly on cultivated pelargoniums in Germany. Pape named it "Kräuselkrankheit" (leaf curl). Verplancke (57) in 1932 reported leaf curl in Belgium, Pethybridge and Smith (37) in England, as a very common disease, Blattny (3) in 1933 in Czechoslovakia, Jones (22) in 1938 in the United States, Berkeley (2) in Canada, Calvino (9) in Italy, Parievskaya (35) in 1948 in the U. S. S. R., and Schreier in 1953 (45) in Austria. The Index of Plant Diseases in the United States (21) lists curly top on P. domesticum and P. hortorum in California; leaf curl on P. hortorum

in Minnesota, New Jersey, Ohio, Pennsylvania, and Washington; mosaic on P. domesticum, and P. graveolens in California and Texas. Jones (23) later reported mosaic on P. hortorum varieties in Washington. It is not known that these diseases are caused by single viruses; rather there is evidence to indicate that in many cases virus complexes may be involved. Blattny (3), Parievskaya (35), and others support this view on the basis of complex symptom-expression. Successful transmission of certain viruses, including southern celery virus (62) and lily rosette virus (49) to pelargonium plants under specified conditions also indicate that pelargoniums may harbor many viruses.

Leaf curl (Marmor pelargonii Holmes, Pelargonium virus 1 K. M. Sm.) a common virus disease, has variable symptom expression on pelargoniums. Colloquial names like dropsy, measles, crinkle and leaf roll, indicate the variability in symptoms. Irregular to circular chlorotic areas, five-tenth to five mm in diameter, often causing ruffling, crinkling, malformation, and dwarfing of the foliage, are most frequently mentioned as characteristic symptoms (4, 23). The centers of chlorotic spots may become brown with a chlorotic border (23) or elongated, corky, raised necrotic areas may develop on the petioles and stems (23). In other cases pale spots on younger leaves may expand into rounded, stellate

or dendritic blotches of a bright yellow color, sometimes surrounded by one or more concentric rings of a lighter shade (37, 38, 39). Temporary recovery is a striking feature of the disease.

Mosaic (in part Marmor cucumeris Holmes, Cucumis virus 1 K. M. Sm., in part unidentified) is characterized by mottling of the foliage with light-green and dark-green areas. The light-green interveinal areas are surrounded by dark-green areas along the veins (23). The leaves are small and branches shortened, the whole plant appearing dwarfed. The chlorotic areas of affected leaves are thin, palisade cells being shortened, iso-diametrical, with compacted mesophyll. Gigante (16) found that chloroplasts in discolored areas are pale, irregular, or lacking. Mosaic symptoms are masked during the summer months and become most evident in winter or spring (23).

Curly top (Chlorogenus eutetticola Holmes, Beta virus 1 S. M. Sm.) is characterized by clearing of the veins, inward rolling of the leaves, and chlorosis of leaves and stems (13, 23, 47).

Transmission of pelargonium viruses has been an interesting problem since most of the standard methods have failed. Verplancke (57) in 1932 probably was first to find that leaf curl was not transmissible from diseased to healthy pelargonium by wounding, rubbing, or root contact.

Only grafting was successful. Pethybridge and Smith (37) reported also that only graft transmission was possible. Since then grafting has been the main transmission method in virus work with pelargonium, although there have been many attempts to transmit viruses to pelargonium plants by other means. Freitag and Severin (13) succeeded in transmitting curly top of sugar beets to pelargonium by leaf hoppers (Eutettix tenellus); Wellman (62) transmitted southern celery virus by aphids (Aphis sp.) and Smith and Brierley (49) simulated lily rosette symptoms by feeding injury of foxglove aphids (Myzus convolvuli). There is evidence that some viruses may be transmitted through roots. Smith (50) working with tobacco necrosis found that the infective principle occurred in roots of pelargonium plants without showing external symptoms of infection. The infective principle was thought to be soil-borne. Szirmai (53, 54) reported that tobacco root necrosis on pelargonium caused light necrotic lesions on the basal leaves at low temperatures and under poor light conditions in the greenhouse.

In an attempt to explain the mode of action of tobacco mosaic virus in various plants, Hirth (20) found that success in sap transmission was dependent on tannic acid concentration and on pH of virus suspension used. Geraniatannic acids extracted from pelargonium leaves were shown to inhibit the virus at higher concentrations. Further experiments demon-



strated that the tannin-virus complex, resulting from a combination with the protein fraction of the virus, can be dissociated at pH 8.2, allowing the virus to regain its original activity.

No reports are available dealing with interactions of virus diseases and pelargonium stem rot. However, there have been reported some instances where pathogenic organisms were associated with saprophytes, and even with viruses in nature. In some, the presence of the saprophyte intensified the virulence of the pathogen (synergism) as in the bacterial disease of ivy (7); while in others, the reverse happened (antagonism) as in halo blight of beans in Australia (1). Hedges (17, 18) investigating the association of common bean mosaic with the bean-wilt pathogen, showed that the virus not only masked the effects of the bacterium, but also caused it to become less virulent. Similar synergistic and antagonistic associations between viruses and microorganisms are known from animal pathology (11).

## MATERIALS AND METHODS

Pelargonium hortorum variety Ricard was used in most experiments since it was found to be highly susceptible to bacterial blight. Better Times and Radio Red varieties and P. hortorum seedlings were used in some experiments. In order to eliminate plants with latent bacterial infections, rooted cuttings growing in three-inch pots were kept for two weeks at 27°C night temperature. During the pre-experimental period all plants were treated uniformly. They were watered daily with distilled water and fed weekly with the basal nutrient solution equal to one Hoagland (14).

Xanthomonas pelargonii isolates were obtained from naturally infected pelargonium stems. In most of the experiments isolate 182 was used. Stock cultures were kept under refrigeration on autoclaved potato plugs from which fresh potato dextrose broth cultures were prepared for inoculations. Pathogenicity of cultures was checked at intervals. Isolates most frequently used were kept also in stock plants for reisolations when needed. Fifty ml of potato dextrose broth in 250 ml Erlenmeyer flasks were inoculated, kept for 48 hours under constant shaking and used as inoculum. In some cases bacteria were thrown down by centrifuging at 4400 times gravity (R. C. F.) for 30 minutes, washed with physiological fluid, filtered through sintered glass filter, resuspended in physiological fluid, and used as inoculum.

For inoculation, stems of test plants were punctured with a needle and a small cotton plug dipped in bacterial suspension was inserted and left in the wound. In some cases leaves were inoculated by spraying bacterial suspension on the leaves with a glass atomizer, keeping the plants thereafter for 48 hours in a fog chamber.

Composition of solutions used in experiments to determine the effect of mineral nutrition on disease development is given in Tables II and III. The salt concentration of the basal nutrient solution (1H), which was identical to that used by Gallegly and Walker (14) was reduced to one-tenth (.1H) and one-half (.5H) and increased twice (2H), and three times (3H). High nitrogen, high phosphorus, and high potassium solutions were prepared by increasing the amounts of the respective ions contained in the basal solution by three times. Solutions for low nitrogen, phosphorus, and potassium were prepared by decreasing the amounts of these ions contained in basal solution to one-third of normal. All other constituents were kept at 1H level. Osmotic pressure of the solutions were kept on the same level by adding calculated amounts of NaCl to the stock solutions. All solutions contained the same amount of microelements (Table II). Basal (1H) solution was considered as high calcium level. For the medium level Ca was reduced to one-fifth and for the low level to one-seventeenth the Ca level of the normal solution (Table III).

TABLE II

COMPOSITION OF HOAGLAND'S SOLUTION USED FOR STUDYING HOST NUTRITION IN  
RELATION TO DEVELOPMENT OF BACTERIAL BLIGHT OF PELARGONIUM<sup>a</sup>

Stock Solution (1 M)	Ml of Stock Solution per 10 l. of Nutrient Solution <sup>b</sup>						
	Basal	High N	Low N	High P	Low P	High K	Low K
Ca(NO <sub>3</sub> ) <sub>2</sub> · H <sub>2</sub> O	50	50	--	50	50	50	50
KNO <sub>3</sub>	50	50	15	50	50	50	--
KH <sub>2</sub> PO <sub>4</sub>	10	10	10	10	1	10	--
MgSO <sub>4</sub> · 7H <sub>2</sub> O	20	20	20	20	20	20	20
CaCl <sub>2</sub> · 2H <sub>2</sub> O	--	--	50	--	--	--	--
NaNO <sub>3</sub>	--	300	--	--	--	--	50
KCl		--	35	--	9	120	6
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	--	--	--	20	--	--	10
NaCl <sup>c</sup>	300	--	300	270	304	180	292

<sup>a</sup>After Gallegly and Walker (14)

<sup>b</sup>One ml of minor element stock solution (H<sub>3</sub>BO<sub>3</sub>, 2.818 g.; CuCl<sub>2</sub> · 2H<sub>2</sub>O, 0.042 g.; ZnCl<sub>2</sub>, 0.0434 g.; MnCl<sub>2</sub> · 4H<sub>2</sub>O, 1.815 g.; FeCl<sub>3</sub> · 6H<sub>2</sub>O, 5.0 g.; distilled H<sub>2</sub>O, 1000 ml) added to one l. of nutrient solution.

<sup>c</sup>Used only in studies with unbalanced solutions for adjusting osmotic values equal to that in the high N solutions.

TABLE III

COMPOSITION OF HOAGLAND'S SOLUTION USED FOR STUDYING Ca  
NUTRITION OF HOST IN RELATION TO DEVELOPMENT  
OF BACTERIAL BLIGHT OF PELARGONIUM

Stock Solution	Ml of Stock Solution per 10 l. of Nutrient Solution <sup>a</sup>		
	High Ca	Medium Ca	Low Ca
$\text{Ca}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$	50	10	3
$\text{KNO}_3$	50	50	50
$\text{KH}_2\text{PO}_4$	10	10	10
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	20	20	20
$\text{NaNO}_3$	--	80	94

<sup>a</sup>One ml of minor element stock solution (see Table II) was added to one l. of nutrient solution.

Sodium sequestrenate, which is often used as a source of iron, was found to be very toxic to pelargoniums. For this reason  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (see Table II) was used as the iron source.

For nutrition experiments, cuttings (var. Ricard) were selected for uniformity and were grown under uniform conditions preceding inoculation. Roots were washed and the plants were set in clean white silica sand (grade no. 4) in eight inch varnished clay pots. Each pot was provided with a siphon for drainage in such a way that about 250 ml of undrained space was left in the bottom, as was described by Gallegly and Walker (14). Four rooted cuttings were planted per pot. After four weeks of feeding daily with half Hoagland balanced nutrient solution and weekly flushing with distilled water, plants were once more selected for uniformity and numbered. Scions were taken from each plant for virus indexing, and plants were arranged for tests on the greenhouse bench. After 30 days of feeding separately at the various nutrient levels all plants were stem inoculated with isolate 182. In all nutrient experiments plants were fed daily and watered once a week with distilled water to minimize the possible salt accumulation in pots. Disease readings were taken 10, 20, 30, 40, 50 and 110 days after inoculation.

The experimental design used was a randomized block with three

to seven replications depending on the number of treatments. Each treatment thus contained nine to 15 pots, with 36 to 60 plants, and the disease readings were subject to statistical analysis. Disease readings taken 50 days after inoculation were analyzed statistically. These readings were based on the following classification: 0, healthy; 10, slight but positive symptoms; 20, slight darkening of stem, occasional partial wilting of leaves; 40, blackening of stem, shedding of lower leaves; 60, black stem rot with occasional tip necrosis; 80, severe stem rot; 100, dead.

The presence of viruses in experimental plants was determined by grafting to virus-free seedling plants. Scions were taken from plants under question, inserted into stems of seedling plants by veneer grafts and bound with latex bandage. Two seedlings were grafted in each case and each assay was repeated two or three times. Virus infections, if present, caused symptoms on newly formed leaves of understocks about 20 to 40 days after grafting.

## EXPERIMENTAL RESULTS

### Etiological and Disease Factors

#### Inoculation Methods

Several methods of inoculation were tried to find the most reliable method for further experimental work. P. hortorum seedlings and Ricard rooted cuttings and bacterial isolate 182 were used. Stem puncture, leaf spray, leaf injection, inoculation through the leaf scars, through uninjured epidermis, and through decapitated stem were tried. Ten seedlings and ten Ricard rooted cuttings were used for each inoculation method. Stem puncture and leaf spray techniques were described in a previous section. For leaf injection the bacterial suspension was forced into the main vein of the leaf through a hollow glass microneedle. In other methods latex bandage was used to attach small pieces of rubber tubing filled with bacterial suspension, over leaf scars, over the epidermis at internodes or over freshly decapitated stems. Only stem inoculation gave uniformly positive results. Leaf scar and decapitated stem inoculations occasionally produced stem rot symptoms 50 days after inoculation, but leaf spray, leaf injection, and uninjured epidermis failed to produce symptoms (Table IV).



TABLE IV  
EFFECT OF INOCULATION METHOD ON DEVELOPMENT OF STEM ROT IN  
PELARGONIUM

Inoculation Method	Percent <sup>a</sup> Diseased Plants in	
	Seedlings	Ricard Plants
Stem inoculation	100	100
Leaf spray	0	0
Leaf injection	0	0
Leaf scar	30	20
Uninjured epidermis	0	0
Decapitated stem	0	20

<sup>a</sup>Percent of total plants developing disease.

## Host Range Studies

The main purpose of these experiments was to test the possible susceptibility of common flowers, vegetables, and weeds, which might harbor the organism as a source of inoculum for pelargonium. Hellmers (19), Munnecke (28), and Starr et al (51) have previously determined the hosts in the Geranium family and found susceptibility of various degrees in the following species: Pelargonium hortorum Bailey, P. domesticum Bailey, P. peltatum Ait., Geranium maculatum L., G. sanguineum L., G. pratense L., G. sylvaticum L., and G. yedoense Franch. et Sav.

Plants of 93 species belonging to 40 families were stem inoculated and re-isolations were made after one to two months. Inoculations were made to greenhouse plants and to out-of-door plants in mid-summer. When gram-negative organisms were recovered by re-isolations, pathogenicity was tested by inoculation to stem-rot-free Ricard plants. At least three plants of each species in the flowering stage were inoculated. None of the inoculated plants developed disease symptoms, nor were pathogenic isolates recovered. The following species were tested:

Graminaceae: Zea mays L., Dactylis glomerata L.

Cyperaceae: Carex sp.

Liliaceae: Lilium regale Wils., L. canadense L.

Hostea sp., Hemerocallis sp.

Amaryllidaceae: Narcissus poeticus L.

Iridaceae: Iris kaempferi Sieb., Gladiolus hybridus Hort.

Salicaceae: Salix babylonica L., Populus alba L.

Urticaceae: Urtica gracilis Ait., Ulmus americana L.,

Cannabis sativa L.

Polygonaceae: Rumex acetosella L., Polygonum persicaria L.,

Fagopyrum esculentum Gaertn.

Chenopodiaceae: Chenopodium album L., Atriplex patula L.,

Beta vulgaris L.

Amaranthaceae: Amaranthus sp., Celosia Golden Fleece

Nyctaginaceae: Mirabilis nyctaginea Mac M.

Caryophyllaceae: Dianthus sp.

Portulacaceae: Portulaca oleracea L.

Ranunculaceae: Delphinium sp., Aconitum napellus L.,

Paeonia officinalis L., Nigella sp., Thalictrum sp.

Papaveraceae: Chelidonium majus L., Eschscholtzia califor-

nica Cham., Papaver orientale L.

Cruciferae: Brassica oleracea L., Barbarea vulgaris R. Br.

Crassulaceae: Sedum spectabile Boreau

Saxifragaceae: Ribes sp., Heuchera sp., Hydrangea sp.

Rosaceae: Malus sp., Rosa sp., Fragaria sp.

Leguminosae: Robina pseudoacacia L., Lupinus polyphyllus  
Lindl., Vicia faba L.

Euphorbiaceae: Euphorbia pulcherrima Willd.

Balsaminaceae: Impatiens sp., Balsam sp.

Malvaceae: Althaea rosea Cav., Abutilon Theophrasti Medic.,  
Hibiscus sp.

Lythraceae: Lythrum salicaria L.

Oenagraceae: Oenothera sp.

Umbelliferae: Daucus carota L.

Apocyanaceae: Vinca rosea L., V. minor L.

Asclepiadaceae: Asclepias syriaca L.

Convolvulaceae: Convolvulus arvensis L.

Polemoniaceae: Phlox sp.

Boraginaceae: Cynoglossum sp.

Labiatae: Monarda didyma L., Salvia sp., Leonurus cardiaca L.

Verbenaceae: Verbena sp.

Solanaceae: Petunia hybrida Hort., Nicotiana sp., Solanum  
tuberosum L., Lycopersicon sp.

Scrophulariaceae: Antirrhinum majus L.

Plantaginaceae: Plantago lanceolata L.

Dipsacaceae: Scabiosa sp.

Cucurbitaceae: Cucumis sativus L.

Campanulaceae: Campanula sp.

Compositae: Lactuca scariola L., Cirsium lanceolatum L.,

Ambrosia artemisifolia L., Erigeron sp.,

Arctium lappa L., Achillea millefolium L.,

Cosmos Firestick, Zinnia Peppermint Stick,

Calendula sp., Dahlia sp., Dimorphotheca sp.,

Tagetes sp., Gaillardia sp., Centaurea sp.,

Gerbera Jamesoni Bolus., Chrysanthemum maximum

Ram., Rudbeckia laciniata L., Echinops Ritro L.,

and Heliopsis sp.

## Bacterial Strains

Observations of various isolates of Xanthomonas pelargonii indicated that there were differences in ability to produce stem rot or leaf spot. Therefore single colony isolates 99, 138 and 182 were compared for relative ability in causing stem rot in Radio Red, Ricard, and P. hortorum seedlings. Seventy-five plants, 25 of each host variety, were stem-inoculated with each of the three isolates.

Results taken four weeks after inoculation showed isolate 182 to be

the most virulent, while isolate 99 was the least pathogenic of the three (Figure 1). After several successive dilution plate single-colony re-isolations, the leaf inoculation tests were used on the same host varieties. Isolate 182 again proved to be the most pathogenic of the three, causing leaf spot on all three hosts. Isolate 99 was not capable of causing leaf spot on any of the three (Table V). Variability in producing leaf spot was more striking than variability in causing stem rot. Such variability indicates that X. pelargonii consists of several strains.

#### Effect of Age of Cultures on Virulence of Pathogen

Plant pathogenic bacteria are known to lose virulence in culture (8, 11, 19) making desirable a test of this possibility in isolate 182. Stock cultures in potato dextrose broth were kept at 9°C for one, three, six and 12 months. Fresh potato dextrose broth cultures were obtained from the stock cultures of different ages and stem inoculations were made in Ricard plants. Ten plants were inoculated with each culture. All inoculated plants developed stem rot regardless of the age of bacterial stock cultures used. When the same cultures were used for leaf inoculation, however, there was a noticeable decrease in reaction with increasing age of bacterial stock culture. Plants inoculated with bacterial suspensions from one- and three-month old cultures developed typical bacterial leaf spot, whereas these inoculated with suspensions from six- and 12-month old cultures seldom did so.

TABLE V  
ABILITY OF ISOLATES TO CAUSE LEAF SPOT

Isolate No.	<u>Pelargonium hortorum</u>		
	Radio Red	Ricard	Seedlings
99	a	-	-
138	++	+	
182	+++	++	+

a-, no leaf spots formed.

+, very few leaf spots formed.

++, moderate leaf spot formation.

+++ , heavy leaf spot formation.

### pH Effect on Growth in Culture

The effect of pH on growth in potato dextrose broth was determined by adding a series of phosphate buffers to the broth. Buffers were used at pH 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 6.0, 7.0, 8.0, 8.2, 8.4, 8.6, 8.8, and 9.0. The criterion of growth was turbidity of cultures as measured with a Klett-Sumerson turbidimeter. Isolate 182 was found not to grow in potato dextrose broth below pH 5.6 and above pH 8.4, which agrees closely with previous findings (5). Although the bacterium is sensitive to acid, it still grows in pelargonium plants with a sap pH well below the lower limit for bacterial growth in culture (Table VI). All pH values of sap were below the lower limit for growth of the causal organism in culture media (Table VI), although there was some variation from part to part, for example sap from leaf samples always showed lower pH readings than sap from stems. Lower parts of stems were less acid than top parts of stems. Pith sap had lower pH than cortex sap, which may be caused by the higher cell sap content in the former. In spite of the unfavorable acidity, the bacterium was able to grow, and determinations showed that sap of diseased portions was alkaline.

### Tissues Invaded and Movement of Bacteria in Plants

There is confusion in the literature whether X. pelargonii is a systemic pathogen or a parenchyma invader. Leaf spot is believed to involve strictly parenchyma invasion (19), but for stem rot some authors believe that



TABLE VI  
pH VALUES IN SAP OF PELARGONIUM SP.

Species	Tissues and Plant Parts Used	Average pH
<u>P. hortorum</u> <sup>a</sup>	Diseased stem	8.0
	Healthy plants:	
	Leaf blade	3.9
	Top stem	4.8
	Middle stem	5.0
	Bottom of stem	5.6
	Center of stem:	
	Pith	4.8
	Xylem	5.1
	Cortex	5.3
<u>P. melissimum</u>	Leaves	3.3

<sup>a</sup> 18-month-old seedlings were used.

the pathogen is mainly vascular (28). Since the question has some importance in a study of nutritional relationships of disease development, a few exploratory tests were made.

Ninety Ricard cuttings (average weight 15 gm each) from stem rot free and virus free stock plants were allowed to take up bacterial suspensions (100,000 cells per ml) of isolate 182 by transpirational pull for 24 hours, after which they were placed in sterile sand in three-inch pots. Another group of 45 cuttings were kept as uninoculated controls. Isolations from treated cuttings immediately after inoculation showed that bacteria were in vessels. A week after treatment half the inoculated cuttings were perforated at the 2.5 and 5 cm levels above the soil line. Five weeks later thirty-eight inoculated, but unwounded plants showed basal stem rot, but no rot at other places. In the wounded plants, 35 out of 45 cuttings had butt-end rot, 21 out of 45 had rot at the 2.5 cm wound, and eight out of 45 had rot at the 5.0 cm wound. All isolations from the vessels of treated cuttings were positive. Microscopic examinations of diseased cuttings showed deterioration from parenchyma in pith and cortex. All 45 check plants formed roots and were normal.

In another test two groups of 25 cuttings each (var. Ricard) were allowed to take up bacterial suspensions for 24 hours. Basal ends of cuttings in one group were washed, surface sterilized with mercuric chloride (1:1000),

and placed in three-inch pots containing sterilized soil. The other group of 25 cuttings served as inoculated controls. After five weeks, seven cuttings from the group treated with mercuric chloride had typical basal stem rot and 18 were normal, while 16 plants from the control group had stem rot and nine were normal. However, the pathogen was present in the symptomless plants, as determined by isolations. These tests suggest that even though X. pelargonii is present in vessels, it must be in contact with parenchyma before symptoms result.

The distribution of bacteria in vessels following inoculation was determined by isolations. Cuttings from known stem rot free plants (var. Ricard) about 15 cm long, were allowed to take up bacteria from suspensions by transpirational pull. After taking up inoculum for six, 12 and 24 hours, isolations were attempted from stems, petioles, and leaves. In most cases bacteria were present in the stem vessels after the cuttings were left for six hours in bacterial suspension. In a few cases the pathogen was found in petioles of lowest leaves after staying in suspension for 24 hours, but it was never found in leaf blades.

In another test the movement of bacteria in stem-inoculated plants (var. Ricard) was determined. Isolations were made 50 and 100 days after inoculation with the results illustrated in Figure 2. The pathogen moved in stem vessels independently of stem rot symptom expression, and was found well above rot. Petioles were seldom invaded.

Attempts to isolate the organism from petioles of leaves with spot-infections were not successful. X. pelargonii seems unable to move from leaf spots into the stem through petioles. In a second attempt another 87 isolations were made from green leaf tissue surrounding bacterial leaf spots and 123 isolations were made from petioles of heavily spotted leaves. In all cases results were negative, showing that X. pelargonii does not become systemic from leaf spots.

#### Root Inoculation Tests.

P. hortorum seedlings were grown in three-inch pots so that the main root penetrated through the hole of the pot (Figure 15, C). Roots of 25 such plants were inoculated by dipping the roots in a bacterial suspension (100, 000 cells per ml) in hyacinth forcing flasks. Roots of 25 control plants were treated likewise, except that no bacteria were put in the flasks. Seventeen plants developed positive stem rot symptoms as a result of root inoculation.

#### Effect of Pelargonium Debris on Susceptibility of Plants to Stem Rot

One part of fresh stem and leaf choppings from stem rot free P. hortorum seedlings was mixed with 100 parts (by weight) of pasteurized soil, and 25 three-inch pots were filled with the soil mixture. Another

group of 25 pots were filled with pasteurized soil and used as controls. All pots were planted with Radio Red rooted cuttings and two weeks later were stem inoculated with isolate 182. Four weeks after inoculation plants in pots with pelargonium debris showed stem rot in advanced stage, whereas controls in soil were not yet showing positive symptoms (Figure 15, B). This could have resulted from a deleterious effect of pelargonium debris in soil on growth of pelargonium plants.

### Environmental Factors

#### Relation of Night Temperatures to Disease Development

Frequent observations indicated that cultivated pelargoniums at our latitude suffer from stem rot mostly during the summer months (e. g. July and August) when night temperatures are at their peak. Later, with declining temperatures the disease is slowed down also, and the general appearance of the plants is considerably improved. These observations indicate that temperature has a decisive influence on disease development. Experiments were devised to gather more exact data on this interaction.

In an exploratory test 25 P. hortorum seedlings in three-inch pots were kept at 27° C night temperature, and another group of 25 plants at 21° C night temperature. After four weeks of treatment, the general appearance and growth of the plants were compared. Plants at the higher night temperature showed considerably less growth and developed chlorotic

leaves (Figure 13, C) similar to iron deficiency symptoms, whereas plants kept at 21°C were normal in appearance.

In a second test, 30 plants (var. Ricard) in five-inch pots were kept at 27°C night temperature, and another equal group at 21°C night temperature. After four weeks of treatment, both groups were stem-inoculated and kept at 21°C night temperature. Four weeks after inoculation the average disease index for the first group was 42, and for the second group 19, showing that plants predisposed by high night temperature were more susceptible.

Based on this information four groups of rooted cuttings (var. Better Times) 25 plants each, were stem-inoculated with isolate 138, and exposed to night temperatures after inoculation at 10°C, 16°C, 21°C and 27°C. Four other groups of 25 plants each were stem punctured and the wounds were treated with sterile potato dextrose broth. These four groups were used as control at the four temperature levels. Disease readings were taken one, two, three, four, five and 11 weeks after inoculation (Figure 3, Table VII). Plants kept at 27°C showed first symptoms seven days after inoculation, and all were dead in four weeks. In contrast, plants at 16°C required three weeks for symptoms to show. At 10°C disease development was still slower at the beginning, but increased rapidly after the fifth week. No control plants became diseased.

Although plants at 10°C night temperature were slow to show initial symptoms, the disease later developed rapidly (Table VII), probably because of an unfavorable temperature for plant growth. Average weights of pelargonium plants at the end of the fifth and eleventh weeks of growth at the several temperature levels showed that 16° to 21° C was optimum for growth (Table VIII). On the other hand, the causal organism was found to grow best on potato plugs at 24° to 26° C (Table IX).

#### Relation of Nutrient Concentration to Disease Development

This greenhouse experiment lasted from June to November, 1955. Cuttings (var. Ricard) were rooted in pasteurized sand, and selected for uniformity. Plants were then pre-treated by growing in sand culture at the 0.5 Hoagland nutrient level for 30 days, after which they were again selected for uniformity, and arranged in randomized blocks with five nutrient levels as treatments. After growing in the test nutrient levels for 30 days, they were stem inoculated with isolate 182. Nutrient levels were balanced Hoagland's solutions at one-tenth, five-tenths, one, two and three times normal. Uninoculated plants grew best at the one and two H levels, and poorest at the one-tenth H level (Table X) as determined by weight at the time of inoculation and 50 days later.

Final disease indices taken 50 days after inoculation show that

TABLE VII  
EFFECT OF NIGHT TEMPERATURE ON STEM ROT DEVELOPMENT IN  
INOCULATED PLANTS (VAR. BETTER TIMES)

Weeks After Inoculation	Average Disease Indices <sup>a</sup> At			
	10° C	16° C	21° C	27° C
1	0	0	0	18
2	0	0	2	37
3	2	3	8	84
4	3	10	12	100
5	8	19	59	100
11	62	58	83	100

<sup>a</sup>Disease indices:

0, healthy; 10, inoculation positive; 20 slight darkening of stem, occasional partial wilting of leaves; 40, blackening of stem, shedding of lower leaves; 60, black stem rot with occasional tip necrosis; 80, severe stem rot; 100, dead.



TABLE VIII

EFFECT OF NIGHT TEMPERATURE ON GROWTH OF PELARGONIUM,  
VAR. BETTER TIMES, FOR 5 AND 11 WEEKS  
(Values are average fresh weight in grams of ten plants)

Weeks	Night Temperatures			
	10° C	16° C	21° C	27° C
5	16.3	62.1	54.7	28.9
11	19.7	86.3	73.2	37.5

TABLE IX

EFFECT OF TEMPERATURE ON GROWTH OF X. PELARGONII ISOLATE 182  
ON AUTOCLAVED POTATO PLUGS FOR 20 DAYS

Temperature	Estimate of Growth
12° C	very weak
16° C	weak
20° C	moderate
24° C	good
26° C	abundant
28° C	good
32° C	weak
37° C	very weak

disease developed most rapidly at nutrient levels below optimum for growth, somewhat more slowly at levels above optimum, and slowest at one and two H levels. In other words, disease development increased when plant growth decreased, and became less severe with conditions favoring growth. This inverse correlation of growth and disease development is shown in Figure 13, A and B. There were no significant differences between disease indices at one-tenth and five-tenths H levels, and between one and two H levels. Average plant weight at five-tenths H level is considerably higher than at one-tenth H, but disease index is insignificantly lower.

#### Relation of Nutrient Balance to Disease Development

The effects of low, normal and high levels of nitrogen, phosphorus and potassium on stem rot development were studied in one experiment, and the effects of three different calcium levels were determined in a second.

The N, P and K effects were determined in the greenhouse from January to May, 1956. High and low N, P and K levels were compared with one H solution as described in "Materials and Methods". Plants were grown for 30 days in their respective treatments before being inoculated. Night temperature in the greenhouse during the pre-inoculation period was kept about 21°C, and was raised and held at 24°C following inoculation. Average weight of plants at the time of inoculation was 29, 22, 19, 28, 26, 28, and 22

TABLE X

WEIGHT OF UNINOCULATED CONTROL PLANTS AT TIME OF INOCULATION  
AND AT THE END OF THE NUTRIENT CONCENTRATION EXPERIMENT

Nutrient Levels	Average Weight in Grams at	
	Inoculation Time	Fifty Days After Inoculation
0.1 H	12.8	39.1
0.5 H	20.3	74.8
1 H	33.2	85.3
2 H	30.3	85.0
3 H	26.0	65.0

TABLE XI

RELATION OF NUTRIENT CONCENTRATION TO DEVELOPMENT OF  
BACTERIAL BLIGHT IN PELARGONIUM

Nutrient Levels	Average Disease Indices <sup>a</sup> 50 Days After Inoculation
0.1 H	81.2
0.5 H	80.0
1 H	51.2
2 H	53.1
3 H	62.4
L. S. D. 19:1	3.3
99:1	4.6

<sup>a</sup>Disease indices are explained in Table VII. They were taken 50 days after inoculation.

grams for plants growing in basal, high N, low N, high P, low P, high K and low K respectively. Average weights of uninoculated control plants 110 days after inoculation day were 136 grams for basal solution, 123 for high nitrogen, 107 for low nitrogen, 123 for high phosphorus, 120 for low phosphorus, 125 for high potassium, and 115 for low potassium. Disease indices were recorded 50 days after inoculation when first plants in some of the treatments reached a disease rating of 100. The experiment, however, was continued to observe the disease development to its final stage, and to re-index virus diseases in plants showing slow stem rot development. Final disease readings and scions for virus re-indexing were taken 110 days after inoculation.

Statistical analysis of disease indices 50 days after inoculation showed significant differences between balanced and unbalanced treatments (Table XII), with disease developing more slowly in plants with the balanced nutrients. Of the unbalanced solutions, high nitrogen and low potassium caused highest disease development at this point, followed by low nitrogen and high phosphorus (Figures 5, 6, 7 and 8). High potassium and low phosphorus were not significantly different and showed the slowest disease development of all the unbalanced treatments. Sixty days later (110 days after inoculation) all plants were dead in high nitrogen and high phosphorus treatments, whereas those of basal, high potassium, low phosphorus, and low nitrogen were still alive (Figure 14, A, B, C). During the second half of the

TABLE XII

RELATION OF N, P, AND K BALANCE TO STEM ROT DEVELOPMENT IN  
 PELARGONIUM (VAR. RICARD) PLANTS  
 (Values are the Indices of Disease<sup>a</sup> Ratings Taken 50 Days After Inoculation)

Treatments	Replications							Mean
	1	2	3	4	5	6	7	
Basal	22.8	23.4	18.9	27.2	23.8	20.4	26.0	23.2
High N	46.5	46.6	46.7	47.2	55.5	38.5	49.5	47.2
Low N	40.1	41.7	40.6	31.1	42.0	45.0	47.2	41.1
High P	41.1	41.0	36.2	46.7	42.5	38.5	44.5	41.5
Low P	36.5	36.6	33.0	40.4	35.0	40.0	32.6	36.3
High K	32.4	32.8	33.0	34.0	30.7	30.6	34.6	32.6
Low K	40.0	44.4	46.6	45.0	41.6	47.3	45.3	44.3
L. S. D 19:1								3.4
99:1								4.5

<sup>a</sup>Disease indices are explained in Table VII.

experiment disease development was faster in high phosphorus plants than in any of the other treatments. Assays for the presence of viruses showed that plants carrying mottle, crinkle, and leaf curl viruses developed stem rot faster than plants free of viruses or those with ring spot virus alone. This aspect is treated in more detail later.

The effect of calcium was determined in the greenhouse from March to August, 1956. Ricard rooted cuttings were grown in silica sand at low, medium, and high Ca level as described before. At the time of inoculation average weight of the plants was 12, 21 and 37 grams for low, medium and high Ca plants, respectively. The experiment was terminated 50 days after inoculation, when the average weights of uninoculated plants were 16, 36, and 97 grams for low, medium and high Ca, respectively. The weight of Ricard plants increased linearly with increasing calcium in the nutrient solution.

Statistical analysis of disease indices showed significantly less disease development at high Ca level than at the two lower levels, which were not significantly different (Table XIII). The disease indices in comparison to average plant weights 50 days after inoculation are illustrated in Figure 9, and the disease development up to 110 days after inoculation in Figures 10 and 15, A. The development of disease increased with decreasing plant growth in this experiment as in the previous experiments (Figure 9).

TABLE XIII

RELATION OF CALCIUM BALANCE TO STEM ROT DEVELOPMENT IN PELARGONIUM  
(Values are the Average Disease Indices<sup>a</sup> 50 Days After Inoculation)

Treatment	Replications			Mean
	1	2	3	
Low Ca	80.0	71.7	66.0	72.6
Medium Ca	53.3	70.9	68.3	64.2
High Ca	31.0	30.0	35.0	32.0
L. S. D. 19:1				18.9
99:1				31.3

<sup>a</sup>Disease indices are described in Table VII.

In summarizing the results of experiments of environmental factors it is evident that stem rot development is delayed in plants showing good growth, and speeded up as conditions become less favorable for growth, except in high N and high P treatments.

### The Virus Factor

Pelargonium plants of the same variety, age and appearance were often observed to vary greatly in their response to X. Pelargonii. Since pelargonium is known to harbor latent virus infection (20, 50), this possibility was considered as an explanation of the non-uniform responses. To detect the possible presence of viruses in experimental plants, veneer grafts were made to P. hortorum seedlings which were presumed to be virus-free. Following grafting, plants were kept in the greenhouse long enough for possible virus symptoms to appear in the seedling stock. Such experiments showed that unusual or complex symptoms often appeared on new leaves of the stock, both above and below the graft. Symptoms resembling those of ring spot (Figure 16, D), necrotic ring spot (Figure 17, A, C), crinkle, mottle (Figure 17, B), mosaic, spotted wilt, curling of leaves (Figure 17, D), and their modifications alone or in combinations were (Figures 16, C; 17, B, D) observed. Because of the variation in symptom expression, it was difficult or even impossible to identify these as leaf curl, mosaic, or curly top, by



using Jones (23) terminology. Therefore, they were grouped according to their most prevalent symptoms as ringspot, necrotic ringspot, crinkle, mottle, mosaic and curly top. The prevalence of these conditions is indicated by an indexing survey of six varieties from commercial sources, consisting of 100 plants each of Ricard, Radio Red, Olympic Red, Irvington Beauty, Enchantress Supreme, and Fiat. This survey showed that about 62 per cent of these plants carried virus diseases in latent form (Figure 11).

Several of these reactions have not been described in the literature, so more knowledge about the possible virus conditions was needed before continuing the stem rot interaction experiments. Attempts were made to transmit pelargonium viruses by mechanical means, and by dodder (Cuscuta arvensis Beyrich). Sap from leaves, petals and roots of known virus diseased pelargonium plants was rubbed on leaves of Nicotiana glutinosa L., Cucumis sativus L. (var. National Pickling), Vigna sinensis Endl., Gomphrena globosa L., Zinnia elegans Jacq., tomato (varieties Bonny Best and Jefferson), and pelargonium (P. hortorum) seedlings. No index plant showed virus symptoms six weeks after attempted inoculation. Following some difficulty in getting dodder to grow on pelargonium, a strain was selected which was successful. Dodder established on known virus diseased pelargonium plants was allowed to grow from pelargonium to tomato, zinnia, cucumber, carrot, tobacco, geranium (Geranium

maculatum) and pelargonium seedlings. No virus symptoms were observed after 12 weeks.

Symptom-expression following use of several scions from the same stock plants grafted to seedlings was not uniform. Scions from lower stem portions had a tendency to cause more pronounced symptoms in seedling stocks than those from the upper part of the stem. Understocks grafted with two or more scions showed more pronounced symptoms than those grafted with one scion. Cuttings taken from the lower stem portion of known virus diseased stock plants showed virus symptoms after root formation, when growth was resumed, regardless of time of year, whereas those from upper part of stock plants showed symptoms less distinctly and less frequently.

Effect of light and temperature on virus expression was examined. Known virus diseased and virus free stock plants were kept for 10 days under continuous light of low intensity (100-400 foot candles) at a constant temperature of 9°C. A duplicate group of 35 plants was kept under ordinary greenhouse conditions at 21°C night temperature. Older leaves of virus-infected plants under low temperature and low light lost chlorophyll and green rings, dots, and mottling appeared on yellow background of leaves. No virus-free control plants reacted in this manner.

Based on this information, experiments were devised to study the possible interaction between stem rot and virus diseases. As a first test, two sets of rooted Ricard cuttings, 100 plants each, were indexed. Scions from the first group were veneer-grafted to seedlings, presence of various virus diseases was determined, and the parent plants were grouped as ring-spot, necrotic ringspot, and crinkle, according to the most characteristic symptoms appearing in seedlings. Plants of group two were inoculated with X. pelargonii isolate 182 and 50 days later the 37 plants with least evidence of stem rot (rating 40 or less) were indexed for virus diseases. Results showed that nine per cent of group one plants were free of both viruses and stem rot, while 22 per cent were latently infected with X. pelargonii (Figure 12). The other 69 per cent carried viruses in some form--32 carried ringspot, 12 carried necrotic ringspot, and 25 carried crinkle. In group two, ten plants out of the 37 survivors (27 per cent) were virus free. Virus carrying plants in group two totaled 27 (73 per cent) of which 17 (46 per cent) carried ringspot, eight (22 per cent) carried necrotic ringspot, and two (five per cent) carried crinkles (Figure 12). The data indicate that plants carrying crinkles latently are much more sensitive to stem rot, since the percentage of plants carrying this virus was reduced from 25 to five as a result of inoculation with X. pelargonii. The small number of plants plus the complicating effect of latent X. pellargonii infections in

many plants of group two preclude any conclusion regarding the other viruses.

In a second test, 120 P. hortorum seedlings were grafted with scions from virus-free plants, and 237 were grafted with scions from known virus diseased stock plants. Virus symptoms were determined 40 days after grafting, and stems were inoculated with isolate 182 in order to observe and compare the development of stem rot in virus free and virus infected seedlings. Slowest stem rot development occurred in virus free seedlings, fastest in plants with crinkle and mottle symptoms, and intermediate in groups with ringspot and necrotic ringspot symptoms (Table XIV).

In a third test, rooted cuttings from virus free and from known virus diseased stock plants (var. Ricard) were stem inoculated with isolate 182, and stem rot development was determined. Stem rot development was much slower in virus free than in virus diseased plants. Plants with leaf mottle symptoms showed fastest disease development, those with necrotic ringspot less, and plants with ringspot symptoms were the slowest among virus diseased pelargonium plants (Table XV) to develop stem rot.

TABLE XIV  
RELATION OF VIRUS DISEASES TO STEM ROT DEVELOPMENT IN  
P. HORTORUM SEEDLINGS  
(Plants were Classified 50 Days after Inoculation with X. Pelargonii)

Virus	Total No. Plants	Number of Plants with Stem Rot Rating of			
		0	Slight	Moderate	Severe
Virus-free	102	34	52	8	8
Ringspot	130	24	50	30	24
Necrotic ringspot	22	2	6	4	10
Mottle	44	6	8	12	18
Crinkle	36	2	4	10	20
Total	334	70	120	64	80

TABLE XV

RELATION OF VIRUS DISEASES TO STEM ROT DEVELOPMENT IN ROOTED  
CUTTINGS (VAR. RICARD)

(Plants were Classified 50 Days after Inoculation with X. Pelargonii).

Virus	Total No. Plants	Number of Plants with Stem Rot Rating of			
		0	Slight	Moderate	Severe
Virus-free	25	18	5	1	1
Ringspot	25	9	11	3	2
Necrotic ringspot	25	5	2	10	8
Mottle	25	2	4	7	12
Total	100	34	22	21	23

## DISCUSSION

All varieties of P. hortorum have been subject to stem rot caused by X. pelargonii when tested by stem inoculations. However, varieties differ in relative resistance, since disease develops much more slowly in some varieties than in others. In commercial culture, the difference in resistance is sometimes more apparent than real. For example, many growers are sure that the variety Radio Red has a high resistance, while the varieties Better Times and Ricard are extremely susceptible. Inoculation experiments by Munnecke (28) showed that Radio Red was highly susceptible, Ricard was moderately so, and Better Times was the most resistant variety tried. Present observations confirm this, as far as Radio Red and Ricard are concerned. Differences between observed resistance and experimental results may be explained as a difference in latent infections. Better Times, being somewhat resistant, may under certain conditions carry a high level of latent infections, which result in many diseased plants when conditions favor disease. Radio Red is very susceptible, and under the proper conditions may have few latent infections, since diseased plants would tend to be eliminated.

Variation between bacterial strains in ability to cause leaf spot is even greater than variation in ability to cause stem rot. Several factors

may attribute to the more uniformly positive results from stem inoculation. Inserted cotton plugs undoubtedly act as reservoirs of inoculum; they also may absorb acidic cell sap of crushed cells, thereby raising the pH of the wounded area and making it more suitable for bacterial attack. Success in leaf inoculations, on the other hand, depends more on environmental conditions. Such factors may account, in part, for the experimentally demonstrated decrease in virulence of an isolate when tested by leaf inoculations, while the same isolate did not lose virulence after prolonged maintenance in culture when tested by stem inoculations.

Formerly, leaf spot and stem rot were assumed to be caused by different species of bacteria (5). More recently, experiments have shown that leaf spot can be caused by the stem rot pathogen (19, 28), although some workers never find leaf spot. The fact that certain strains of the pathogen will cause leaf spot and stem rot, while other strains cause only stem rot, may help to explain some of the past confusion. In addition to this qualitative difference in strains, there are also quantitative differences in virulence.

Inoculation studies on a wide variety of common flowers, vegetables, and weeds showed that X. pelargonii has a very narrow host range confined to the Geranium family. As the bacterial blight organism can be grown on a variety of simple culture media, it was concluded that the causal agent was



very sensitive to some non-nutritional host conditions.

Cell sap pH values of all parts of pelargonium plants were lower than the lowest limit for growth of X. pelargonii in culture. The pH of crushed tissue, however, is not necessarily the same as the pH of protoplasm and intercellular areas of intact plants. The acidity of intercellular spaces is more critical, since this is where the pathogen is first established. It is difficult to determine the true pH of middle lamella and intercellular spaces in situ, but it is presumably less acidic than cell sap. Nevertheless, there are some indications that pH may be a factor in initial establishment of infection. All X. pelargonii isolates from blighted pelargonium plants caused stem rot if stem inoculations were used, but some isolates were not able to cause leaf spot regardless of cell concentrations used as inoculum. In stem inoculations the fastest and most pronounced stem rot development was obtained by using a small portion of diseased pelargonium tissue as inoculum. Slower disease development occurred when broth culture or centrifuged and washed bacterial suspensions were used. Inoculations with less virulent isolates have been relatively more successful on lower stem portions than on the upper, especially when inoculum from centrifuged bacterial precipitates was used. From these observations it could be concluded that some inhibitory effect increases as the pH decreases, or that the bacterium produces certain metabolic products which raises the pH of the surrounding tissue.

The current experiments indicated that the bacterial blight organism, although present in vessels, produced disease only on invasion of parenchyma. It moved with the transpiration stream in vessels and caused disease whenever parenchyma tissue was reached, such as occurred at wounds. This may explain why discoloration of vessels, such as is found in typical vascular wilt diseases, was not observed in latently infected pelargoniums.

Stem rot increased with increasing temperatures from 10° to 27°C during the first five weeks after inoculation. Later, the disease increased more rapidly in plants kept at 10°C night temperature than in the others. Since the optimum temperature for growth of X. pelargonii is about 27°C and the optimum temperature for host growth somewhat below 20°C, disease development during the first few weeks following inoculation seems to be primarily an effect of temperature on the pathogen. Later, however, plants at the lowest night temperatures were increasingly affected. This may be due to an unfavorably low temperature which weakens the host. This is partially supported by the comparison of growth rates of var. Better Times rooted cuttings and disease indices. These data would indicate a correlation between night temperatures and average disease indices during the first period of test and inverse correlation between average plant weight and disease indices during the final period of test.

The effect of high (27°C) night temperature on increasing stem rot development is useful for detecting latently contaminated pelargonium plants. Several weeks of such treatment usually suffices.

Data on nutrient concentration effects indicated that slowly growing plants developed disease more rapidly than plants under optimum condition of growth. Typical vascular diseases, such as cabbage yellows, Fusarium oxysporum f. conglutinans (Wr.) Snyder et Hansen, tomato wilt, F. oxysporum f. lycopersici (Sacc.) Snyder et Hansen, and bacterial wilt of tomato, Pseudomonas solanacearum E. F. Sm. decrease with increasing nutrient levels. On the other hand, the effect of nutrient concentration on disease development of bacterial canker of tomato, Corynebacterium michiganense (E. F. Sm.) Jensen, a parenchyma invader, is found to be almost diametrically opposite (14). Walker and Foster (60) who studied the problem of xylem and phloem invaders, postulated that with xylem invaders (fusaria), using nitrates as a source of nitrogen, the pathogen may be able to "out compete" the host for minerals in the xylem vessels, but as the concentration increases the host is able to grow more rapidly and escape wilting. Phloem invaders may be dependent upon nitrogen compounds elaborated by the host so that the amount of available food depends upon the growth of the host plant.

Bacterial stem rot of pelargoniums behaves like vascular invaders in its response to nutrients, the disease expression being approximately

inversely proportional to plant growth. Isolations from diseased pelargonium plants usually yielded X. pelargonii in vessels, but in order to cause stem rot symptoms, the causal organism must reach parenchyma tissue of host. Biochemical tests (5, 12, 19, 27, 51) showed that X. pelargonii utilized organic nitrogen, sugars, various organic acids, and pectin, but did not reduce nitrate, which may put it in the class of parenchyma invaders. This seeming contradiction may be more logical if the method of parenchyma invasion is considered. X. pelargonii is not able to attack salicin, which has a  $\beta$ -glucosidic linkage, and is not known to hydrolyze cellulose. However, in diseased pelargonium stems the first visual symptom often appears at points where stipuli have been attached to the stem, and at leaf-scars, which suggests that broken ends of vessels serve as exits for bacteria. Once in the parenchyma, the causal organism destroys tissue at a relatively constant rate and a weaker host succumbs first.

Disease in plants grown in unbalanced nutrient solutions was found to be correlated with decreased plant growth. In unbalanced solutions, the bacterial stem rot development was decreased in low P, high K, and high Ca solutions, and increased in low Ca, high P, and in high N solutions.

Ca plays a vital role in metabolism of plants generally and in pelargoniums particularly. It is known to be permanently fixed as calcium salt of pectic compounds in the middle lamella, to form salts with organic

acids, to combine with protein molecules, to be necessary for the growth of apical meristem, to be required for synthesis of organic acids, and to have a role in nitrogen metabolism. Nightingale (31) showed that some plants were unable to absorb and assimilate nitrates in the absence of calcium. Since Ca is not known to be essential for bacteria (40) it may be assumed that the effect of calcium in stem rot development is mainly due to beneficial action on plant growth.

Pelargonium plants grown in high potassium solution behaved similarly to high calcium plants in stem rot development. It is generally known that actively growing young tissues are always rich in potassium and that there is an accumulation of soluble organic nitrogen (amino acids and amides) in K deficient plants (61). Since the protein content of potassium deficient plants is relatively low, it is believed that this element is involved in protein synthesis. Amino acids and amides on the other hand, are utilized readily by X. pelargonii, thus potassium deficiency may support the growth of the pathogen and inhibit the growth of the host simultaneously.

Plants in high P solution first developed disease slightly faster than those in low P, but disease later increased rapidly in high P plants. Nightingale (32) found that the absorption of inorganic nitrogen was depressed when available phosphates in the rooting medium were high. Maturity of such plants was reached earlier. Current experiments have shown that older pelargonium

plants and lower stem portions are somewhat more susceptible to X. pelargonii. Faster aging and onset of senility may have been the reason for faster stem rot development in high P plants during the latter part of the experiment.

Plants in high N solution developed symptoms more rapidly than plants in low N. In biochemical tests (5, 19, 27, 51) X. pelargonii utilized nitrogen in the organic but not in the inorganic form. Everything else being equal, one could thus expect faster disease development in plants higher in organic nitrogen. Nightingale (30) working with tomato plants found these contained very little organic nitrogen and no nitrate or ammonium when grown in minus-nitrate solutions, while plants grown in plus-nitrate solutions had substantial amounts of organic nitrogen. Gallegly and Walker (14), working under similar conditions with tomato plants, found that at 24° C plants in high N solutions contained more nitrogen compounds and were more susceptible to bacterial wilt. X. pelargonii is primarily dependent for food on parenchyma tissue, therefore one could expect it to be benefitted by higher organic nitrogen content in this tissue. This may explain the higher disease ratings in high N solutions.

The fact that plants in all unbalanced nutrient solutions show significantly higher disease ratings would indicate that besides the interactions just described, plant vigor and other unexplained factors are involved. Reactions of plants in low balanced nutrient solution further support this

conclusion. It is possible that auxin relationships are involved here.

About 62 per cent of plants of commercial pelargonium stocks carried virus diseases in a latent form. Plants carrying viruses causing crinkle, mottle, mosaic or combinations of these, were most susceptible to stem rot, as compared to disease development in virus-free plants. Plants carrying viruses causing ringspot and necrotic ringspot behaved as intermediates. Knowledge of virus diseases of pelargoniums is scant, and nothing was previously known about the interaction between X. pelargonii and viruses in pelargonium. Associations between microbes, and between microbes and viruses, as reviewed by Waksman (58, 59) and by Hedges (17, 18) show that various types of interactions may be possible. However, certain viruses may simply weaken pelargonium plants, making them more sensitive to bacteria; other factors which weaken this host are known to increase susceptibility. Whatever the association between viruses and X. pelargonii in pelargonium may be, it is purely speculative until further studies are made.

The data presented here may have practical as well as theoretical implications. X. pelargonii may remain latent in its host under conditions favorable for growth of pelargonium. However, when plants are exposed to environmental conditions unfavorable for host growth, such as high night temperatures or unfavorable nutrient conditions, the latent infection will

be expressed as stem rot, and infected plants can be eliminated. This knowledge should be useful for producing propagating stocks free of X. pelargonii. Viruses also exist in a latent form in commercial stocks; these appear to be of practical importance because they make plants more sensitive to X. pelargonii. Latent virus infection can be detected by grafts to virus free clones, which react in a distinctive manner, although they usually recover later and the virus again assumes its latent character. Once free of viruses, greenhouse stock should remain free if ordinary precautions are taken, since the viruses are not spread mechanically and insects do not go to pelargoniums until temperatures are lowered. To keep stock free of X. pelargonii, however, may be more difficult, since it can be spread by water, soil, equipment, tools and mechanically during propagation.

That bacterial and virus infections can be eliminated from propagating stocks, and that stocks can be kept disease-free, has been proven. Such stocks of varieties Ricard, Radio Red, Olympic Red, Irvington Beauty, and Fiat, previously contaminated with both X. pelargonii and viruses, have been on hand at Michigan State University for over a year.



## SUMMARY

Experimental data showed that commercially available Pelargonium stocks carried bacterial blight in a latent form. Twenty-six per cent of 600 plants of six such varieties, kept under conditions favoring disease development, but precluding disease spread, developed stem rot within three months. X. pelargonii did not attack plants outside the Geranium family. Strains of the causal organism were found to differ qualitatively and quantitatively in effects on host plants. It was isolated from vessels of inoculated plants, but caused stem rot only when parenchyma was invaded. Root invasion was demonstrated. Disease was favored by high temperatures. Symptoms were almost absent at 10°C night temperature, developed slowly at 21°C, and rapidly killed plants at 27°C. Mineral nutrient levels below and above optimum for growth enhanced disease development, as did high N, high P, and low Ca. Symptoms were inhibited by high K, low P, high Ca, and balanced nutrient solution at optimum concentrations for growth.

Sixty-two per cent of 600 commercial pelargonium plants of six varieties carried latent virus diseases. The viruses were not transmitted mechanically or by dodder, but were graft transmissible to seedlings, where ringspots, necrotic ringspots, crinkle, mottle, mosaic, spotting, and necrotic wilting were produced. The presence of certain virus diseases in pelargonium plants enhanced stem rot development.

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Figure 1

Relative virulence of X. pelargonii isolates 99, 138, and 182 to varieties Radio Red and Ricard and P. hortorum seedlings. Zero indicates no stem rot development, and 100 indicates death of the plants. Disease indices were taken 50 days after inoculation.



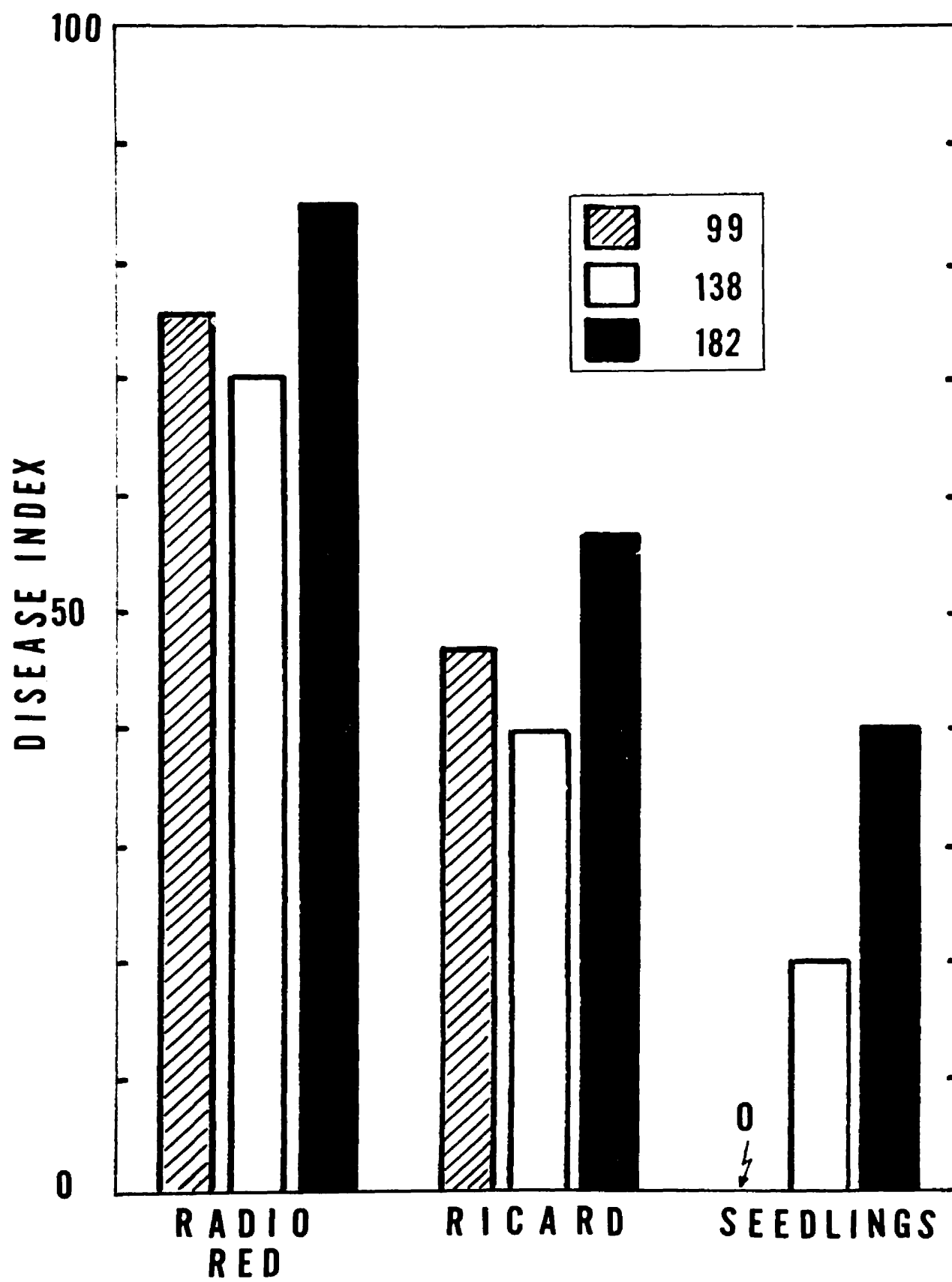


Figure 2

Rates of movement of X. pelargonii in stems (var. Ricard) as determined by per cent re-isolations at 5, 15, and 25 cm above the point of inoculation.

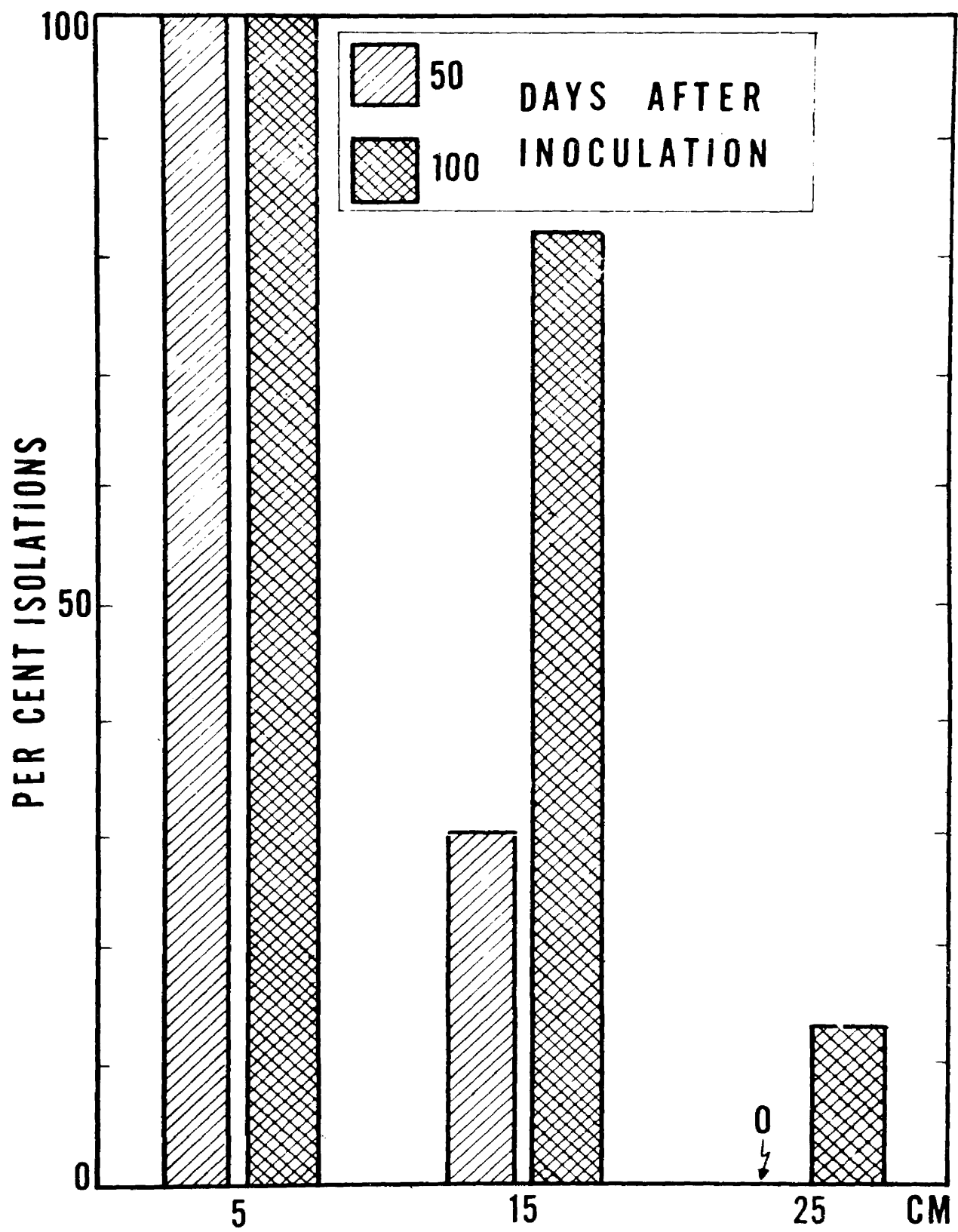


Figure 3

Effect of night temperatures on stem rot development in  
pelargonium (var. Better Times).

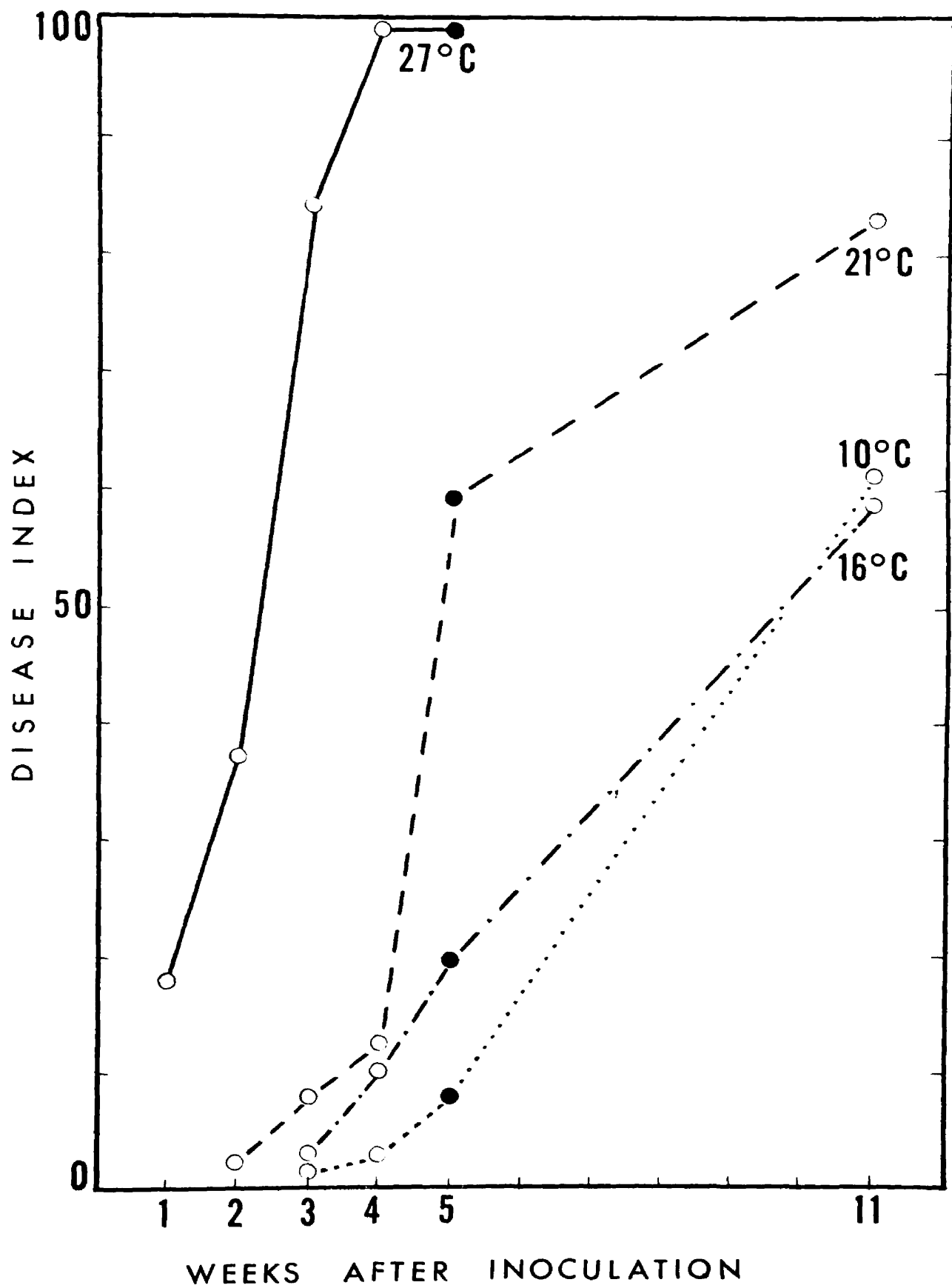


Figure 4

Comparison of disease development and growth of pelargonium as affected by nutrient concentrations from 0.1 to 3.0 Hoagland levels.

Disease indices were taken 50 days after inoculation. Fresh weights are those of uninoculated controls.

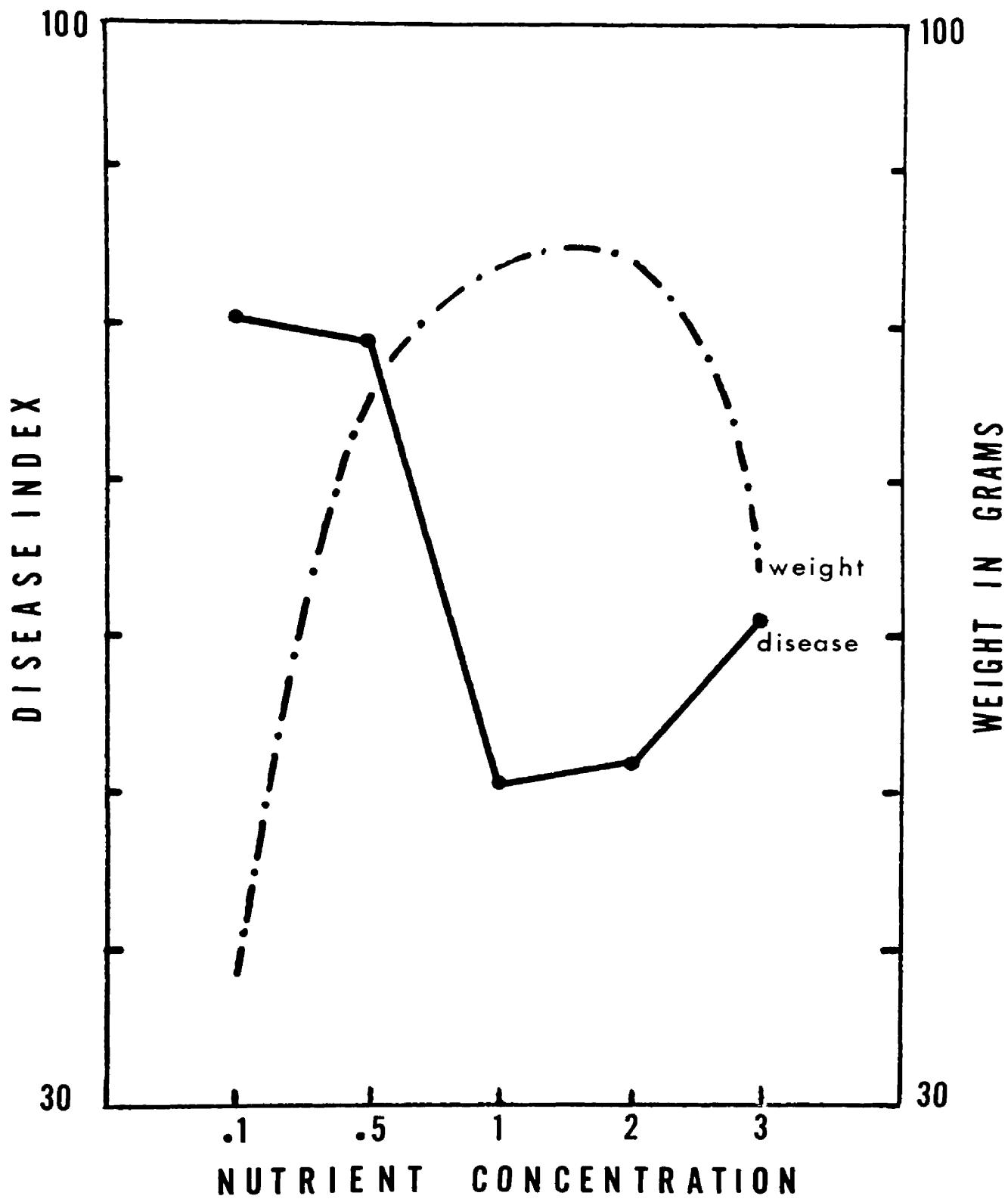


Figure 5

Effects of high and low levels of N, P, and K on stem rot development in pelargonium. The basal solution was 1.0 Hoagland's (1H). Disease indices were determined 50 days (black), and 110 days (dotted) after inoculation.



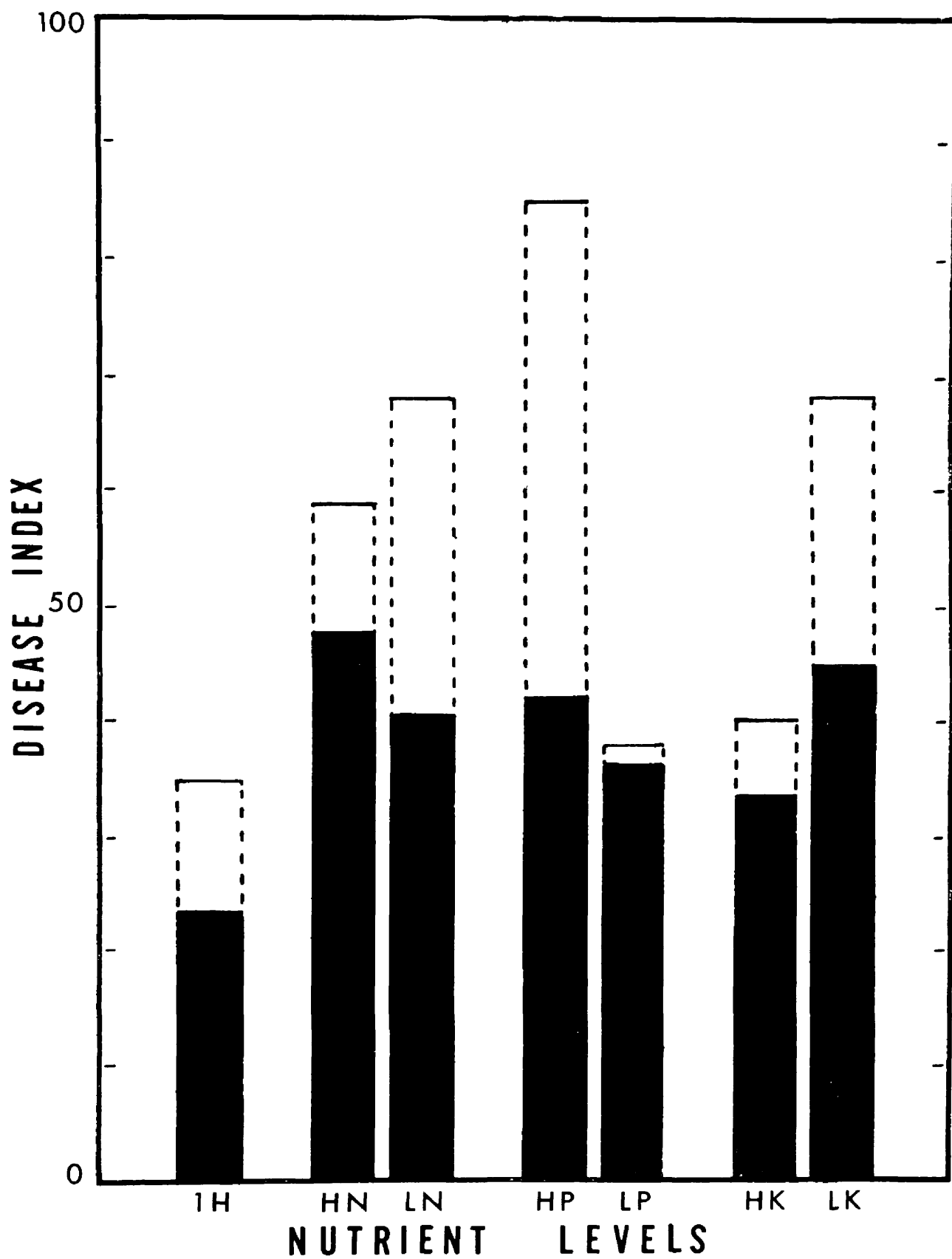


Figure '6

The effect of low (LN) and high (HN) nitrogen levels on rate of stem rot development, as compared with balanced nutrient levels (1H). Disease indices 50 days after inoculation (dots) were analyzed statistically.

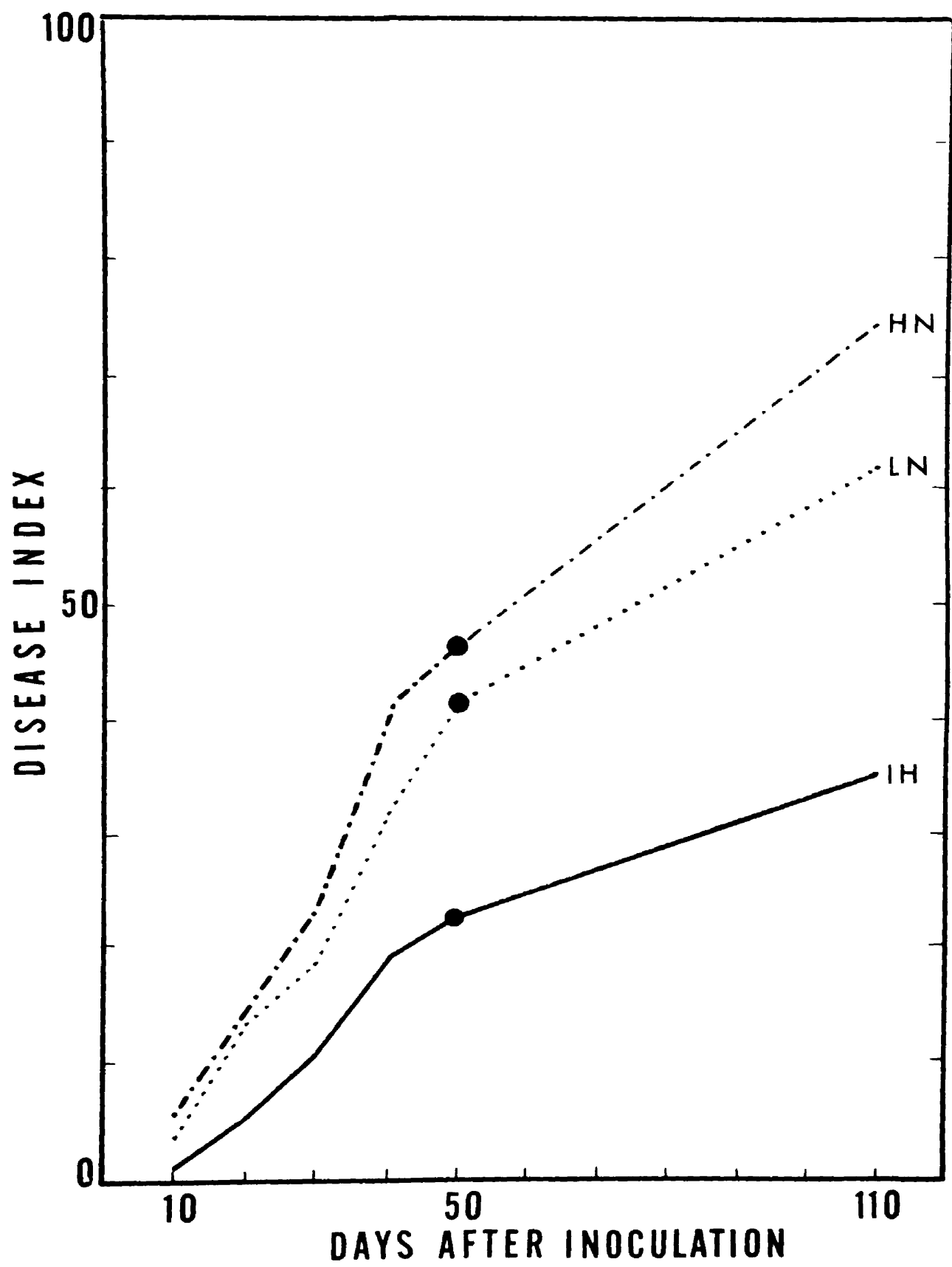


Figure 7

Effect of P levels on rate of stem rot development. LP = low phosphorus; HP = high phosphorus; 1.0 H = 1 Hoagland's solution. Disease indices 50 days after inoculation (dots) were analyzed statistically.

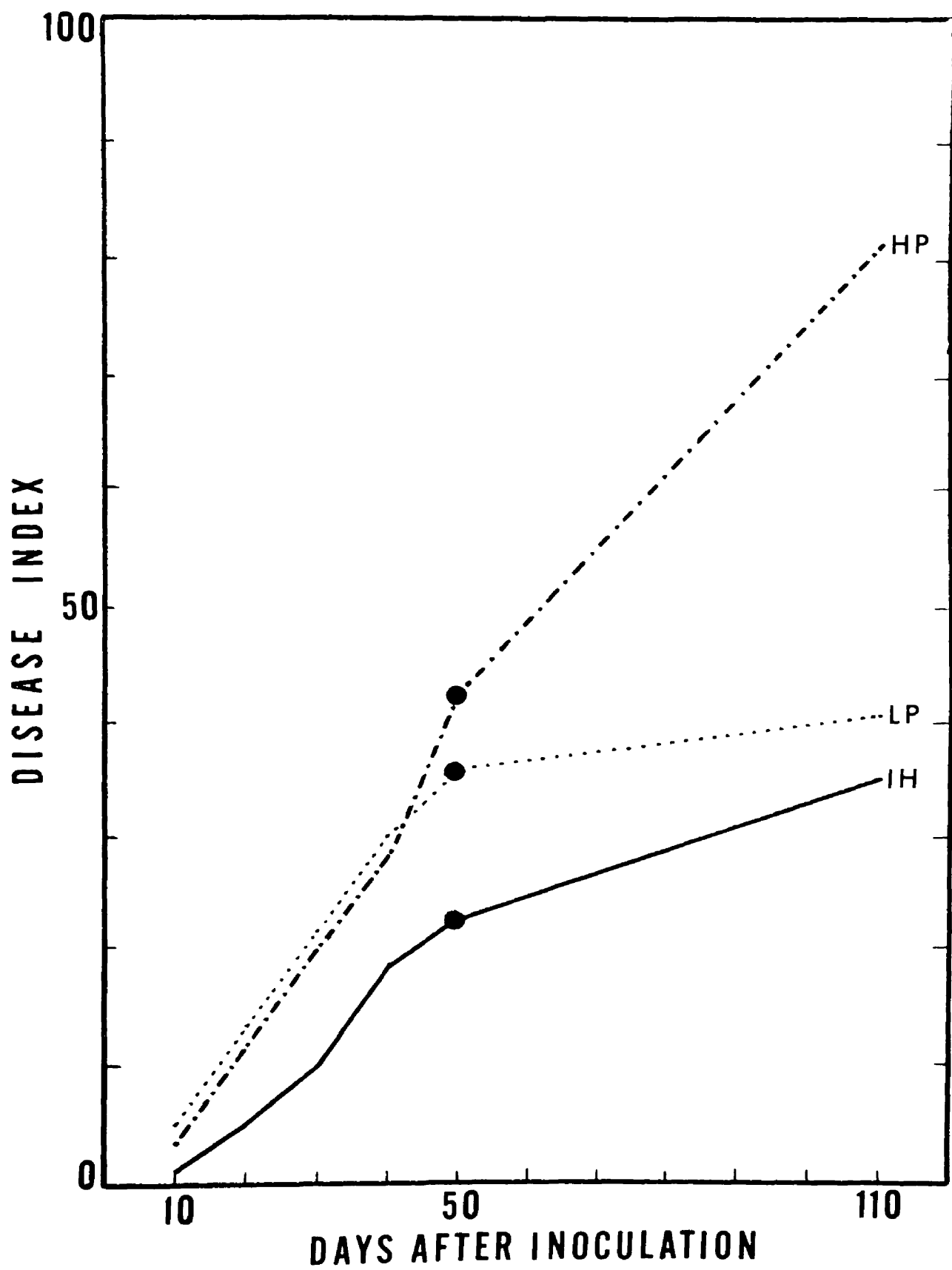
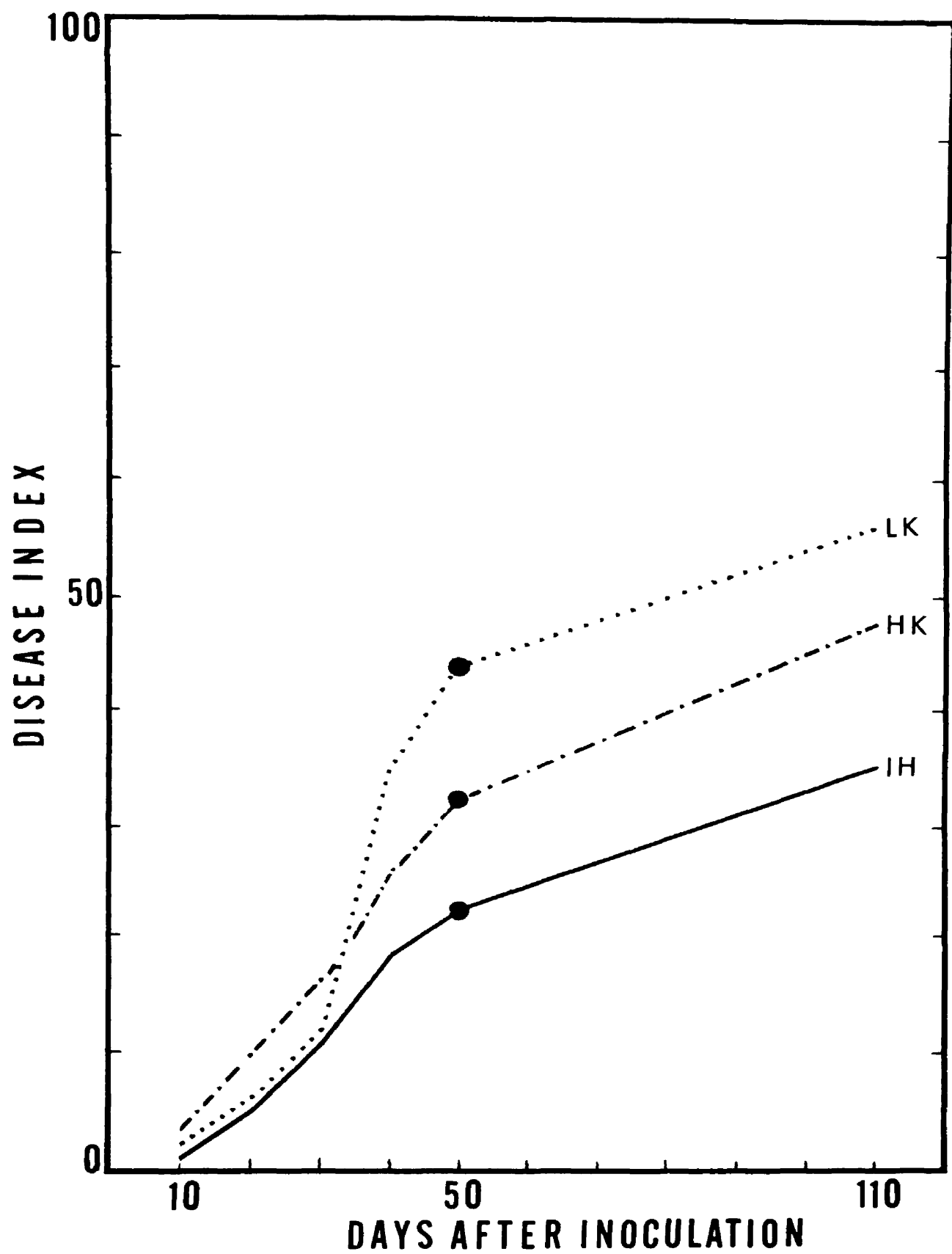


Figure 8

Effect of K levels on rate of stem rot development. LK = low potassium; HK = high potassium; 1 H = 1 Hoagland's solution. Disease indices 50 days after inoculation (dots) were analyzed statistically.



### Figure 9

Disease development and growth of pelargonium as affected by Ca concentration in the nutrient solution. Disease readings and green weights were taken 50 days after inoculation. Weights are those of uninoculated controls.



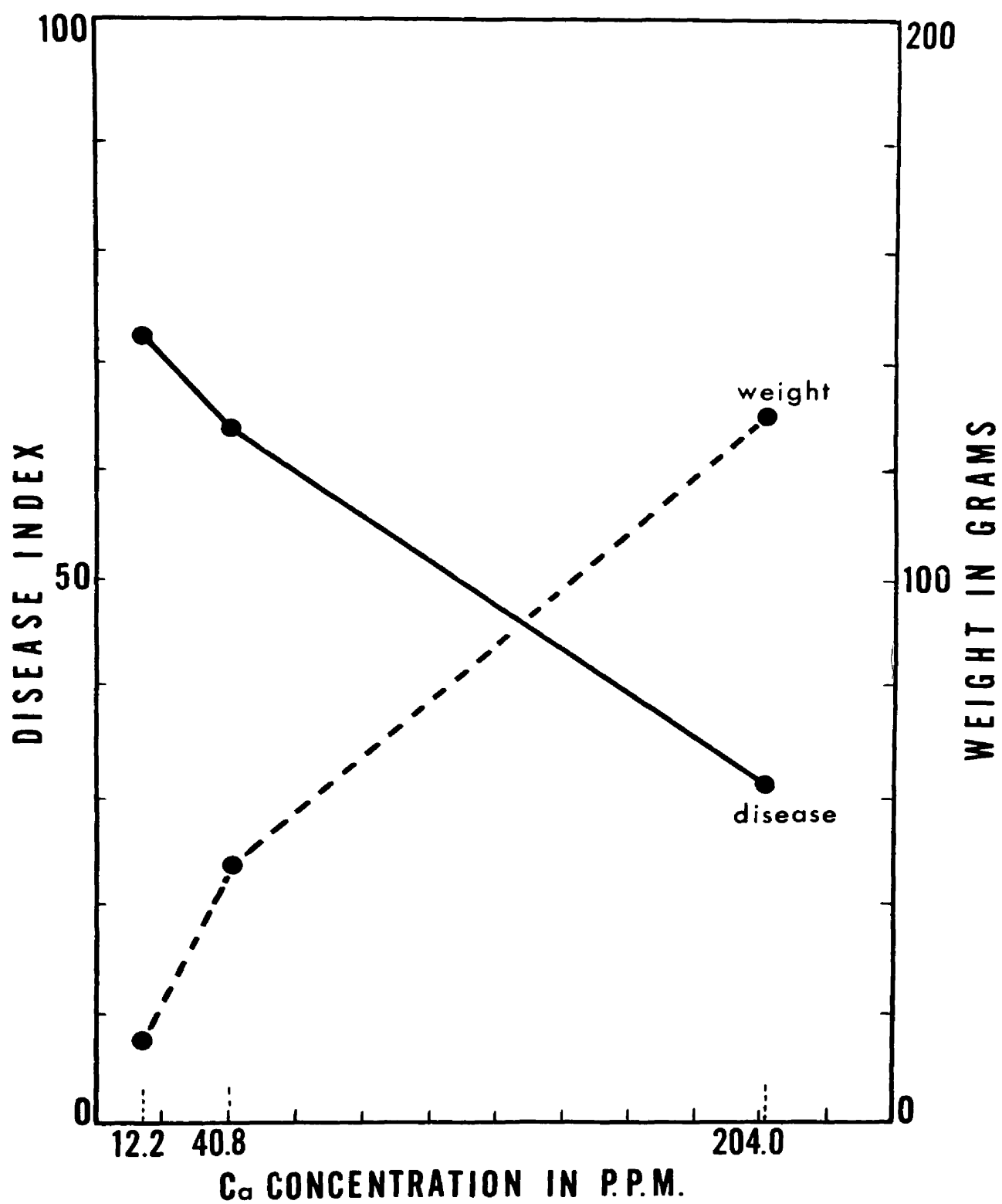


Figure 10

Effects of Ca levels on rate of stem rot development. Low (LCa), medium (MCa), and high (HCa) calcium solutions were used. Disease indices 50 days after inoculation (dots) were analyzed statistically.

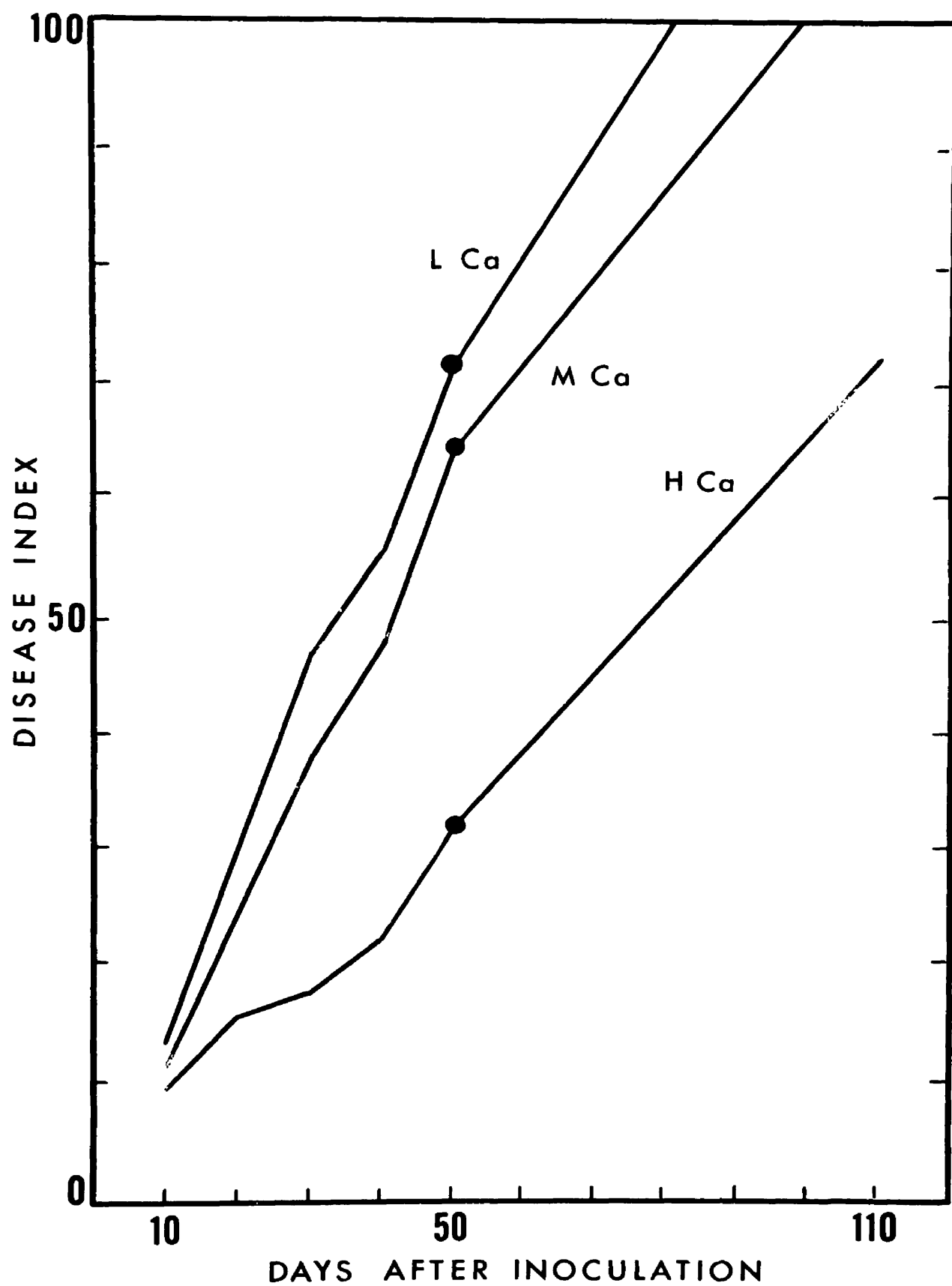


Figure 11

Plants carrying latent X. pelargonii (black) and latent virus infections (shaded) in pelargonium varieties Ricard (R), Radio Red (RR), Olympic Red (OR), Irvington Beauty (IB), Enchantress Supreme (E), and Fiat (F) from commercial sources.

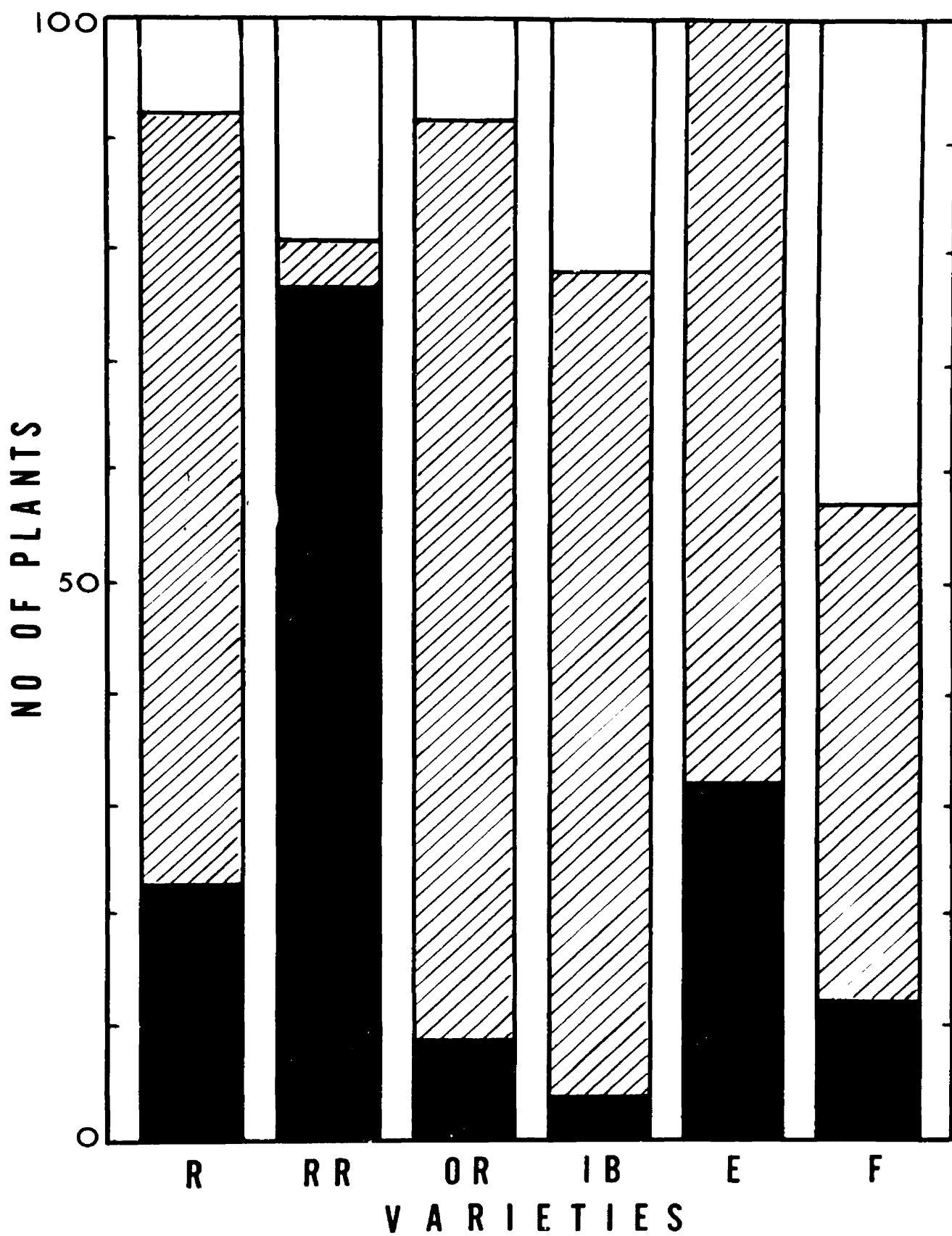


Figure 12

Per cent infection by several viruses and X. pelargonii in a commercial stock (var. Ricard) (left) and in a similar sample 50 days after inoculation with X. pelargonii (right). RS = ringspot; NRS = necrotic ringspot; CR = crinkle; SR = stem rot.

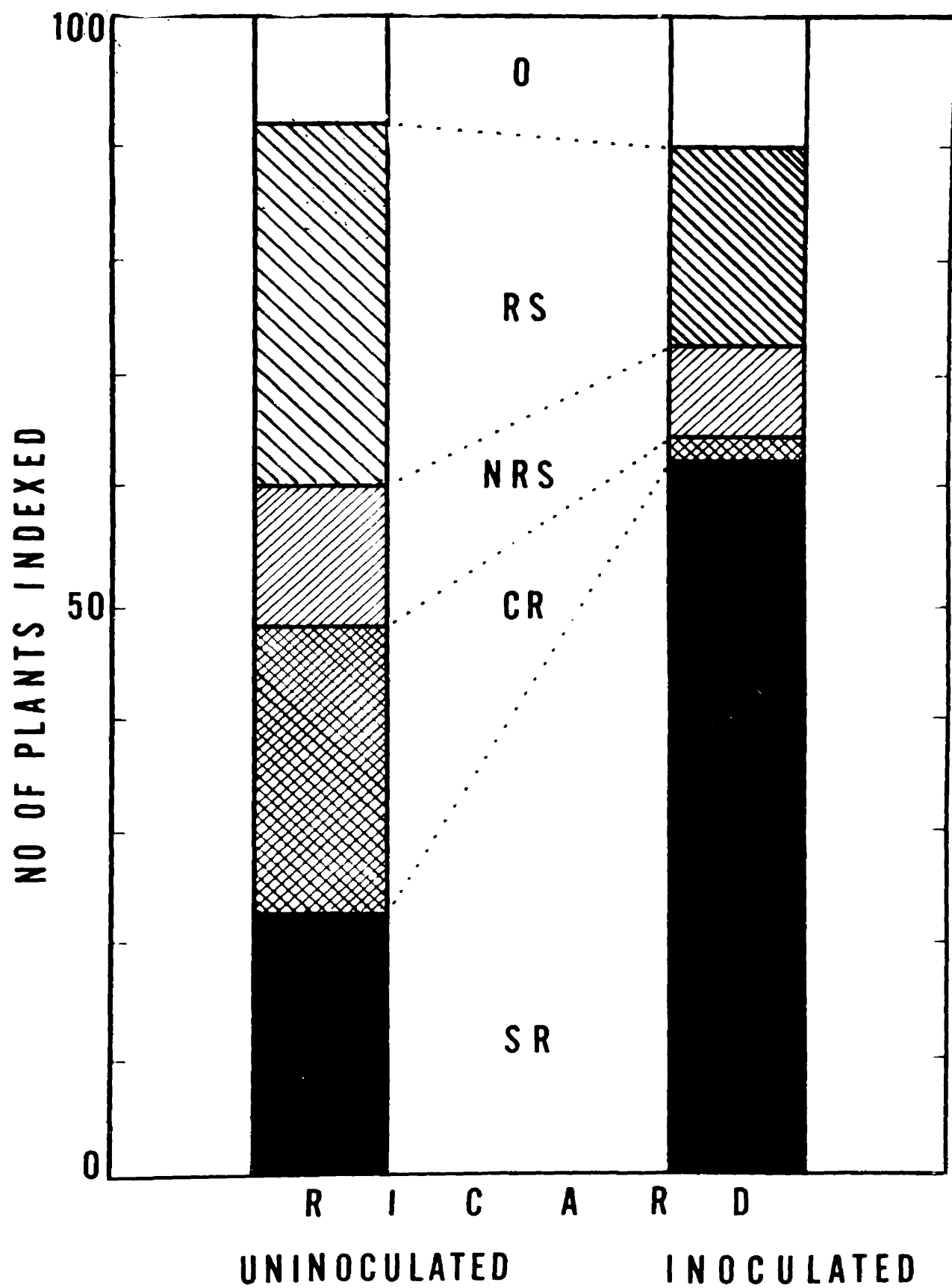


Figure 13

A. Effect of balanced nutrient concentrations (left to right) 0.1, 0.5, 1.0, 2.0, and 3.0 Hoagland's levels on growth of pelargonium.

B. Comparative stem rot in plants grown in balanced nutrient concentrations of (left to right) 0.1, 0.5, 1.0, 2.0 and 3.0 Hoagland's levels.

C. Etiolation of new leaves of pelargonium seedlings caused by 2 weeks exposure to high night temperature (27° C).



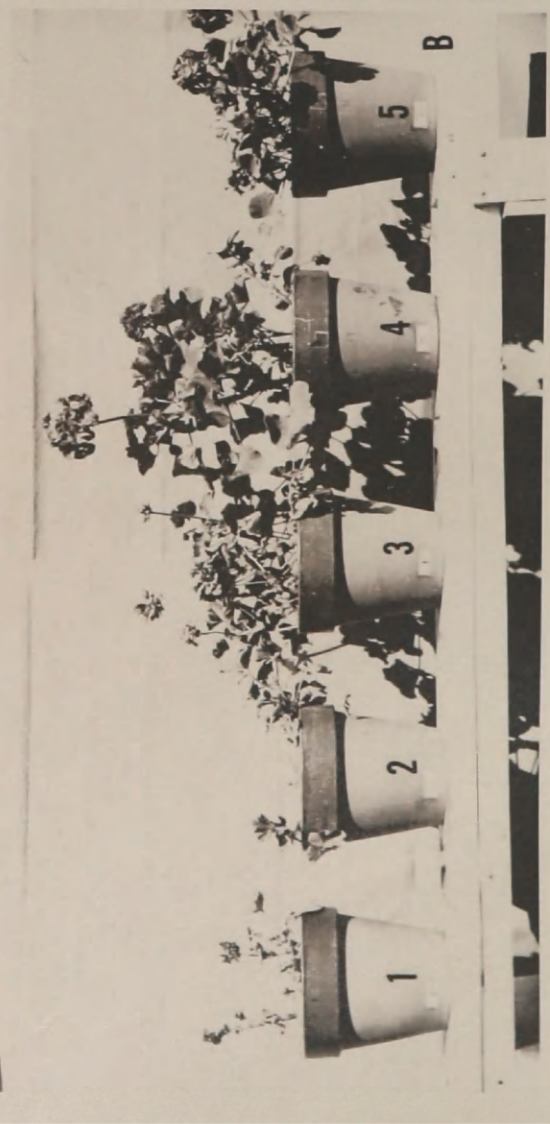
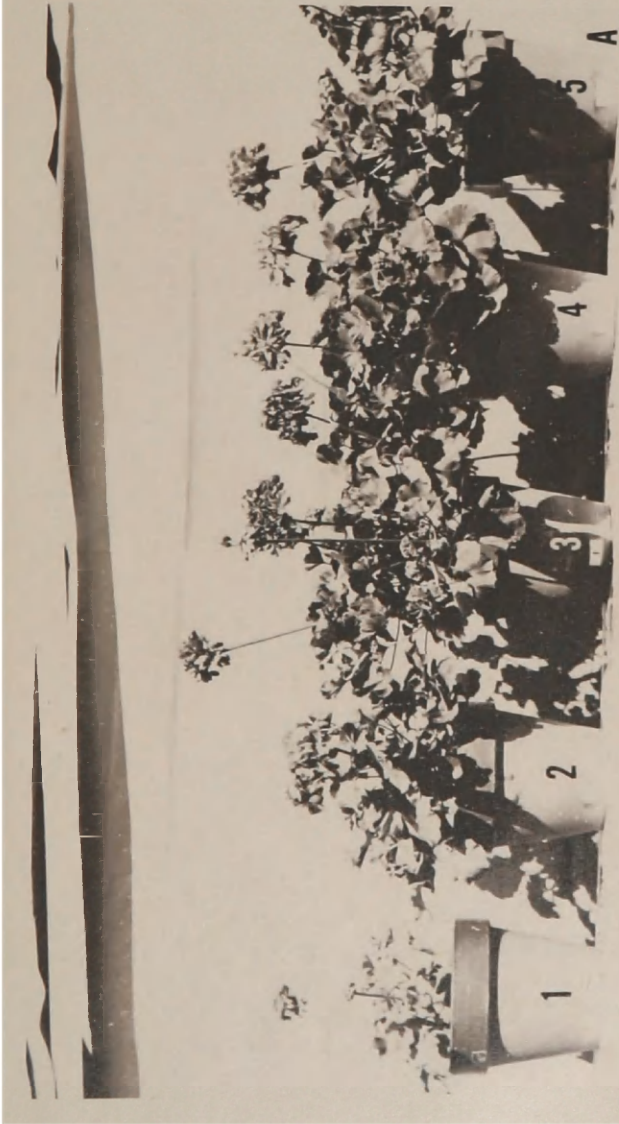


Figure 14

A. Comparative stem rot in plants grown in (1) high N, (2) low N, and (3) 1.0 Hoagland's solutions. 110 days after inoculation.

B. Comparative stem rot in plants grown in (1) high P, (2) low P, and (3) 1.0 Hoagland's solutions. 110 days after inoculation.

C. Comparative stem rot in plants grown in (1) low K, (2) high K, and (3) 1.0 Hoagland's solutions. 110 days after inoculation.





Figure 15

A. Comparative stem rot 110 days after inoculation, in plants grown in (1) low Ca, (2) medium Ca, and (3) high Ca solutions.

B. Effect of pelargonium debris in soil on stem rot development. Left, plants grown in pasteurized soil; right, plants grown in pasteurized soil plus debris. Picture was taken four weeks after inoculation.

C. Right, pelargonium seedling inoculated by root dip; left, control plant.



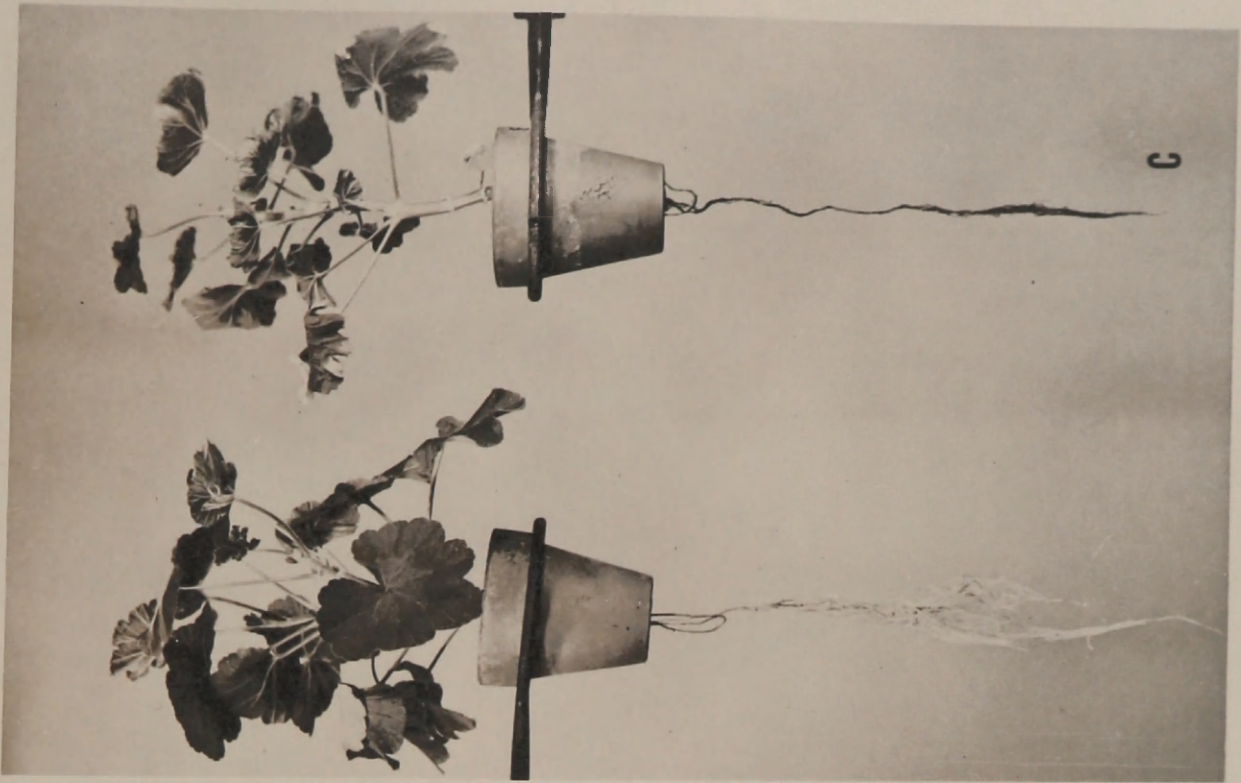
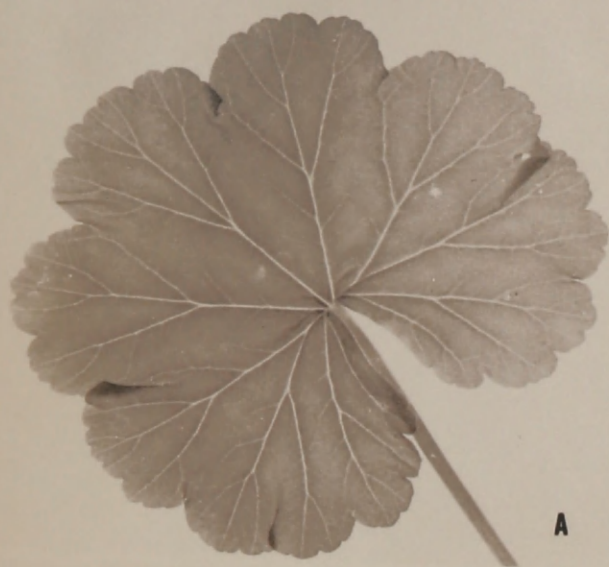
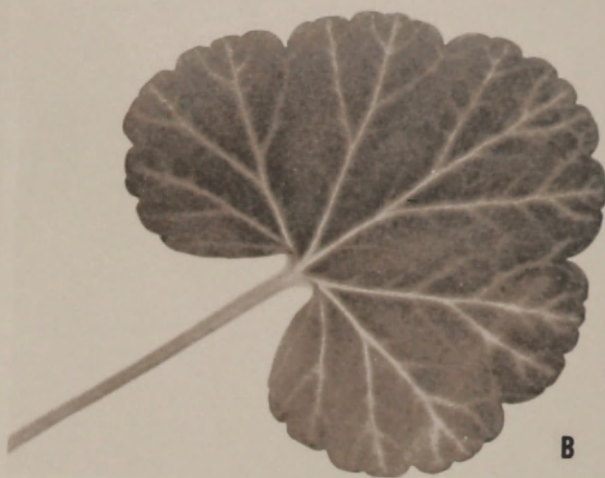


Figure 16

Virus disease symptoms in pelargonium leaves. A, healthy control; B, vein clearing; C, mottle; and D, ringspot.



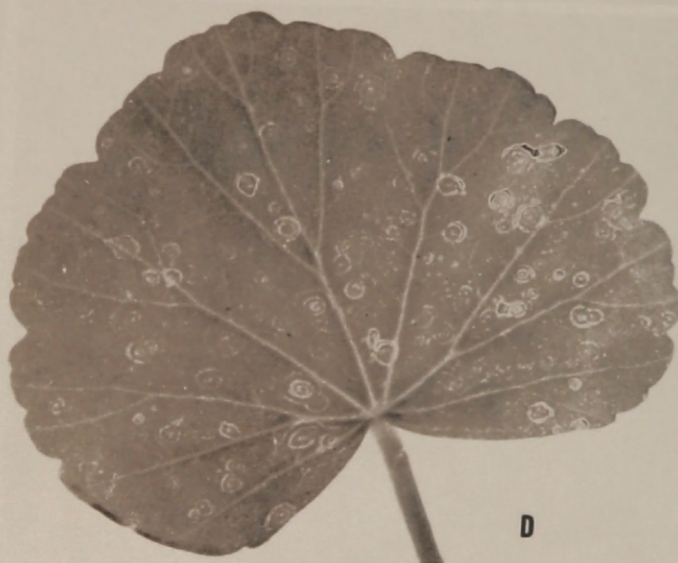
A



B



C



D

Figure 17

Virus disease symptoms in pelargonium. A, necrotic ring-spot; B, necrosis; C, brown, elongated, corky, raised necrotic stripes on stem, associated with leaf curl and necrotic ringspot viruses; D, curly top.





A



B



C



D