GROWTH, MINERAL UPTAKE AND PHOSPHORUS METABOLISM OF *PISUM SATIVUM* L. AS INFLUENCED BY AIR AND SOIL TEMPERATURES, PHOSPHORUS NUTRITION AND GROWTH RETARDING CHEMICALS

by

NURUDEEN OLORUN-NIMBE ADEDIPE
B.S.A., University of British Columbia, 1966

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department
of
PLANT SCIENCE

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April, 1969

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and Study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Plant Science

The University of British Columbia
Vancouver 8, Canada

Date April 29, 1969

Supervisor: Professor Douglas P. Ormrod

ABSTRACT

In greenhouse and controlled environment experiments, the influences of temperature, P nutrition, and foliar sprays of 3 growth retarding chemicals on the growth, yield and mineral composition of Pisum sativum L. cv. Dark Skin Perfection were investigated. The utilization of P under 4 air and soil temperature regimes within the physiological range was also studied. The dwarfing effect of high temperature was related to that due to relatively high concentrations of growth retarding chemicals.

Banded P fertilizer, applied at rates of up to 352 lb. per acre, increased plant growth, pea yield and the uptake of N, P, K, Ca and Mg. P increased the total contents of all 5 minerals in all 3 tissues (vine, pod and pea seed), but had multiple effects on mineral concentrations. Efficiency of P in producing pea yield increase was maximum at the 44 lb. per acre rate.

The high air temperature of 30° decreased growth, pea yield, and total mineral uptake, compared with a temperature of 21°. The high soil temperature of 18° increased these 3 groups of variables, as compared with a temperature of 10°. Increases in mineral concentration at the high air temperature were largely due to "concentration effects" resulting from

smaller plants. Increases due to the high soil temperature were absolute because they occurred even in bigger plants. Increase in mineral uptake at the high soil temperature was not due to increased root growth, but was a result of increased metabolic activity. The effect of soil temperature on total absorption was greater than on translocation into the pea seed.

(2-chloroethyl) trimethylammonium chloride (Cycocel) at 1 ppm was the most effective in terms of growth and yield stimulation. 2,4-dichlorobenzyl tributylphosphonium chloride (Phosfon) at 100 ppm was the most effective with respect to growth retardation, but markedly decreased pea yield. N-N-dimethylamino succinamic acid (B-Nine) at concentrations of 1 and 100 ppm was ineffective in altering growth pattern. Effects of the growth retarding chemicals on mineral uptake largely reflected plant size differences, and were not absolute effects. Cycocel and Phosfon at low concentrations are promising for use in arresting excessive vegetative growth and its attendant problems, and in increasing pea yield without deformative effects.

The effects of relatively high concentrations of Cycocel and Phosfon were similar to those of high temperatures with respect to plant dwarfing, changes in mineral composition and alteration in the levels of Glucose, G-1-P, G-6-P, F-6-P, Fl,6-P, ADP and ATP. It appears that high concentrations of growth retarding chemicals and high temperatures depress plant growth by reducing the utilization of ATP in the phosphorylation of sugars, in the glycolytic sequence.

The nearest-optimal air and soil temperature regime for plant growth and mineral uptake was the 21/13/18° day/night/soil. For uniformity in the nomenclature of plant growth regulators, it is suggested that growth retarding chemicals be called "RETARDINS".

TABLE OF CONTENTS

Section		Page
INTRODUCTION		1
1.	REVIEW OF LITERATURE	4
A.	TEMPERATURE AND PLANT GROWTH	4
1.1	General Aspects of Temperature and Plant Growth Processes	4
1.2	Soil Temperature and Plant Growth	6
1.3	Temperature and the Growth of the Pea Plant	10
1.4	Soil Temperature and the Growth of Some Plants	12
1.5	Field Manipulation of Soil Temperature	13
1.6	Apparatus for Controlling Soil Temperature	14
B.	MINERAL NUTRITION OF PLANTS	15
1.7	Soil-Plant Relationships in Plant Nutrition	15
1.8	Mechanism(s) of Ion Uptake	16
1.9	Mineral Uptake and Transport in Plants	19
1.10	Mineral Uptake as Influenced by Temperature	21
1.11	Efficiency of Phosphorus Fertilizer Placement	23
1.12	Case for Plant Analysis and Intact-Plant Nutrition	25
C.	THE PHYSIOLOGY OF GROWTH RETARDING CHEMICALS	27
1.13	General Aspects of Growth Retarding Chemicals	27
1.14	Structural Requirements for Activity	28
1.15	Possible Mode(s) of Action	29
1.16	Effects of Growth Retarding Chemicals on Plants	30

Section		Page
D.	BIOCHEMICAL ASPECTS OF TEMPERATURE, PHOSPHORUS AND GROWTH RETARDING CHEMICAL RESPONSES	32
1.17	Biochemistry of Temperature Response	32
1.18	Carbohydrates and Mineral Uptake	36
1.19	Role and Metabolism of Phosphorus in Plants	36
1.20	Growth Retarding Chemicals and Plant Metabolism	43
2.	MATERIALS AND METHODS	46
2.1	Experiments Conducted	46
2.2	Control of Air and Soil Temperatures and Soil Moisture	47
2.3	Soil, Fertilizer Application, Planting and Harvesting	53
2.4	Application of Growth Retarding Chemicals	55
2.5	Analytical Techniques	56
2.6	Statistical Analyses of Data	60
3.	RESULTS	62
3.1	Plant Response to Phosphorus Fertilizer in the Greenhouse	62
3.2	Effects of Phosphorus Nutrition on Growth and Mineral Uptake at 3 Pre-fruiting Growth Stages as Influenced by Air and Soil Temperatures	65
3.3	Effects of Phosphorus Nutrition on Yield Factors at Estimated Marketable Maturity of Peas as Influenced by Air and Soil Temperatures	74

Section			Page
	3.4	Mineral Translocation Patterns	81
	3.5	Comparative Growth, Plastid Pigment and Mineral Responses of the Plant to Foliar Applications of Cycocel, Phosfon and B-Nine at Low Concentrations	81
	3.6	Effects of Temperature and Applied Phosphorus on Glucose, Hexose phosphates and Adenosine phosphates in Pea tissues	88
	3.7	Effects of Cycocel and Phosfon on Glucose, Hexose phosphates and Adenosine phosphates in 5-day old Pea Radicles	91
4.	DISCUSSION		95
	4.1	Growth Responses to Phosphorus Nutrition	95
	4.2	Mineral Uptake Responses to Phosphorus Nutrition	98
	4.3	Comparative Growth, Plastid Pigment and Mineral Responses of the Plant to Foliar Applications of Cycocel, Phosfon and B-Nine at Low Concentrations	102
	4.4	Phosphorus Metabolism as Influenced by Temperature, Phosphorus and Growth Retarding Chemicals	104
5.	SUMMARY AND CONCLUSIONS		113
6.	BIBLIOGRAPHY		117

LIST OF TABLES

Table No.		Page
1	Effects of phosphorus fertilization on growth and yield factors of pea plants grown in the greenhouse. Values are for one plant.	63
2	Effects of phosphorus fertilization on the concentrations and total contents of N, P, K, Ca and Mg in the vine, pod and pea of greenhouse-grown plants.	64
3	An example of a Combined Analysis of Variance Computer Output for Main Effects.	66
4	F Values and the significances of Main Effects at each growth stage, and of each plant tissue at crop maturity. Part I. Dry matter and mineral concentrations.	67
5	F Values and the significances of Main Effects at each growth stage, and of each plant tissue at crop maturity. Part II. Total mineral contents.	68
6	Effects of air and soil temperatures on Growth Response of pea plants at 3 pre-fruiting stages to phosphorus fertilization.	69
7	Effects of air and soil temperatures and phosphorus nutrition on the concentrations of 5 minerals in the pea vine at 3 pre-fruiting growth stages.	71

Table No.		Page
8	Effects of air and soil temperatures and phosphorus nutrition on the total contents of 5 minerals in the pea vine at 3 pre-fruiting growth stages.	73
9	Growth characteristics and yield factors in peas as influenced by air and soil temperatures and phosphorus.	76
10	Mineral uptake and distribution in the root, vine, pod and pea seed as influenced by air and soil temperatures and phosphorus. Mineral concentrations, percent of dry matter.	78
11	Mineral uptake and distribution in the root, vine, pod and pea seed as influenced by air and soil temperatures and phosphorus. Mineral contents, milligrams per plant.	79
12	Absorption and translocation of N, P, K, Ca and Mg as influenced by phosphorus nutrition at 4 air and soil temperature regimes.	82
13	Effects of Cycocel, Phosfon and B-Nine on growth and yield factors in peas.	84
14	Effects of Cycocel, Phosfon and B-Nine on plastid pigment contents of pea plants.	85
15	Mineral composition and total contents in pea plants as influenced by two concentrations of Cycocel, Phosfon and B-Nine.	86

Table No.		Page
16	Effect of temperature on Glucose, Hexose phosphates, ADP and ATP levels of 5-day old pea radicles. Fresh weight (F.W.) in g., compounds in μM/g F.W.	90
17	Tissue levels of Glucose, Hexose phosphates, ADP and ATP as influenced by air and soil temperatures and applied phosphorus. Levels of compounds in μM/g fresh weight.	92
18	Effects of Cycocel and Phosfon on the levels of Glucose, Hexose phosphates, ADP and ATP in 5-day old pea radicles. Fresh weight (F.W.) in g, compounds in μM/g F.W.	93

LIST OF FIGURES

Figure No.		Page
1	Exterior of growth cabinet showing time switch, thermostats and pilot light bulbs.	48
2	Interior of growth cabinet showing pot placement	48
3	Interior of growth cabinet showing waterbath, cold water coiled copper tube, thermostat and thermistors.	49
4	Schematic diagram of Soil Temperature Control Equipment	50
5	Refrigerated Water Tank.	51
6	Bouyoucos Moisture Meter and materials for soil moisture calibration.	51
7	Effects of air and soil temperatures on growth at 0 and 44 lb. P/A.	75
8	Effect of phosphorus fertilization on growth of the plant at the nearest-optimum air and soil temperature combination.	77
9	Effects of air and soil temperatures and phosphorus on the number and size of peas per pot (4 plants).	77
10	Effects of B-Nine, Cycocel and Phosfon at 1 and 100 ppm on growth of the plant.	87

Figure No.		Page
11	Typical absorption spectra of glucose (glu), hexose phosphates (HP) and adenosine phosphates (AP) of 5-day old pea radicles grown at 25°. 1 g of fresh tissue was used.	89
12	Comparative rate effects of Cycocel and Phosfon on P utilization patterns in 5-day old pea radicles grown at 25°.	109

ACKNOWLEDGEMENTS

I wish to thank Dr. Douglas P. Ormrod, Professor, Department of Plant Science, University of British Columbia, under whose supervision this study was undertaken, for his academic and technical advice during research, and for his guidance in the preparation of this thesis.

Deep appreciation is extended, for individual and collective advice, and for the review of this thesis, to the members of my graduate committee:

Dr. V.C. Brink, Chairman, Department of Plant Science

Dr. G.W. Eaton, Department of Plant Science

Dr. L.E. Lowe, Department of Soil Science

Dr. W.B. Schofield, Department of Botany

Mr. A.R. Maurer, Vegetable Physiologist, Agassiz Research Station

I am also indebted to Dr. H.F. Fletcher and Mr. V.W. Case, formerly of the Soil Research Unit of the Agassiz Research Station, for providing experimental soil and soil analysis data.

I am grateful to Messrs. I. Derics and L. Scrubb, technicians, for help with laboratory equipment. Valuable assistance in the computer programming of data was given by Dr. G.W. Eaton.

I thank Mrs. N.O. Adedipe for her help with laborious manual operations and Miss Diane Sherwood for her accurate typing of this thesis.

Financial aid, gratefully acknowledged, was provided by Canada Department of Agriculture and the National Research Council of Canada through support of the project and through Postgraduate Scholarship.

INTRODUCTION

The pea (Pisum sativum L.) is a cool weather species, grown extensively in the Lower Mainland of British Columbia, in northwestern Washington State, Great Britain and Australia. Combined acreage in British Columbia and Washington is over 60,000. Two important factors limiting pea yield are warm temperatures and deficiency of plant nutrients, notably phosphorus.

The climate of the pea-growing areas of British Columbia and Washington is variable, not only from east to west but also from year to year. It has been established with experiments at the Agassiz Research Station and at the University of British Columbia, over 3 seasons, that a small difference in the mean temperature of the growing season can affect yields significantly. This means that the cooler weather near the sea coast allows for a longer growing season than does the weather of areas farther inland where higher temperatures may decrease yields. On the other hand the latter may be more favourable for early spring planting.

Phosphorus fertilization has led to considerable increases in yield because most soils in the pea-growing areas are phosphorus-deficient. Higher pea yields due to phosphorus fertilization are also accompanied by slightly higher pea:vine ratio, so that less vine is handled per unit of peas harvested.

Phosphorus is frequently deficient in the soils of the Lower Mainland of British Columbia, and values lower than

10 pounds available P per acre have been reported. Experiments are therefore necessary to clarify the separate effects of air and soil temperatures on the response of peas to phosphorus added to a deficient soil. For example, it is possible that early planting in colder soils may require more phosphorus fertilizer than for warmer soils, or that phosphorus fertilization could offset some of the adverse effects of high temperatures in warm pea-growing areas.

Many experiments have been carried out to establish optimum air or optimum soil temperature for the growth of peas. However, the combined effects of air and soil temperatures, and the response to phosphorus fertilization as an interacting factor have had little investigation. Much of the information about the relative importance of the shoot and root temperatures has been obtained, therefore, indirectly. Also, most of the experiments reported hitherto were not carried out at different stages of growth, but usually at one growth stage; and have also scarcely been carried out to crop maturity to elucidate mineral distribution patterns in the different parts of the WHOLE, SOIL-GROWN plant.

It is possible that growth retarding chemicals may be used to achieve a reduction of the plant vine and consequently to reduce problems associated with excessive vegetative growth without reducing pea yield, or perhaps promoting yield.

The garden pea was chosen because of its relative homogeneity of populations, easily observable morphological

characters, elongate stem growth useful for nutrient absorption and transloation studies, and its increasing economic importance in the agricultural industry of the Lower Mainland of British Columbia and elsewhere. The cultivar Dark Skin Perfection was used because of its wide commercial acceptance based on a relatively high pea:vine ratio, medium height and tenderness of peas.

The specific objectives of the present studies were: to determine the effects of air and soil temperatures on:

1. Growth characteristics and yield factors.
2. Phosphorus uptake at 3 pre-fruiting stages, and distribution in the different parts of the plant at crop maturity.
3. The influence of applied phosphorus on the uptake and distribution of N, K, Ca and Mg; and also to determine:
4. Optimum air and soil temperature combination for efficient production of peas and utilization of plant nutrients.
5. Comparative responses of the plant to low concentrations of 3 growth retarding chemicals: Cycocel, Phosfon and B-Nine.
6. Possible differences in phosphorus utilization as reflected by some specific metabolic phosphorylated compounds.
7. Possible relationships between temperature responses and growth retarding chemical responses, since the dwarfing effects of high temperature on the pea plant are morphologically similar to those of growth retarding chemicals on many plants.

1. REVIEW OF LITERATURE

A. TEMPERATURE AND PLANT GROWTH

1.1 General Aspects of Temperature and Plant Growth Processes

An increase of temperature almost invariably increases the rate of a chemical reaction. For a homogeneous system it is known that the rate is doubled or trebled for each 10° rise of temperature, and even for many heterogeneous purely chemical reactions. The application of information obtained with isolated chemical reactions to the complex chemistry of a living plant is difficult; it is in fact dangerous to think of plant growth in terms of simple chemical reactions, or to apply directly information obtained with isolated enzyme systems (Nielsen and Humphries, 1965).

Nevertheless, all reactions occurring in plant cells follow the basic laws of thermodynamics and rate theory; that is, an increase in plant growth processes with increase in temperature, from a minimum where there is relative inactivity up to a maximum beyond which growth is hampered mainly due to the denaturation of proteins. However, the plant being a living entity made up of complex constituents, survives in most cases, variations and fluctuations in temperature. It is thought that one of the main defence mechanisms plant cells may possess to resist the deleterious effects of low or high temperatures, is the development of protoplasm which can resist the chemical and physical aspects of these temperatures.

To add to the complexity of plant response to temperature, it is known that optimum temperature for growth is affected as much by the medium used to grow plants as it is by the supply of nutrients available and their placement in soils (Knoll et al., 1964; Brouwer, 1959).

An optimum has therefore been considered to be the result of 2 opposing mechanisms at work in a plant, one synthetic and the other degradative. At cool temperatures the degradative mechanism, largely respiration, is slowed down more than the synthetic, largely photosynthesis. As temperatures are raised, the rates of both the synthetic and degradative processes increase, the latter more rapidly until a compensation temperature is attained above which there is a net loss of dry matter. Exceptions to this general rule will, of course, occur in the case of plants such as corn and sugarcane which have no respiration in the light (Downton and Tregunna, 1968).

Inactivation of certain enzyme systems may occur at temperatures well below that at which proteins are denatured (about 60°). As a result certain metabolites may not be produced and plant growth will be reduced (Bonner, 1957). Hence the supply of carbohydrates and essential metabolites may well be the determining factors in establishing optimum temperatures for plant growth. Weissman (1964), for example, suggested that leaves are dependent to a considerable extent on amino acid constituents produced in the roots, for building up protein. He further suggested that

amino acids are synthesized only in the root. From this it is reasonable to suggest that soil temperature is as limiting as, if not more limiting than, air temperature, for plant growth.

1.2 Soil Temperature and Plant Growth

The soil is a dynamic natural body consisting of the weathered and biologically molded upper part of the regolith (Buckman and Brady, 1960). Soil temperature exerts its influence on plant growth through its physical, chemical and biological effects on root growth. Some of the chemical reactions in the soil which are influenced by temperature are hydration, hydrolysis, oxidation, carbonation and solution.

One of the most important physical factors influenced by soil temperature is the viscosity of water. As the temperature is increased the number of hydrogen bonds in water diminishes because of thermal movements of the molecules, and therefore the energy of activation will decrease. In other words the lower the temperature the more hydrogen bonding there is, and the more the hydrogen bonding, the greater is the viscosity (Nielsen and Humphries, 1965).

Entry of water into plant roots depends partly on metabolic activity, and therefore on temperature. Some water enters the root passively, and cold may slow its entry by increasing its viscosity (Nielsen and Humphries, 1966).

Kramer (1949) attributed reduced uptake of water by transpiring plants in cold soils to increased resistance to water movement across the living cells of the root, and he reported (1956) that the additive effects of temperature on viscosity and permeability of protoplasm decreased the uptake of water at 5° to a quarter of that at 25°.

Much is known about the effect of temperature on mineral absorption both by isolated roots and intact plants. Uptake of ions depends on energy supplied by oxidation of carbohydrates and absorption is therefore slowed by cold temperature. Nutrient uptake by intact plants is optimal at lower temperature than is that of excised roots, which shows that the shoot influences uptake by the root at higher temperatures. Nielsen et al. (1960) found ions to be more concentrated in the roots of lucerne at 5° than at 12°, although total uptake was greater at 12°. This they attributed to either the fact that transport from the roots is impeded at 5°, or because sugars are more concentrated in roots at 5°. However, the gross size of the root system - measured as total weight or length - is not an estimate of its absorbing capacity because a large and changing proportion of the system ceases to absorb (Nielsen and Humphries, 1966). They also contended that when roots are at a low temperature they use less carbohydrates, allowing carbohydrates to accumulate in the leaf, thus slowing photosynthesis and shortening the life span of the leaf.

Temperature affects ion uptake and transport through the plant similarly because both are energy-dependent processes, and are thus much slower in the cold. Shtrausberg (1955) suggested that cold roots diminished movement of nutrients to the shoot of cucumber plants and so inhibited growth.

Gas exchange in the soil atmosphere is also a function of temperature. Temperature affects not only the rate at which oxygen is used in metabolic processes, but also the rate at which it reaches the root system. A supply of oxygen in the root zone is necessary to maintain respiration. Since the rate of respiration varies with temperature, the supply of oxygen necessary to maintain optimum plant metabolism might be expected also to vary with temperature. Cannon (1925) attributed the necessity for higher oxygen concentrations in the soil atmosphere in order to maintain normal root growth at higher temperatures, to the decreasing solubility of oxygen in the soil solution with temperature increase. Letey et al. (1961) however were of the opinion that increased diffusion rate of oxygen to the root surface is more important because of a higher diffusion coefficient through both gas and liquid. Oxygen diffusion rates at high soil temperatures were found to be higher than those at low temperatures. They also showed that the accumulation of Ca and P in cotton and sunflower was stimulated by increased oxygen supply. In a subsequent report (Letey et al., 1962)

oxygen diffusion rates were shown to increase 1.8% per degree rise in soil temperature for a given oxygen concentration.

One of the most important biological effects of soil temperature is its influence on microbial activities. This is particularly important with the pea plant, a legume, most if not all of the nitrogen nutrition of which is dependent on the activities of the symbiotic bacteria, Rhizobium leguminosarum. Generally, it is to be expected that a rise in temperature, within limits, would lead to increased nodulation and greater nitrogen-fixing capacities of the bacteria. Meyer and Anderson (1959) found that a moderately high temperature of 30° inhibited symbiotic N fixation in subterranean clover grown in nutrient agar. Later, using soil cultures, Mes (1959) reported that plants of Vicia faba, Lupinus luteus and Pisum sativum reacted in accordance with the results of Meyer and Anderson. An increase in day temperature from 18, 19 or 21° to either 25 or 27° generally decreased N percentage and the total N content of the plants. An increase in the night temperature from 10 to 21° generally also decreased the total N content although actual N percentage often increased. It was emphasized that response would depend on whether the species is a warm- or cool-temperature plant.

It is thus evident that the effects of temperature on plant growth are as complex as the plant itself. The effects of temperature are therefore not only on the plant

per se but also on the physical and chemical status of, and biological activities in, the soil on one hand, and the complex metabolic reactions in the plant on the other hand.

1.3 Temperature and the Growth of the Pea plant

Some of the early reports on the effects of temperature on the growth and development of peas were summarized by Beattie et al. (1942). The pea is a cool weather species. High temperature checks its growth and causes it to flower and form pods before the plant has attained enough size to bear a good crop, while cool weather permits a long continued growth and the formation of many pods that do not reach the harvest stage prematurely. Brenchley (1920) showed that the maximum rate of increase in the weight of the pea plant was obtained prior to flowering. The rate fell off after reaching this maximum, but an abrupt rise was observed for a period which may have been connected with the initiation of sexual reproduction.

Later, Went (1957) found that germination at a high temperature of 26° was faster but the plants were less uniform than when germinated at 23 or 20°. However, the optimal temperature for stem elongation decreased in the course of development. At the highest temperature hardly any seeds were set, and pea weight was low. Only one or a few seeds developed per pod with one or two pods per plant; while at the lower temperatures all ovules grew into seeds. Fresh and dry weights of the

whole plant were affected by the environment in a similar way to stem elongation.

Highkin (1960) showed that a lack of day/night temperature fluctuation was inhibitory for growth of the pea plant. The experiments indicated the need for certain environmental fluctuations during the entire life cycle of the plant. One explanation given by Highkin was that an environment with fluctuations on a 24-hour basis is in better agreement with endogenous diurnal rhythms which occur in plants as well as in animals and a constant temperature environment was considered "incompatible" with the "biological clock" operating in the plant. This had led to the wide use, in experimental work, of different temperatures in the day and night portions of the daily cycle.

Wang and Bryson (1956) further pointed out that the response of peas to temperature varies with the plant's stage of development. They divided the life cycle into underground, seedling, vegetative and reproductive stages, and showed that the optimum temperature range changed from about 14-24° to 18-31° for the first two stages, and to 10-21° as the plant passed through the last two stages.

Relatively high yields of peas are obtained in the Lower Mainland of British Columbia as a result of generally favourable climatic conditions. Studies by Fletcher *et al.* (1966) however indicated that yields vary widely between locations and planting dates. They established optimum seasonal mean temperature for peas, in field experiments at Agassiz and Vancouver.

At Agassiz where temperatures exceeded optimum they obtained a negative correlation of temperature with total dry matter yield, peas per pod and pea yield. At Vancouver where temperatures were suboptimum, they obtained a positive correlation of temperature with total dry matter yield, pea yield and some other growth characteristics. The optimum temperature for peas was suggested to be about 21-22°. In controlled environment studies, Stanfield et al. (1966) found that the combination of high day and high night temperatures caused an increase in the number of nodes to the first flower. Tillering was most prolific at the lower temperatures and was absent at 32° day temperature. Pea yield decreased as temperature increased above 16/10° day/night temperatures, due mainly to a reduction in the number of pods per plant. On a dry matter accumulation per day basis, vine growth decreased above and below a temperature optimum which shifted from 21/16 to 16/10° in the course of plant development.

1.4 Soil Temperature and the Growth of Some Plants

There has not been much research in the area of the interaction of air and soil temperatures on the growth of peas. One of the few reports on the effect of soil temperature on pea growth is that of Mack et al. (1964). In a greenhouse experiment with peas, during which air temperature fluctuated between 30° maximum and 13° minimum, soil temperatures were maintained at 13, 17, 21 and 25°. They found that dry weights of pea plants at early bloom were usually highest at soil temperatures of 21 and 25°.

The influence of soil temperature on the growth of other plants has been widely studied. Tomato (Lingle and Davis, 1959), snapbean (Mack et al., 1964; Singh and Mack, 1966), and barley (Power et al., 1963) growth have been found generally to increase with increase in soil temperature from about 10 to 30°. Dormaar and Ketcheson (1960) and Nielsen et al. (1961) also obtained increases in the yield of corn as root temperatures were increased from 5 to 27°.

1.5 Field Manipulation of Soil Temperature

Most of the work on influencing soil temperature in the field has been with mulches. Unfortunately the natural mulches of plant residues lower the soil temperature and delay germination and early growth of crops planted in them (Nielsen and Humphries, 1965). Anderson and Russell (1964) obtained a decrease of 0-3° for each 1000 lb./A of bright wheat straw. The effect of such mulches is to delay maturity and to reduce yields where appreciable quantities of crop residues are left on the surface.

Translucent plastic films warm the soil by transmitting much insolation to the soil beneath. Black films absorb the insolation, heat up and conduct much of the heat into the atmosphere leaving the soil beneath at nearly the same temperature (Clarkson, 1960).

Larson and Willis (1957) showed that the direction of rows can affect soil temperatures, north-south ones permitting more radiation to reach the soil with resultant heating than east-west ones.

1.6 Apparatus for Controlling Soil Temperature

While there have been many different physical arrangements of apparatus for controlling root temperatures, the use of water as a cooling or heating medium has been a common feature. Thus Campbell and Presley (1945), Cooper et al. (1960) and Willis et al. (1963) have described operations using water to control soil temperature.

Mederski and Jones (1963) controlled soil temperature in the field, using heating cables imbedded under rows of plants. They were thus only able to work with temperatures above the normal soil temperature. Mack and Evans (1965) however excavated their plot area and laid in pipes to carry both heated and cooled water to condition the soil placed over the pipes. This type of work gives a better understanding of the practical implications of soil temperature effects, but more controlled environment work is required to reduce the number of treatments needed for field trials (Nielsen and Humphries, 1965).

B. MINERAL NUTRITION OF PLANTS

1.7 Soil-Plant Relationships in Plant Nutrition

If other factors such as light, temperature and water are not limiting growth, then the appropriate supply of nutrients may be limiting. Plant growth requires a continuous net shift of ions from the soil system into the plant; that is, there is a steady input of ions from the solid phase into the soil solution, and a continuous metabolic removal of ions from the soil solution by the plant (Fried and Shapiro, 1961).

Ion uptake by plants from a soil system may be divided into 4 steps:

(a) the release of the ion from the solid phase into the soil solution

(b) the movement of the ion from any point in the soil solution to the vicinity of the root

(c) the movement of the ion from the vicinity of the root into the root

(d) the movement of the ion to the shoot of the plant

The overall process of ion absorption and transport is thus considered a multi-step phenomenon in which various steps may be rate limiting, depending on external (soil) and internal (plant) conditions, the latter being especially related to a series of physiological processes concerning growth and energy supply (Brouwer, 1965).

The transfer of ions between the soil phase and the solution phase is apparently not rate limiting for either

phosphorus (P) or cations in the overall ion uptake process. Thus for two acid soils and one calcareous soil, Fried et al. (1957) demonstrated that 13 to 15 lb. P/A can be released each hour. The rate of formation of soil solution P was found to be greater than the rate of absorption of P by the plant from the soil solution by a factor of at least 250.

Ohlrogge (1962) considers nutrient uptake as a rate process and is therefore best expressed as a rate, that is grams/plant/day or hour. This establishes the delivery capacity of a soil per unit of time to maintain an adequately nourished crop. He also remarked that each phase of plant development deserves greater attention. Only after the phases of plant development have been completely described and understood can they be assembled into an understandable cycle.

1.8 Mechanism(s) of Ion Uptake

"Passive" and "active" mechanisms of the entry of ions into the root have been proposed. Passive entry is supported by the work of Hylmo (1958), and Epstein (1960). This envisions a continuation of the soil solution into the root into the so-called "outer" or "free" space. But if diffusion were rate-limiting, ion uptake should be directly proportional to concentration. Generally, it is not. The high temperature coefficients of ion accumulation for monovalent anions including phosphate, and cations also indicate that diffusion is not rate-limiting (Fried and Shapiro, 1961).

Active entry of ions, which implies the use of metabolic energy, is supported by the work of Brouwer (1956), and van den Honert et al. (1955). It is generally known that the partition of ions between solution and plant tissue deviates considerably from the partition which is to be expected on the basis of free diffusion (Brouwer, 1965). Although much effort has been devoted to the study of the mechanisms underlying transport of ions, there is still no clear insight into the process. The only statement that may be made with certainty is that this transport depends on metabolically produced energy. Such a conclusion is arrived at from the influence of oxygen supply, temperature, and inhibitors of metabolism, notably dinitrophenol (Butler, 1953). The way in which this energy is transferred to the transport process, however, remains unclear.

According to the electrochemical theory of anion respiration as developed by Lundegardh (1955), a direct relationship exists between respiration and ion uptake, and particularly between anion respiration and anion transport. The anions are assumed to be transported along adsorption tracks, while at the same time electrons produced in respiration move in the reverse direction. The cations move inwards by exchange against the H ion, also a product of respiration. Most of the criticisms against the theory are that it is based on the assumption of only one transport mechanism for anions and cations.

Although the electrochemical theories of anion respiration (Lundegardh, 1955), and redox pump (Conway, 1953) have had many supporters, much attention is now being paid to the carrier theory, especially after the work of Epstein and Hagen (1952). The essential features in the functioning of carriers are the combination of ion and carrier molecules outside the membrane, the movement of the ion-carrier complex across the membrane, and the subsequent discharge of this complex inside the membrane. After the dissociation of the ions from the carrier, the ions are prevented from back diffusion by the impermeability of the membrane to free ions (Epstein, 1960). This theory is believed to be the most satisfactory one based on data of kinetic analysis (Thomas, 1956).

A number of groups of compounds have been suggested as carriers although the chemical nature of the carriers is still unknown. Some postulated compounds include phosphorylated energy-rich nitrogenous compounds (Steward and Street, 1947) and ribonucleoproteins (Tanada, 1956). Tanada suggested that the nucleic acid portion binds the cations while the protein moiety binds the anions. There seems to be complete agreement that ion-binding compounds might be capable of readily undergoing oxidation and reduction, or of undergoing some other change in energy level such as would be involved with phosphorylated compounds (Gauch, 1956). In view of the increasing amount of evidence that "energy-rich" organic phosphates such as adenosine triphosphate (ATP) act as intermediates in energy transfer, it seems not unreasonable to conclude that active transport, like other endergonic processes, depends on energy derived more or less

directly from such substances. The effects of phosphorylation inhibitors such as DNP, on salt absorption supports this conclusion, but so far there is no direct experimental evidence (Sutcliffe, 1962).

The position of the rate-limiting step or steps in the overall process of nutrient absorption and transport is not known for all environmental conditions and plant species. It can conceivably be during movement of the ion to the vicinity of the plant root, at the turn-over of the ion-carrier complex, or at the transport step to the plant top. (Fried and Shapiro, 1961).

1.9 Mineral Uptake and Transport in Plants

The mineral composition of a tissue is dynamic since it is subject to the physico-chemical changes manifest in growth processes. Some elements are present in high concentrations in young tissue and are diluted as the tissue enlarges. Others are present in low concentrations in young tissue and gradually increase. The accumulation of dry weight dilutes all elements unless an influx of minerals offsets this effect. There are instances in which additions of elements lowered or had no effect on element concentration in the plant, but when total element per leaf instead of concentration was considered, there was a good correlation with yield (Smith, 1962).

Next to the supply of elements, the physiological age of the tissue is probably the most important factor affecting the mineral composition of a given species. Concentrations of

N, P and K decrease with age in apple (Rogers et al., 1953) and vegetables (Bradley and Fleming, 1960). N also decreases with age in Japanese mint (Singh and Singh, 1968). Ca and Mg increase with age in vegetables. With respect to pea plants, MacLean and Byers (1968) found that Ca and Mg remained relatively constant. They established tentative levels of N, P and K for the stem apex at the 5th to 8th-node stages as 3.5, 0.20 and 2.5-3.0% respectively.

Redistribution of elements within the plant is a continuous process and one tissue gains as another loses (Rogers et al., 1953). Also, different parts of a plant will reflect the P status of the plant to different degrees (Ulrich and Berry, 1961). Studies with tracer P showed that P is continuously circulating up and down the plant (Biddulph et al., 1958), but not laterally (Rinne and Langston, 1960). Many plants show a loss of leaf K concomitant with fruit development. N and K are regarded as mobile (Lundegardh, 1947), but Bowling and Weatherley (1964) reported that 99% of K absorbed by Ricinus communis roots was accumulated in the root tissues. Ca and Mg are usually relatively immobile, but Mg can move under stress of deficiency.

The supply of one element may result in a simultaneous increase in the tissue level of that element as well as in another. This is referred to as a synergistic effect, some examples of which are: the increase in leaf Ca of avocado from applied N (Embleton et al., 1958), the increase in leaf Mg of tung also resulting from applied N (Neff et al., 1958), and the decrease in total N content of Japanese mint due to P deficiency (Singh

and Singh, 1968). Effects of such ions as nitrate, sulphate and phosphate in stimulating the absorption of other ions is probably due to enhancement of metabolism (Sutcliffe, 1962).

Antagonistic effects, that is where an increase in one element leads to a decrease in another, have also been reported, notably the decrease in leaf P resulting from an increase in N supply to Valencia orange (Reitz and Koo, 1960).

Multi-element effects, that is where the supply of one element may affect the tissue contents of other elements are also common. For example, in citrus, an increase in B has no effect on N, but increases P and Ca while it decreases K and Mg (Smith and Reuther, 1951).

1.10 Mineral Uptake as Influenced by Temperature

Mineral uptake by plants may be influenced by environmental factors, prominent among which are nutrient availability as well as shoot and root temperatures.

Increased soil temperature may raise the concentration of soluble soil and fertilizer P by increasing the rate of mineralization of organic P or the chemical decomposition of insoluble inorganic forms of P. The equilibrium concentration of P in solution is also raised by higher soil temperatures (Arambarri and Talibudeen, 1959). However, higher soil temperatures may reduce P solubility by increasing the rate of immobilization and chemical fixation of P in the soil. Thus, the net result of increased soil temperature on P solubility will depend on

the relative rates at which these processes change with temperature. These rates may vary considerably from one soil to another (Hinman et al., 1962).

In addition, variations in soil temperature may also affect nutrient uptake through changes in the amount of root extension. When temperature is reduced below optimum, root growth and extension are also reduced. This may be due to reduced translocation of carbohydrates from the tops, or to reduced nutrient uptake from the soil, or both (Richards et al., 1952).

A number of studies have been carried out to determine the interactive influences of soil temperature and P fertilization on P uptake. In a greenhouse experiment with peas and beans, Mack et al. (1964) maintained soil temperatures of about 13, 17, 21 and 25° with rates of P application ranging from 0 to 280 lb. per acre. P increased in the bean and pea plants with temperature and applied P. During the early stages of the growth of corn, an increase in soil temperature increased the uptake of N, P and K, but did not significantly influence the composition of corn plants sampled at 60 days or at maturity (Mederski and Jones, 1963). The P content of annual range forage legumes also increased with temperature and P fertility (McKell et al., 1962). Singh and Mack (1966) found that while P and K contents of snapbean shoots were increased at high soil temperatures of up to 24°, there was no consistent effect of soil temperature on N, Ca and Mg contents. Generally, P was highest in shoot and pod, K in pod, Ca in shoot, and Mg in root and shoot. In the tomato

plant, Locascio and Warren (1960) reported increase in P content with increase in soil temperature up to between 21 and 30°. Lingle and Davis (1959) also found increase in P, but Ca and Mg concentrations of the tomato plant tended to decrease at high soil temperatures. Nielsen and Cunningham (1964), however, found that within the range of 11 to 28° increased soil temperature greatly increased percent Ca and Mg but had little influence on the concentrations of N, P and K in Italian ryegrass.

The effect of soil-applied P in increasing the percentage P in the plants was significantly greater at a lower soil temperature than at a higher soil temperature, for pole beans (Apple and Butts, 1953), corn (Ketcheson, 1957), and red clover (Robinson et al., 1959).

1.11 Efficiency of Phosphorus Fertilizer Placement

Some of the methods of application of fertilizers are banding, surface broadcasting and leaving on soil surface or discing into the soil, foliar application, and adding to irrigation water. Some fertilizer materials may be satisfactorily added by several of the above application methods. Other fertilizer materials are rather specific in that they must be applied by a certain method if good results are to be obtained.

Phosphorus is classified as an "immobile" nutrient because it does not readily move in the soil. While "mobile" nutrients, for example nitrogen, may become positionally available for plant absorption by two forces: by virtue of its moving into the root zone, or by virtue of root growth to the

nitrogen, P is positionally available to the plant primarily because of root extension into the zone of fertilizer P. Only one action force therefore makes P positionally available to plants (Welch et al, 1966).

Radioactive P has permitted the determination of the quantity of fertilizer P absorbed by plants with different placement methods. Nelson et al. (1949) reported that fertilizer P absorbed by plants was less for broadcast than for placement with the seed or for mixing in the row. The effects on plant uptake of banded compared with broadcast P are dependent on such factors as soil moisture, temperature, and the chemical form of P.

Olsen et al. (1961) found that P uptake by corn seedlings was inversely related to soil moisture tension. Simpson (1960) also observed that the lowering of soil moisture tension to field capacity (achieved by the daily watering of plants) greatly increased the uptake of fertilizer P from superphosphate by oats. This means that during dry periods broadcast and ploughed-under P might be more readily absorbed than banded P. Robinson et al. (1959) established the superiority of band placement over P mixed with the soil for red clover, especially at low temperatures. They concluded that the band placement was the more effective because of an increased concentration of P in a portion of the root zone. They emphasized that band application would be particularly important: (1) for seedings made during periods of low temperature, (2) for crops that make most of their growth during cold weather, and (3) for

soils low in available phosphate, particularly if they are high in fixing capacity.

Nelson et al. (1949) stated that 200 lbs. of fertilizer P in the row was frequently as effective as 400 lbs. broadcast P per acre, in increasing corn yields. Welch et al. (1966) working with three different soils at three locations concluded that the relative efficiency of broadcast as compared with banded P appears to be related to the initial P status of the soil to which P is applied. As the fertility status of a soil is raised to a higher level by fertilization, the advantage of band application as compared with broadcast application would be expected to decrease.

1.12 Case for Plant Analysis and Intact-Plant Nutrition

The fact that the uptake of salts from the soil is influenced by many factors makes soil chemical analysis questionable as a universal method of determining soil fertility (Lundegardh, 1947). Soil analysis includes the use of acids, bases, and neutral salts for extracting particular nutrients and predicting nutrient needs. This method may be misleading because many cases have been cited pointing to prediction errors made, including cases where soil analysis for P have shown a complete range of error and unreliability. There are cases where high available P (60-145 lbs. per acre foot at soil pH 4.6) are known to give a strong response to phosphate fertilizer, and low P (28-30 lbs. per acre foot) where response was not obtained (Clements, 1964).

Thus soil analysis may give readings which show no relationship either to plant absorption or response, and Clements concluded that the occasional correlation of soil analysis with crop yield can only be regarded as fortuitous. A comparison between soil analysis and leaf analysis has resulted in a higher degree of reliability for the latter (Lundegardh, 1947). Tissue analysis is thus very widely used as a guide to nutrient adequacy, since only those nutrient salts which have entered the plant determine growth and fruiting. However a combination of plant and soil analyses may be the ideal approach.

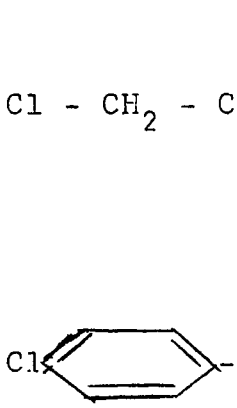
Even where tissue analysis is used as a guide to nutrient requirement, reliability is strengthened by the use of intact plants for nutrient studies. Thus it has been suggested by Williams (1955) that much caution is necessary when using results from abstract procedures such as tissue culture for the interpretation of growth processes within the intact plant. Sutcliffe (1962) is also of the opinion that while much can be learned about the mechanism of ion movements at the cellular level by the study of homogenous tissues, the relevance of such knowledge to the processes going on in the intact plant must ultimately be demonstrated rather than assumed.

C. THE PHYSIOLOGY OF GROWTH RETARDING CHEMICALS

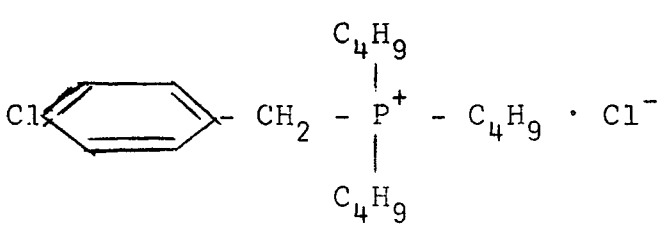
1.13 General Aspects of Growth Retarding Chemicals

The term "growth retardant" is used for all chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects. The physiological action required for a chemical to be a growth retardant excluded many kinds of growth regulators such as auxins, herbicides of growth regulator type, growth inhibitors like maleic hydrazide, and germination inhibitors (Cathey, 1964).

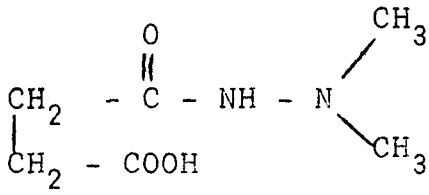
Several groups of growth retarding chemicals have been reported. They belong to quite distinct chemical classes but have similar effects on plant growth. They include quaternary ammonium carbamates (Halevy and Cathey, 1960), phosphonium compounds (Preston and Link, 1958), and succinamic acid derivatives (Riddell et al., 1962).



(2-chloroethyl)trimethylammonium chloride (Cycocel)



2,4-dichlorobenzyltributylphosphonium chloride (Phosfon)



N-dimethylamino succinamic acid (B-Nine)

While some of the compounds are particularly active on some groups of plant species, there is no obvious correlation between taxonomic classification and plant response to a particular compound. Cathey and Stuart (1961) found that 19 out of 55 species tested were responsive to soil applications of Cycocel.

1.14. Structural Requirements for Activity

Substituted Cholines. (2-chloroethyl) trimethylammonium chloride (Cycocel, CCC) is the most active. It was considered an analog of choline. The bromide and chloride salts are active compounds. The trimethyl quaternary ammonium cation is necessary for activity; any substitution for even one methyl group has been found to produce nearly inactive compounds. For optimal activity, the carbon chain which contains the substituent at the end should be 2 carbons in length.

Phosphoniums. The most active structure is that of 2,4-dichlorobenzyl tributylphosphonium chloride (Phosfon). The tributyl quaternary phosphonium cation is necessary for activity; any substitution of shorter alkyl groups or phenyl groups for even one butyl group leads to loss of activity. For optimal activity the benzene ring should have a substituent in the 4-position and be small in size, nucleophilic and non-ionizable.

Succinamic acids. N-dimethylamino succinamic acid (B-Nine, Alar) is unique in its chemical structure as a growth retardant. It does not contain a benzene ring, quaternary ammonium or phosphonium cation, or substituents that are of small size, nucleophilic and non ionizable like the substituted cholines and phosphoniums. B-Nine is a free, ionizable acid with the C-C-N-N system found in B-hydroxyethyl hydrazine and maleic hydrazine. The unsaturated form, N-dimethylamino maleamic acid (CO_{11}) was unstable in aqueous solutions. The analogous compounds derived from phthalic acid were inactive. The amides of B-Nine were active compounds but less so than the acids. The metal and alkanolamine salts of B-Nine were readily formed and functioned as growth retardants (Cathey, 1964).

1.15 Possible Mode(s) of Action

Certain growth retardants have been shown to reduce gibberellic acid (GA) production in Fusarium moniliforme (Hinneman et al., 1965) and pea seeds (Badlev et al., 1965). Other reports show an interaction with auxin rather than GA. Cleland (1965) and Kuraishi and Muir (1963) showed an interaction with auxin. Kuraishi and Muir observed a decrease in diffusible endogenous auxin content in plants treated with growth retardants. The effect on auxin has been suggested to operate through a decrease in biosynthesis as shown for tall and dwarf peas by Reed et al. (1965) or by an increase in auxin degradation as shown for cucumber by Halevy (1963), or by both.

Because the effects of growth retardants are in many aspects opposite to those of gibberellins, they were generally designated antigibberellins. Lockhart (1962), however, suggested that they be considered antimetabolites, since the route to ultimate expression by the plants may vary with species in regard to the sequence of metabolic steps leading to such expressions. It thus seems that the primary effects of the growth retardants are not necessarily restricted to the hormonal level. More basic aspects of metabolism may be involved. Further work on the possible mechanism of action will be reviewed under "Biochemical Aspects" (1.20).

1.16 Effects of Growth Retarding Chemicals on Plants

The plant growth-regulating properties of (2-chloroethyl) trimethylammonium chloride (Cycocel) were reported for wheat by Tolbert (1960) who found that concentrations ranging from 0.13 to 1300 ppm led to a reduction in size, which was accompanied by a darker green colour than in untreated plants. Adedipe et al. (1968) found 1.3 ppm to stimulate the growth of pea plants, to reduce the chlorophyll concentration of pea leaves, and to increase pea yield. Knavel (1968) reported that Cycocel-treated tomato plants contained more N, P, Ca and Mg than other plants. Untreated plants contained more K than the treated plants.

2,4-dichlorobenzyl tributylphosphonium chloride (Phosfon) was reported by Preston and Link (1958) to retard the

growth of a large number of species including soybean, snapbean, mungbean and sweet pea.

The growth-retarding capabilities of N-dimethylamino succinamic acid (B-Nine) have been reported by Jaffe and Isenberg (1965) for petunias and cucumbers, and by Batjer et al. (1964) for apples, pears and sweet cherries. Generally, concentrations between 500 and 2000 ppm retarded the growth and caused a marked increase in the amount of bloom on the fruit trees the following spring. Applications over two consecutive years were found to have no appreciable influence on the foliar levels of N, P, K, Ca, Mg, Mn, Zn, B; fruit set and total yield of apples (Southwick et al., 1968), but Knavel (1968) reported increases in N, P and Ca in tomato plants. When applied to peas, B-Nine at a concentration of 2000 ppm reduced shoot length about 40% and acted as a general inhibitor of growth rather than by suppressing growth of particular organs (Sprent, 1967).

In comparative studies by Cathey and Stuart (1961) with buckwheat and sweet pea, and by Moore (1967) with cucumber hypocotyls, Phosfon was found to be more effective in growth retardation than quaternary ammonium compounds (including Cycocel). Growth retardation due to B-Nine did not persist as long as that due to Phosfon (Majumder, 1968); and Cycocel was more effective than B-Nine for reducing tomato plant growth. Both Cycocel and B-Nine increased N, P and Ca and decreased K (Knavel, 1968). These growth retardants have hitherto most frequently been used at high concentration.

D. BIOCHEMICAL ASPECTS OF TEMPERATURE, PHOSPHORUS AND GROWTH RETARDING CHEMICAL RESPONSES

1.17 Biochemistry of Temperature Response

Went (1944) gave one of the early reports concerning the effects of temperature on physiological processes. In the tomato plant, translocation of sugars was found to be greater at 18° than at 26.5°C, while the uptake of radiophosphorus from the soil was greater at 26.5 than at 18°. Bonner (1943) reported a set of experiments in which thiamine was applied to Cosmos plants grown at different temperature regimes. The responses obtained were taken to indicate a case of a chemically reparable low temperature lesion (Bonner, 1957).

Galston and Hand (1949) presented data to provide evidence for a high-temperature induced reduction of the ability of the pea plant to synthesize adenine. These results were obtained from experiments on the addition of adenine alone, and in the presence of IAA to sub-apical sections of etiolated pea epicotyls at 25 and 35°. Galston (1957) later reported the response to adenine of intact green plants of pea. All plants were kept at 23° during the day but different groups were subjected to different night temperatures ranging from 2 to 30°. The responses listed for stem height and stem fresh weight are less pronounced than for leaf fresh weight, where adenine appears to exert its greatest effect. However, even in this case, the stimualtion by adenine is much the same over the entire temperature range, indicating that temperature is not inducing an adenine deficiency. Langridge and Griffing (1959),

in a statistical analysis of the pea data concluded that there were no significant temperature-adenine interactions as far as growth in epicotyl length was concerned. Ketellaper and Bonner (1961) also observed temperature-induced inhibition of plant growth. Plants were grown at different temperatures, including the optimal temperature, and sprayed regularly with a mixture of B vitamins, vitamin C, casein hydrolysate, sucrose or ribosides. Application of 10% sucrose solution to pea plants grown in artificial light at 23° day and 17° night temperatures (23/17°) caused a 56% increase in dry weight, which made the dry weight equal to that of plants grown in optimal conditions (17/17°). Treatment of pea plants kept at 30/23° with a vitamin B mixture or ribosides gave up to 40% increase in dry weight.

Many of these growth stimulations reported for flowering plants were however of poor reproducibility. More clear-cut results have been obtained with Arabidopsis thaliana, using aseptic culture techniques, closely controlled environmental conditions and statistical tests for temperature-supplement-growth interactions. Langridge and Griffing (1959) detected high temperature lesions in the plant. They grew 43 races at constant temperature of 25, 30 and 31.5°. Five of these races were depressed in growth, and morphologically abnormal when cultured at 31.5°. Further experiments established that in two races the temperature lesion was completely prevented if biotin was supplied at the rate of 3 ug per plant. The temperature

lesion in the third race was partially alleviated in the presence of cytidine at 250 µg/plant, while the other two races did not respond to supplements. It was concluded that there are genetic implications in the response of plants to temperature; in this case, there are two gene functions.

Generally, the organic compounds most often used to counteract high temperature effects are glutamic acid, thiamin, biotin (Langridge, 1963) and adenine. With respect to the differences in, and muliplicity of, compounds required, Langridge offered some explanations. At temperature extremes the rate of growth may be limited by the velocity of a single reaction. In many microorganisms growth ceases at temperatures usually only slightly above the optimum, but may be restored by the addition of a single substance. If the growing temperature is raised a little higher, a further substance becomes necessary and these requirements become progressively more numerous with increasing temperature. Thus, temperature lesions do not form a homogeneous class as far as mechanisms responsible for the requirements are concerned. The possible causes for high temperature growth requirements include (a) accelerated breakdown of metabolites (b) occurrence of rate imbalance (c) non-formation of adaptive enzymes due to heat destruction of ribonucleic acid (RNA) and (d) reversible and irreversible heat inactivation of enzymes.

Another approach to the temperature lesion problem is to analyze plants subjected to different temperatures. This would show which compounds or groups of compounds become

limiting. In such a study, Potts and Ormrod (1969) in their phosphorus-compounds fractionation work with the pea plant showed that there was no change in the levels of organic, lipid and total phosphorus for up to 6 days when plants were transferred abruptly from 25/15° to 35/25° (day/night temperatures). Only after 6 days was there a definite increase in inorganic phosphorus, the contributor to which could not be accounted for, but contributions possibly from nucleic acid or protein fractions were suggested. They concluded that their results do not preclude the possibility that the concentrations of compounds within the organic phosphorus fraction did vary. Such specific compounds include adenylic acid, hexose phosphates and adenosine phosphates in Euglena (Albaum, 1952) and other plants.

Impaired respiration and photosynthesis could obviously constitute a significant factor in declining growth at high temperatures. Ormrod and Bunter (1961) reported increases in seedling respiration rates followed by rapid declines in 4 cultivars of rice, with increase in temperature up to 37 or 43°. Beevers and Hanson (1964) pointed out that rapid oxidation of substrate does not necessarily imply an efficient production of ATP. Working with mitochondria obtained from the roots and shoots of etiolated corn seedlings, they found that high temperatures did uncouple oxidative phosphorylation. This was evident between 40 and 45° where substrate oxidation increased but P/O ratios declined. At 50° both respiration and phosphorylation were impaired, phosphorylation to

a higher degree. Root mitochondria appeared to be somewhat more sensitive to high temperatures than were shoot mitochondria. Between 30 and 50° root mitochondria suffered 80% inhibition of phosphorylative efficiency, compared with 58% for shoot mitochondria.

El-Sharkawy and Hesketh (1964) reported gas-exchange experiments for a species each of Sorghum, Helianthus, Gossypium and Thespesia. Net photosynthetic rates were depressed within 20 minutes by high temperatures of up to 60°. The effects of temperature on plant metabolism are thus effects on the many component processes and reactions in the plant.

1.18 Carbohydrates and Mineral Uptake

There are a number of substances which either stimulate or inhibit salt uptake by an influence on metabolism. Among those which promote absorption at suitable concentrations are soluble sugars and other respiratory substrates (Sutcliffe, 1962). Humphries (1956) demonstrated a positive correlation between the reducing sugar content of excised barley roots and salt uptake. In the same experiments, sucrose content seemed either to be unrelated or to show a negative correlation with absorption. In intact angiosperms, absorption of salts is sometimes depressed with the onset of flowering, and this is correlated with a fall in the level of carbohydrates in the roots.

1.19. Role and Metabolism of Phosphorus in Plants

General Aspects. In addition to the chemical energy bound in the components of the cell during growth, some components

of all organisms are broken down continuously and must be replaced, a process described as maintenance. Synthesis of material for growth and maintenance, an endergonic process, is possible only if coupled with exergonic reactions. Many plant cells maintain concentrations of ions and metabolites in the vacuoles against a chemical or electrochemical gradient, also a process coupled with exergonic reactions. Another process requiring such coupling, but about which relatively little is known, is protoplasmic streaming or cyclosis (Rowan, 1966).

In the economy of life processes, phosphorylation (which is defined as the biochemical process by which phosphate or phosphoryl radicals are transported to an acceptor by a transfer reaction) occupies a place comparable to that of nucleic acids in protein synthesis and hereditary transmission, of proteins as specific catalysts, and of oxidation-reduction systems in energy transfer. As a consequence of phosphorylation the reactivity of a compound increases. This is due, in many cases, to the capacity for substituting the phosphoryl group with other groups or radicals, to a higher capacity for binding active constituents, e.g. enzymatic proteins, or for forming complexes with metal ions (Marre, 1961).

Phosphorylation(s). The important mechanisms by which orthophosphate is incorporated into organic phosphate are (1) Photophosphorylation and (2) Phosphorylation coupled with respiration.

PHOTOSYNTHETIC PHOSPHORYLATION is defined as the light induced formation of ATP by chloroplasts. The synthesis of ATP by isolated chloroplasts in light without the aid of mitochondria was first described by Arnon et al. (1954). When conditions were arranged so that assimilation of exogenous CO_2 was prevented, isolated chloroplasts used light energy to esterify inorganic phosphate. At least two fundamental differences were apparent which demonstrated that this light-induced ATP formation was not identical with oxidative phosphorylation by mitochondria. ATP formation in the illuminated chloroplasts occurred: (1) without the net consumption of O_2, and (2) without the addition of a chemical substrate to supply the free energy for the synthesis of pyro-phosphate bonds. The only "substrate" thus consumed in photosynthetic phosphorylation is light (Whatley and Losada, 1964).

A large part of the free energy which would be lost from the cell as heat if hexose were oxidized directly to CO_2 and water, is retained as chemical energy through synthesis of ATP.

PHOSPHORYLATION COUPLED WITH RESPIRATION are of two types: (1) "Substrate level phosphorylation" in which the phosphorylation is of an intermediate directly in the pathway of oxidation of the respiratory substrate. Two of such phosphorylations occur, one coupled to the oxidation of glyceraldehyde-3-phosphate in the glycolytic pathway (Beevers, 1961), and the other coupled to the oxidation of succinyl coenzyme A.

(succinyl-CoA) in the citric acid cycle (Kaufmann and Alivisatos, 1955). Substrate level phosphorylations are not uncoupled by DNP (Beevers, 1961).

(2) "Oxidative phosphorylation", is used for the phosphorylation coupled with the steps of the electron transport chain. The phosphorylations are coupled at 3 steps of the chain. Thus, with the acids, pyruvate, citrate and ∝-ketogluterate reacting with NAD, 3 atoms of P are esterified for each atom of oxygen absorbed. In a simple system all ADP would be converted to ATP and the latter would block the acceptance of P by ADP. However, ATP is removed in kinase reactions in which more ADP is formed (Rowan, 1966).

The rate of the glycolytic pathway will be a function of the concentrations of the substrates of the four controlling irreversible reactions (hexokinase, phosphofructokinase, phosphoglycerokinase and pyruvate kinase). ATP at the first two, and ADP at the last two reactions could act as regulators in a feedback mechanism. Concentrations of substrates, not enzymes, have been found to be the rate-limitors in these reaction rates (Rowan, 1966).

The site of activation of the glycolytic pathway by these mechanisms can be detected by using the cross-over theorem of Chance et al. (1958) which states that a control site in a chain of reaction is identified under conditions of increasing flux by the point at which there is a cross-over between relative depletion and relative accumulation of intermediates. But in applying this theorem to the analysis of glycolytic intermediates in yeast (Ghosh and Chance, 1964), the activation

of the pentose phosphate pathway which would lower the concentration of G-6-P must be considered. Despite this, however, the detection of cross-over points appears to be the most fruitful method of examining the regulation of metabolic rate (Rowan, 1966).

Growth and Development. Phosphorus metabolism involves many aspects of plant growth processes. As nucleic acids direct protein synthesis; and precursors of proteins, polysaccharides and fats are formed by the transfer of phosphate or pyrophosphate groups from nucleoside triphosphates, the metabolism of phosphorus is directly concerned in growth and development.

While the dependence of interconversion of carbohydrates and related compounds, amino acid metabolism (Marre, 1961) and fat metabolism (Stumpf, 1955) on phosphate transfer potential energy has been demonstrated at the molecular level, the involvement of phosphorylation in a number of physiological processes is strongly suggested by observations in vivo. Active uptake of solutes, growth by cell wall extension, translocation, and the control of respiration rates are some of the more prominent processes.

Active uptake of solutes. Accumulation of electrolytes by plant cells against an electrochemical gradient or of non-electrolytes against a chemical gradient must be coupled with an exergonic process. Evidence strongly suggests that some common basic phosphate transfer potential energy-requiring mechanisms is required for the active uptake of any solute (Laties, 1959).

Growth by cell wall extension. Growth by cell division and the synthesis of protoplasm are obviously phosphate transfer potential energy-dependent processes in several essential aspects, such as protein and nucleic acid syntheses. If one accepts the idea of a central role of pectin anabolism in cell extension, the necessary dependence of this type of growth appears as a biological corollary because ATP is required in two, or possibly three steps of pectin biosynthesis:hexose (or uronic acid) phosphorylation, UTP synthesis for UDP-sugar formation, and activation of methionine (Marre, 1961).

Translocation. Phosphate transfer has been implicated in the mechanism of translocation. Photosynthate moves in three stages: from chloroplast to bundle-sheath, from bundle-sheath into the sieve tube, and within the sieve tube. Phosphate transfer could be concerned in the mechanism of movement in each stage. The likely mechanisms are phosphorylation of sugars, and movement by protoplasmic streaming (Canny, 1962). The sucrose isolated as the predominant sugar in the phloem from many plants could be in equilibrium with a sugar-phosphate which could be the transit molecule. With respect to the participation of protoplasmic streaming in translocation, evidence suggests that protoplasmic streaming is coupled with dephosphorylation of ATP (Kamiya, 1960).

Phosphorylation and Metabolic control. As essential phosphorylative steps are involved in practically all metabolic pathways, it appears obvious that the efficiency of enzymes and the levels of substrates involved in phosphorylation

reactions closely control any important process in the cell. A number of data indicate that in higher plants, ATP, ADP and P_i levels may control glycolytic and oxidative metabolism. Hatch and Turner (1959) demonstrated that the operation of typical glycolysis in an acetone powder extract from pea seeds, required P_i and adenine nucleotides. Hess (1963) however maintained that P_i does not find a place in the scheme of glycolytic flux control in ascite tumour cells. He demonstrated that under steady-state conditions glycolytic rate is controlled by ATP. The inhibition of glycolysis earlier reported was attributed to a direct inhibition of glyceraldehyde dehydrogenase and of alcohol dehydrogenase (Mossberry et al., 1964).

Levels of Phosphorylated Intermediates in Higher Plants

While the presence of most of the phosphorylated intermediates that occur in animal tissues and in lower organisms have been documented, relatively few data are available concerning the average concentration of the main phosphorylated constituents and their changes in relation to various physiological situations. A survey of recent reports (Marre, 1961) indicates that hexose phosphate and adenosine phosphate, together with P_i are probably the quantitatively more important and widespread components. It appears that hexosemonophosphates predominate among sugar esters, their concentrations ranging between 1 and 6 μM/gram fresh weight. G-1-P and Fl,6-P concentrations are significantly lower, ranging between 0.3 and 2 μM/gram fresh weight. According to a few data available ATP and ADP concentrations vary between 0.2 and 1 μM/gram fresh

weight. A qualitative evaluation of other nucleoside polyphosphates in pea tissues shows that guanosine triphosphate and uridine triphosphate (GTP and UTP) are present in much lower concentrations. Total nucleotide content per unit dry weight in corn seedlings was maximum between 5 and 6 days and then levelled off (Cherry and Hageman, 1960).

1.20 Growth Retarding Chemicals and Plant Metabolism

The effects of growth retardants on gibberellins or auxins or their interactions have received wide attention, but it is still controversial as to which is the more basically influenced. Cleland (1965), however found instances in which there was little or no mutual antagonism between the retardants and gibberellins or auxins. He felt, as some other investigators do, that the inhibitory effects should not be restricted to the hormonal level.

In support of Cleland's view, a few other biochemical aspects of growth retardant effects have been observed. Tanaka and Tolbert (1966) reported that Cycocel (which is also called chlorocholine chloride because it is an analog of choline) stimulated the activity of partially purified choline kinase from spinach and pea leaves, but it inhibited the activity of yeast choline kinase. The activity of different Cycocel analogs on plant growth corresponded to their stimulatory effect on isolated choline kinase. Stimulation of choline kinase of spinach leaves increased with increase in Cycocel concentration from 13 to 1300 ppm. Cycocel thus stimulated choline kinase

activity and the utilization of choline-C^{14} (that is, the incorporation into lipids and insoluble constituents of the plant), which effects were reversed by gibberellin A_3.

Reed et al. (1965) found that the inhibition of shoot elongation in dwarf and tall peas by B-Nine was correlated with the inhibition of the oxidation of tryptamine-2-C^{14} to indoleacetaldehyde-2-C^{14} by way of diamine oxidase, in homogenates prepared from epicotyls of young plants. The growth retarding action of B-Nine was attributed to the formation of 1,1-dimethylhydrazine in vivo. Their studies confirmed earlier work by Dahlgren and Simmerman (1963) which suggested that CO_{11} may retard plant growth because of the formation of 1,1-dimethyl-hydrazine and 1,1-dimethylhydrazinium hydrogen maleate. Both compounds were reported to be produced by the intramolecularly catalyzed decomposition of the growth retardants in aqueous solutions.

Brook et al. (1967) found that Phosfon treatment of the pea plant resulted in a decrease in soluble RNA and an increase in ribosomal RNA. The nucleic acids from treated tissues were more resistant to RNAse degradation and endogenous RNAse activity was lower in treated tissues. It was suggested that these alterations in nucleic acid metabolism could in turn alter a wide variety of metabolic processes resulting in retarded growth.

Ormrod and Williams (1960) found that 50 µg of 2,4-dichlorophenoxyacetic acid (2,4-D) or gibberellic acid per Trifolium plant applied as a spray caused a striking increase

in acid-soluble organic phosphorus and a concurrent decrease in inorganic P as quickly as one minute after treatment. Tuli et al. (1964) suggested that respiration inhibition by N^6-benzyladenine was a consequence of inhibition of respiratory (glycolytic) kinases. The effects of growth retardants may be to induce changes in auxins or gibberellins or both, but these also affect and are affected by other aspects of plant metabolism.

2. MATERIALS AND METHODS

2.1 Experiments Conducted

During the period between June 1966 and February 1969 the following experiments were carried out:

(a) A greenhouse experiment in which the response of peas to 5 levels (0, 44, 88, 176, 352 lb./A) of P_2 was investigated. At the end of this experiment 3 levels (0, 44, 176) were chosen for controlled environment studies.

(b) Effects of air and soil temperatures and the 3 levels of P on mineral uptake and growth at 6th node, 10th node and full bloom stages.

(c) Effects of air and soil temperatures and the 3 levels of P on mineral uptake and distribution in the root, vine, pod and pea seed. Growth characteristics and yield were also observed.

(d) Effects of Cycocel, Phosfon and B-Nine at 1 and 100 ppm on chlorophyll concentration, mineral composition, growth characteristics and pea yield. This experiment was carried out in the greenhouse.

(e) Effects of temperature, Cycocel and Phosfon on the levels of Glucose, Hexose phosphates, ADP and ATP in 5-day old pea radicles. Seeds were germinated in petri dishes.

(f) Effects of air and soil temperatures and P on the levels of compounds in (e) in the leaf and root of 30-day old plants.

2.2 Control of Air and Soil Temperatures and Soil Moisture

For temperature studies, a controlled environment multiple growth chamber with 4 cabinets, as developed and described by Ormrod (1962) was used. Air and soil temperatures were controlled in each cabinet (Fig. 1). Essentially the cabinets were of wood construction. Movement of air through the cabinet was by means of fans which circulated refrigerated in-coming air, and the internal air passed through light panels to the top of the cabinets and thence to the exterior.

Light was provided by a combination of 10 cool-white fluorescent lamps and 2 40-watt tungsten filament lamps. The light panels were 70 cm above plant-container level, and provided a light intensity of about 1600 foot-candles (measured with a Weston Model 756 Illumination Meter without cosine filter) at 50 cm below the lights. The photoperiod, controlled by an "Intermatic" time switch, was 16 hours in a 24-hour cycle.

Soil temperature in each cabinet was controlled by the use of water baths in which the pots were immersed (Fig. 2). This system is similar to that employed by Willis et al. (1963). The water baths measured 90 X 45 X 30 cm. A 180 X 0.5 cm copper tube was coiled in each bath (Fig. 3). To one end of the tube coil was attached a cold-water-inlet rubber tube, while the other end was connected to the warm-water-outlet. Cold water (3-5°) was circulated from a refrigerated tank (Fig. 4, 5). The ascent of cold water to the cold-water-inlet

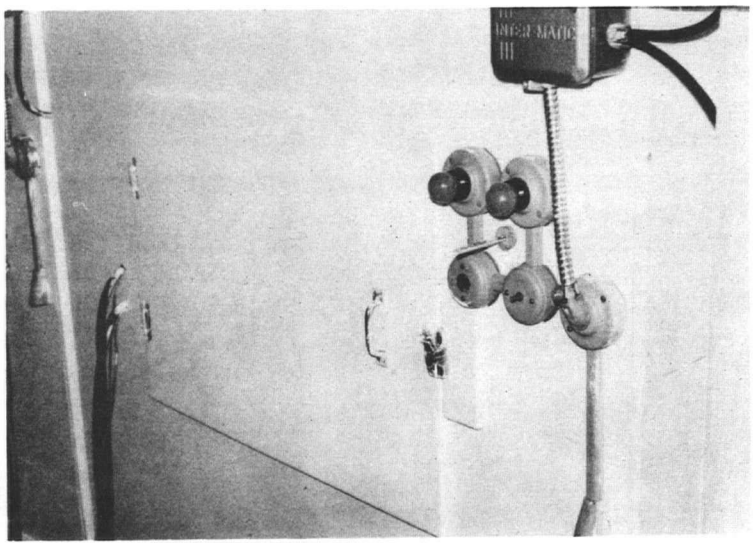


Fig. 1. Exterior of growth cabinet showing time switch, thermostats and pilot light bulbs.

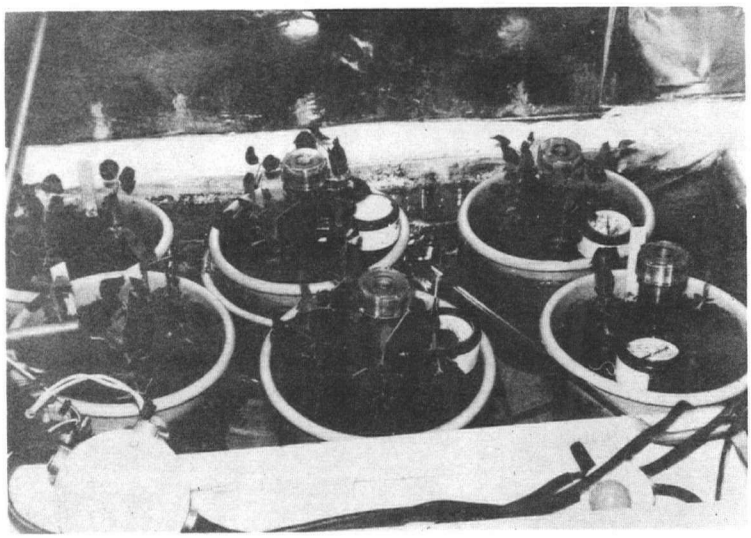


Fig. 2. Interior of growth cabinet showing pot placement.

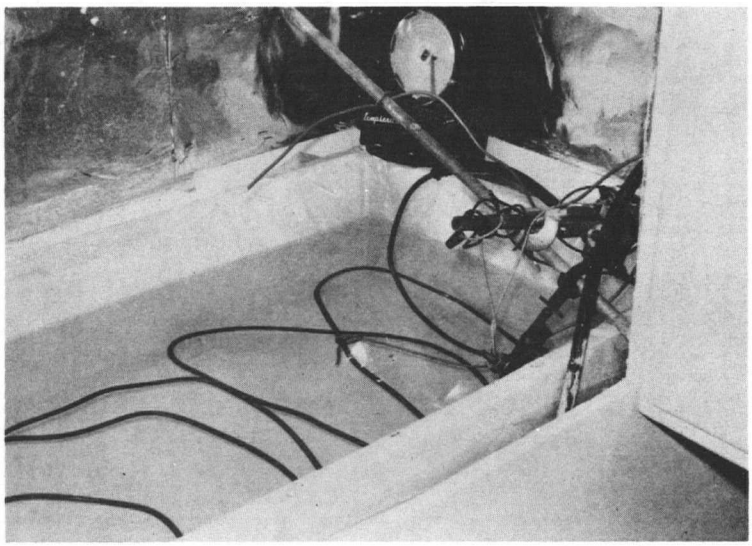
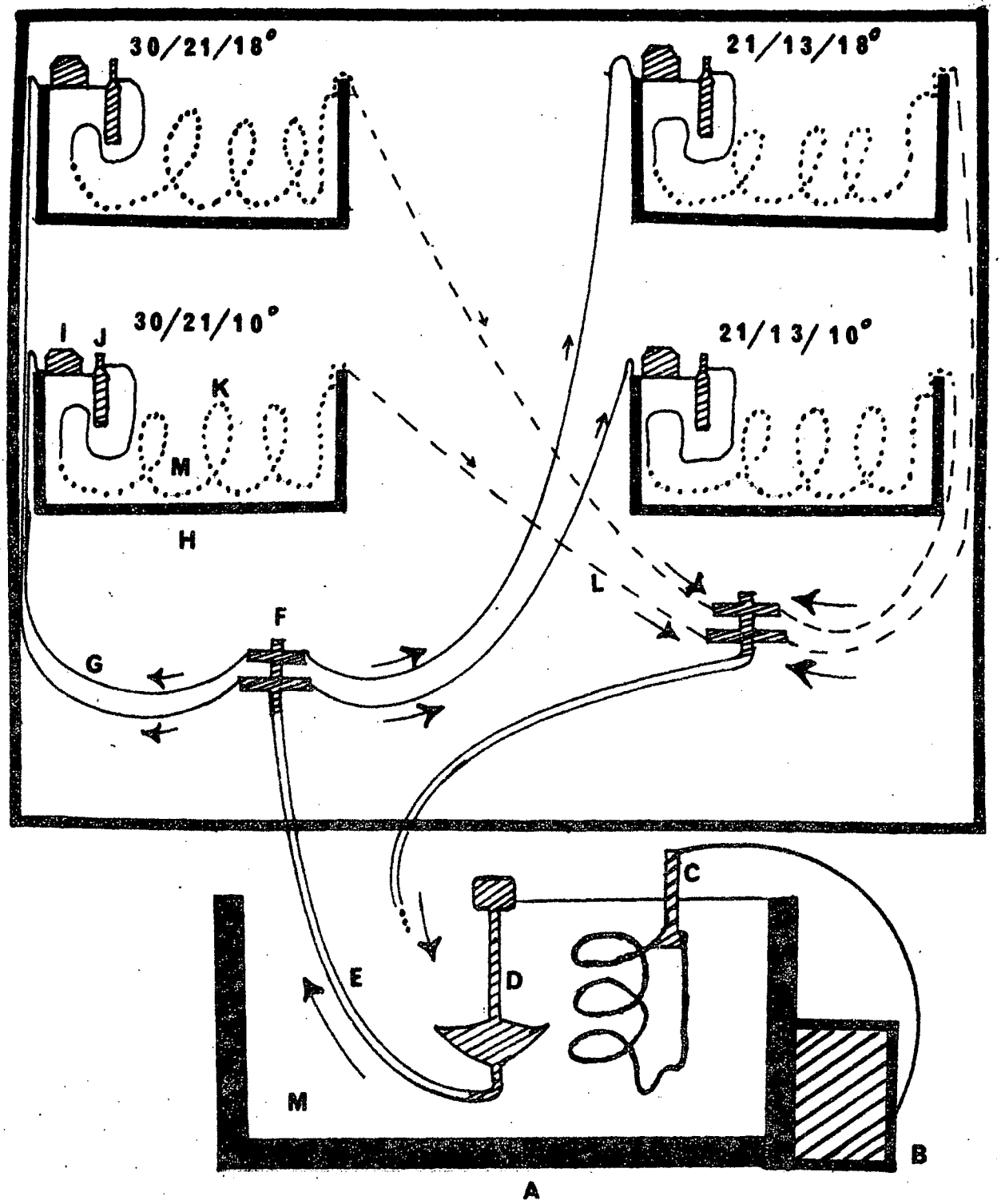


Fig. 3. Interior of growth cabinet showing water bath, cold water coiled copper tube, thermostat and thermistors.



A	Water Tank	H	Water Bath
B	Refrigeration Motor	I	Solenoid Valve
C	Refrigeration Coil	J	Thermostat
D	Water Pump	K	Copper Tube Coil
E	Water Tube from Pump	L	Outlet Water Tube
F	Copper Manifold	M	Water
G	Inlet Water Tube		

Fig. 4. Schematic diagram of soil temperature control equipment.

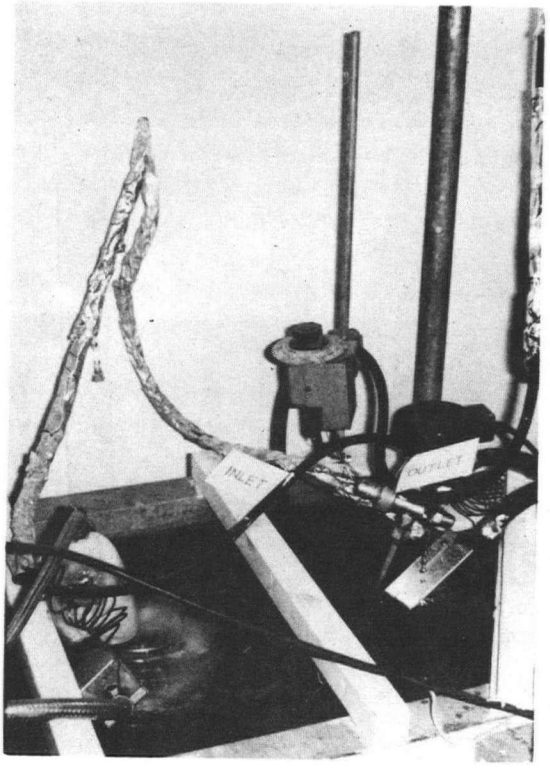


Fig. 5. Refrigerated water tank.

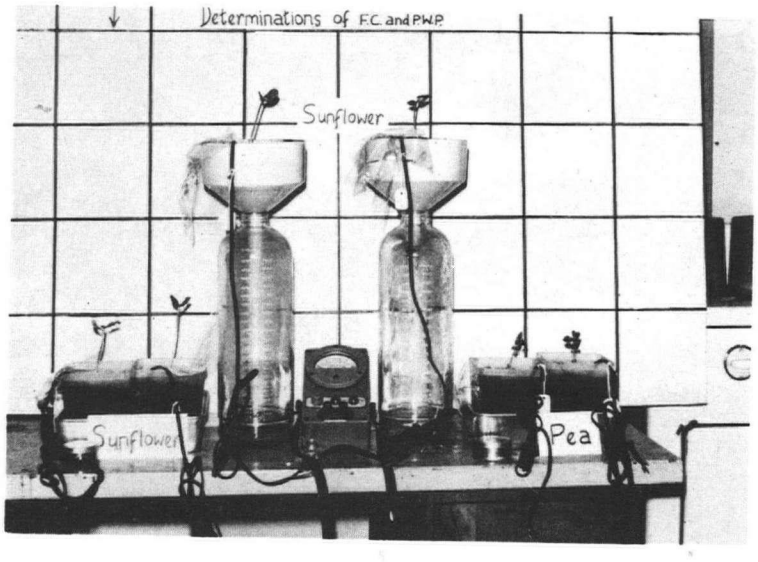


Fig. 6. Bouyoucos moisture meter and materials for soil moisture calibration.

was forced by a pump. The coiled tube ensured continuous cooling of water in the bath, as needed, through a thermostat-operated solenoid valve. Water returned to the refrigerated tank mainly by gravity.

Because soil moisture is known to influence P uptake by plants (Simpson, 1960), it was necessary to keep the soil moisture content close to optimum for P uptake, which is close to field capacity. Also, soil moisture control was desirable to minimize variations due to differences in soil moisture content between temperature regimes and P levels. In addition, the pots submerged in the waterbaths did not have free drainage.

A trial was carried out, using the Industrial Instruments Model BN-2A Bouyoucos Meter and gypsum resistance blocks, to determine the moisture content of the soil at field capacity (F.C.) and permanent wilting point (P.W.P.). Pea and sunflower seedlings were used (Fig. 6). An earlier trial with "Irrometer" tensiometers was unsatisfactory with respect to sensitivity. There were minor differences in the sunflower and pea data. The latter was used.

The moisture content at estimated F.C. (72-hour drainage after saturation) was about 28% of oven dry weight of soil. This gave 102% on the Bouyoucos meter and was regarded as 100%. P.W.P. was about 10% of oven dry weight of soil, which corresponded to 15% on the meter.

To determine the amount of water required to bring 6 kg of soil to 100% on the meter, varying known quantities of water were added and the percentage changes on the meter

recorded. Calculations showed that about 55 ml of water was required to raise the meter reading 1% within the range 20 to 100%. Meter readings were subsequently taken every 3 days up to the 6th node stage, and daily thereafter. The soil was watered according to indicated requirement, which was oftener at the higher temperatures.

Two air and two soil temperatures were combined into four air and soil temperature regimes. These were day/night/soil temperatures of 21/13/10, 21/13/18, 30/21/10, 30/21/18°. The cabinets contiguous to the refrigerated tank carried the 10° soil temperature (Fig. 4).

2.3 Soil, Fertilizer Applications, Planting and Harvesting

A low-P Monroe Silt Loam from the Lower Fraser Valley of British Columbia was used. Chemical analyses indicated the following composition: pH (water), 6.1; N, 0.24%; avail. P, 4 ppm; exch. K, 0.3 m.e./100 g; exch. Ca, 10.8 m.e./100 g; exch. Mg, 1.6 m.e./100 g; total exchangeable bases, 18.4 m.e./100 g. The weight of air-dry soil in the 4 L pot used was 6 kg.

A commercial superphosphate fertilizer (20% P_2O_5) was used as the P source. Each pot, including controls, received a basal dressing of muriate of potash (60% K_2O) required to supply 165 lb. K/A. The weight of fertilizer required to supply 44 and 176 lb. P/A were 1.4 and 5.6 g respectively, while 0.8 g/pot was required for 165 lb. K/A.

A greenhouse study was carried out to establish a response curve of pea plants grown in the experimental soil at 5 levels of P: 0, 44, 88, 176 and 352 lb./A. 3 levels

(0, 44, 176) were subsequently chosen for the air and soil temperature studies. The 44 lb. level was chosen on the basis of maximum efficiency (pea yield per unit of P applied), and the 176 lb. level was chosen to provide a supra-optimal rate.

The basal dressing of K was broadcast over and mixed uniformly with the soil. The pot was filled with 2 kg of soil and a gypsum resistance block put in place. 3.5 kg of soil was poured onto the block. The P fertilizer was then applied in a band, using a 10 cm. dia. funnel as a convenient and reproducible marker for the fertilizer band. The rest (0.5 kg) of the soil was used to cover the fertilizer. After each experiment the soil was discarded and the pot filled with fresh soil.

Seeds of pea, Pisum sativum L. cv. Dark Skin Perfection were treated with a slurry of peat base inoculum "Nitragin" about 30 minutes before planting. 6 seeds were sown 3 cm. outside the periphery of the fertilizer band using a marked peg to obtain a reproducible depth of 2 cm. A 3 cm layer of peat moss was spread on top of the soil to minimize evaporation. 500 ml of water was added to each pot and the pots were transferred into the treatment cabinets. At 2-node stage (between 7 and 10 days after seeding), the seedlings were thinned to 4 per pot, for uniformity. From this stage onward, watering was done according to the requirements of the 4 plants in each pot. Water from a bottle kept in the water bath was used for the 6 pots (2 replicates each of the 3 levels of P) in that bath, to minimize variations in soil temperature. The

departure of soil temperature from water bath temperature was only about 1°. Temperatures were read using a system of thermistors connected to an externally situated Yellow Springs Instruments resistance bridge.

At the pre-defined growth stages (6th node, 10th node, full bloom and estimated marketable maturity of peas) the plants were cut at soil level. Full bloom was regarded as the stage at which each of the 4 plants in a pot was at 0.5 blossom stage according to the classification scheme developed by Maurer et al. (1966). Marketable maturity of peas was estimated by pod-fill. The plant was separated into root, vine, pod and pea seed components. The root was carefully separated from soil by the flotation method of McKell et al. (1961). Fresh weights were determined immediately after harvesting. The tissues were dried in a forced air oven at 60° for 48 hours, weighed, ground in a Wiley mill and dry-ashed in a muffle furnace at 500°. Dry ashing at 500° is within the temperature range given by Clarkson (1966) as being in agreement with results obtained with wet ashing.

2.4 Application of Growth Retarding Chemicals

At the 5th to 6th node stage (15 days after seeding) each plant was sprayed with 10 ml of retarding chemical containing 0.1% "Triton B-1956" surfactant, using a domestic plunger sprayer with 4 jets. Control plants were sprayed with surfactant only. The pots were then placed on the greenhouse bench in a randomized complete block design. There were 6 replicates (blocks) for each treatment, and 2 runs (that is, one repetition of the entire set up) of the experiment.

For radicle studies, 10 seeds were placed in steam-sterilized petri dishes. 10 ml of retarding chemical was added. The petri dishes were transferred into growth cabinets at 25° in the dark.

2.5 Analytical Techniques

PLASTID PIGMENTS. Two days before harvesting, chlorophyll and carotenoid analyses were carried out on representative samples taken from fully expanded leaves at the 2 youngest nodes. Usually, 0.5 g sample, made up of 9 to 15 leaves depending on size, was used. Leaves were cut into pieces and ground with acid-washed sand and $CaCO_3$ in cold acetone using a chilled mortar. Repeated extraction was done, the extracts centrifuged at 2000 rpm, pooled and made to volume. Absorbance was read with a Beckman DU Spectrophotometer using 1 cm quartz cells. Concentrations of chlorophylls a and b were calculated according to MacKinney's (1940) specific absorption coefficients. von Wettstein's (1957) equation was used to calculate carotenoid concentration.

MINERAL COMPOSITION. Chemical analysis was done as follows: semi-micro Kjeldahl for N; P by phospho-molybdate colorimetry using a Beckman C colorimeter; Ca and Mg by atomic absorption; and K by flame emission photometry (A.O.A.C., 1965) using an Evans Electroselenium Atomic Absorption flame photometer, and a Texas Instruments Servo Riter II recorder. Total amounts of minerals in plant tissues were computed from composition and dry weight data.

PHOSPHORYLATED COMPOUNDS. Hexose phosphates, ADP and ATP, as well as glucose, were spearated by ion exchange chromatography, with the use of borate complexing as developed by Khym and Cohn (1953) and later used for the separation of phosphates from Scenedesmus by Goodman et al. (1955).

A combination of cold extraction similar to that suggested by Bieleski (1964) and chemical inhibition (Shaw, 1968; Burston, 1962) was used to inhibit phosphatases because it was found more effective than cold acids (Rowan, 1966).

Liquid N was added to 1.0-2.0 g of fresh plant tissue. This resulted in an immediate freezing of the tissue. The tissue was then homogenized for 5 minutes in cold 80% ethanol containing 0.001M NaF at -20°. The homogenate was centrifuged at 9000 X g for 15 minutes at 0-2°. The extract was concentrated in vacuo to 10 ml. Chlorophyll and phospholipids were removed with hexane, as this was found to be more satisfactory than ethanol-petroleum ether method used by Goodman et al. (1955). The extract was kept at -20° until it was required for analysis.

Separation of the compounds was done by anion exchange using Dowex 1 - X8 (200-400 mesh) in Cl^- form. The exchanger was washed free of fines by repeated decantation and slurried into a 30 X 1.5 cm "Pharmacia" column. The exchanger was converted to the Cl^- form by running 20 bed volumes of 1M HCl through the column. Excess Cl^- was removed by running 20 bed volumes of water through the column.

An LKB Ultrorac 7000 fraction collector was used to separate the effluent. An LKB Peristaltic pump was used to regulate the elution rate at 2.5 ml/minute. The column, fraction collector, pump and eluent reservoir were all in a refrigerator, and temperature was kept at 3°. The column was regenerated after each sample run, and a given resin bed was used for a maximum of 6 sample runs.

Ba and K salts of authentic phosphorylated compounds were obtained from Mann Research Laboratories, New York. The authentic compounds were: glucose-1-phosphate dipotassium; glucose-6-phosphate dibarium; fructose-6-phosphate barium; fructose 1,6-diphosphate barium; adenosine monophosphate dihydrate; adenosine diphosphate barium; and adenosine triphosphate dibarium.

A strong cation exchanger, Dowex 50W - X8 in H^+ form (200-400 mesh) was used batchwise to convert the Ba and K salts of the authentic compounds to their acids. The solutions were neutralized with 1N NH_4OH and stored at -20°. The solutions of the authentic compounds were run through the column to determine the recovery of each compound. A succession of eluting agents were used for selectively desorbing the phosphorylated compounds. The eluting agents and the order of use were as follows:

Compound	Eluting agent	Effluent vol., ml.
Glucose	.001M NH_4OH	150
G-1-P	.025 NH_4Cl + .01M $K_2B_4O_7$	300
G-6-P	.025 NH_4Cl + .0025 NH_4OH + .001M $K_2B_4O_7$	300

Compound	Eluting agent	Effluent vol., ml.
F-6-P	.025 NH_4Cl + .0025 NH_4OH + .00001M $K_2B_4O_7$	300
ADP	.01M HCl	300
Fl,6-P	.02M HCl + .02M KCl	300
ATP	.02M HCl + .20M KCl	300

Recovery percentages of the authentic compounds varied between 94 for G-1-P and 102 for Glucose, and were thus similar to those obtained by Goodman et al. (1955). Recovery was determined by comparing a mixture of the compounds separated on the column with individual compounds not run through the column.

After separation, the very dilute solution of each compound was concentrated in vacuo to 6-8 ml. for the hexose phosphates and 15 ml for the adenosine phosphates. Glucose needed no concentration. The final extract of each compound was neutralized with NH_4OH and analyzed.

For the quantitative determination of glucose and the phosphorylated sugars, Dreywood's anthrone reagent method (Morris, 1948) used by Khym and Cohn (1953) was found to be unsatisfactory with the pea extracts due to the formation of brown colloidal particles. This may have resulted from interference by nitrate or nitrite (Juo and Stotzky, 1967). The method of Scott et al. (1967) employing concentrated sulphuric acid was used instead. This method is based on the formation of ultraviolet-absorbing furan aldehydes in strong sulphuric acid.

To 0.5 ml of sugar solution in a 20 X 2 cm. test tube was rapidly added 40 ml of 95% reagent grade sulphuric acid from an automatic pipette, and the solution mixed. The tube was corked and placed in a 70° water bath for 30 minutes. The tube was then cooled to room temperature. Absorbance was read with a Unicam SP 800 Ultraviolet spectrophotometer, using 1 cm quartz cells. Maximum absorbance of the carbohydrates was found to be at 322 mμ. This method was found to be very satisfactory, particularly with respect to reproducibility. Absorbance of ADP and ATP was read directly after concentration in vacuo. Maximum absorbance at pH 7.0 was at 260 mu. Contents were expressed per unit fresh weight because there were no significant differences in the percent dry matter of the tissues.

2.6 Statistical Analyses of Data

All data were examined by the analysis of variance using an IBM Model 7044 electronic computer.

For the greenhouse experiment in which the response of peas to 5 levels of P was investigated, the randomized complete block design was used. The pots were placed in a manner in which the 5 levels of P were along the bench, while the 4 replicates constituted 4 blocks across the bench. The block effect was not significant, so data were reanalyzed according to the completely randomized design.

In the controlled environment work, the completely randomized design was used within each temperature regime.

Each experiment was conducted twice. There were thus 2 RUNS each of 2 replicates for a total of 4 replicates (pots). In the combined analysis of variance there was a testing term for each of the following: Run, Temperature, Phosphorus and all possible interactions. Significant interactions occurred between temprature and phosphorus in the combined analysis of data for main effects at each growth stage. Therefore, individual analyses were also made for the simple effect of phosphorus at each growth stage and each temperature regime.

For the growth retardant experiment, the 2 concentrations of each of the 3 compounds and the control were regarded as different treatments. There were thus 7 treatments. The randomized complete block design was used with treatments along the bench, and the 6 replicates (blocks, pots) across the bench. There were 2 runs of the experiment so that there was a testing term for Run, Block, Treatment and their interactions.

Treatment means were subjected to Duncan's multiple range test (Duncan, 1955) for determination of significant differences among individual means.

3. RESULTS

3.1 Plant Response to Phosphorus Fertilizer in the Greenhouse

When pea plants were grown in the greenhouse, P fertilizer applied at rates of between 0 and 352 lb./A, increased pod weight, pea weight and total dry matter, but had no effect on plant height and mean internode length (Table 1).

Expressed as percent of dry matter, N and P were generally highest in the pea seed (Table 2). While N was least in the pod, P was least in both the pod and the vine. K, Ca and Mg concentrations were highest in the vine. While Ca and Mg were least in the pea, K was least in the pod.

Expressed as total milligrams of uptake, N in the pea seed was slightly less than in the vine, while P was slightly more in the seed than in the vine. K, Ca and Mg were greatest in the vine.

Applied P increased the concentrations and total contents of N in the vine, pod and pea seed. P in the vine and pod increased with applied P in all tissues except the pea seed. P had no effect on the concentrations of K, Ca and Mg in all tissues with the exception of slight changes in Ca in the pea seed. P however increased the total contents of all minerals in all tissues.

Table 1. Effects of Phosphorus Fertilization on growth and yield factors of pea plants grown in the greenhouse. Values are for one plant.

Lbs. P per acre	Plant height (cm)	Mean inter-node length (cm)	Vine dry weight (g)	Pod		Peas			Total dry matter (g)
				No.	Dry weight (g)	No.	Fresh weight (g)	Dry weight (g)	
0	87.0a*	4.9a	2.97c	2.5c	1.03c	12.5d	4.78d	1.01c	5.01c
44	90.5a	5.0a	3.63b	3.5b	1.46b	19.5c	10.38c	2.19b	7.28b
88	90.8a	5.0a	3.78ab	3.8ab	1.56ab	22.0b	11.71b	2.55b	7.89ab
176	91.0a	5.0a	3.98ab	4.0ab	1.67a	22.8b	12.38ab	2.63ab	8.28a
352	93.5a	5.0a	4.33a	4.3a	1.83a	24.5a	13.20a	2.85a	9.01a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular measurement are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 2. Effects of Phosphorus Fertilization on the concentrations and total contents of N, P, K, Ca and Mg in the vine, pod and pea of greenhouse-grown plants.

Plant tissue	Lbs. P per acre	Per cent of dry matter					Milligrams per plant				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg
Vine	0	2.65c*	0.13d	1.96a	3.25a	0.43a	79c	3d	58b	94c	13b
	44	2.98b	0.15d	2.27a	3.84a	0.48a	109b	5c	82a	139b	17a
	88	3.04b	0.16c	2.07a	3.80a	0.43a	116b	5c	79a	143ab	17a
	176	3.28ab	0.19b	2.18a	3.75a	0.41a	131ab	7b	87a	149ab	17a
	352	3.34a	0.23a	2.11a	3.76a	0.41a	144a	10a	91a	163a	18a
Pod	0	1.18b	0.12b	1.26a	1.57a	0.31a	13c	1d	13b	16b	3b
	44	1.47b	0.17b	1.17a	1.74a	0.34a	21b	2c	17b	25a	5a
	88	1.60a	0.17b	1.24a	1.85a	0.32a	25ab	2c	19ab	29a	5a
	176	1.70a	0.23a	1.37a	1.61a	0.31a	28ab	4b	23a	27a	5a
	352	1.83a	0.27a	1.23a	1.69a	0.30a	33a	6a	23a	31a	5a
Pea	0	3.64b	0.34a	1.58a	0.10d	0.19a	37c	3c	16c	1d	2c
	44	4.33a	0.34a	1.59a	0.11c	0.20a	95b	7b	35b	2c	5b
	88	4.37a	0.35a	1.51a	0.11c	0.19a	112ab	9b	38b	2c	5b
	176	4.39a	0.35a	1.60a	0.12b	0.19a	115a	9b	42a	3b	5b
	352	4.42a	0.39a	1.47a	0.13a	0.21a	126a	12a	42a	4a	6a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular element and tissue are not significantly different at P = 0.05 according to Duncan's multiple range test.

3.2 Effects of Phosphorus Nutrition on Growth and Mineral Uptake at 3 Pre-fruiting Growth Stages as Influenced by Air and Soil Temperatures

The utilization of P in promoting growth and influencing mineral uptake varied with the air and soil temperature combinations. This was evident from the significant interactions between temperature and phosphorus for most of the variables in the combined analysis of variance (Tables 3, 4 and 5). The simple effects of phosphorus at each temperature regime were therefore determined.

GROWTH

Plant Height. Applied P had no effect on plant height and mean internode length at the 6th and 10th node stages (Table 6). At full bloom, P at the 44 lb./A rate increased plant height at all temperature regimes except at the cool air and low soil temperature combination (21/13/10° day/night/soil) where P had no significant effect. The influence of P at the 176 lb./A rate was not significantly different from that at the 44 lb./A rate.

Dry Matter Production. At the 6th node stage, P had no effect on the dry weight of plants at all temperature regimes except at 21/13/18° where P at the 176 lb./A rate increased vine weight 38% (Table 6).

At the 10th node and full bloom stages, P increased the dry weight of plants at all temperature regimes. Increases due to P were greater at the high soil temperature of 18° than at 10°. Also, the increase due to P at the high soil temperature was greater at the cool day/night air temperature of 21/13° than

Table 3. An example of a Combined Analysis of Variance Computer Output for Main Effects

Source	DF	Sum Sq	Mean Sq	Error	F	Prob.
Run	1	0.00047	0.00047		00.01	0.8779
Temp	3	0.76092	0.25364	RXT (A)	61.09	0.0069
RXT (A)	3	0.01246	0.00415		0.11	0.9502
Phosph	2	0.40887	0.20444	ERR (B)	71.11	0.0001
TXP	6	0.18363	0.03061	ERR (B)	10.65	0.0023
ERR (B)	8	0.02299	0.00287		0.07	0.9994
Error	24	0.92475	0.03853			
Total	47	2.31410				

Variable: Dry weight of plants at the 10th node stage

Temp: 21/13/10, 21/13/18, 30/21/10, 30/21/18: Day/night/soil°C

Phosph: 0, 44, 176 lbs. P per acre.

Table 4. F values and the significances of Main Effects at each growth stage, and of each plant tissue at crop maturity. Part I. Dry matter and mineral concentrations.

Variable	Treat. effects	Pre-fruiting stages			Crop maturity			
		6th node	10th node	Full bloom	Root	Vine	Pod	Pea
Dry weight	Temp.	52.9**	61.1**	24.9*	3.5n.s.	63.9**	70.8**	15.0*
	Phos.	18.7**	71.1**	175.3**	106.8**	65.0**	57.2**	61.5**
	TXP	7.5**	10.7**	18.7**	4.9*	5.2*	4.9*	2.0n.s.
%N	Temp.	127.3**	8.8n.s.	6.9n.s.	10.4n.s.	8.2n.s.	2.9n.s.	12.7n.s.
	Phos.	34.2**	28.7**	9.8**	41.0**	11.0**	9.3**	7.9*
	TXP	1.5n.s.	6.2*	4.9*	0.7n.s.	1.7n.s.	0.9n.s.	0.7n.s.
%P	Temp.	178.4**	2.6n.s.	10.6*	4.0n.s.	30.3**	0.1n.s.	2.5n.s.
	Phos.	42.1**	145.1**	262.5**	55.1**	33.4**	4.0n.s.	106.0**
	TXP	1.2n.s.	2.7n.s.	1.6n.s.	4.7*	2.1n.s.	0.6n.s.	12.4**
%K	Temp.	170.0**	65.1**	194.3**	149.7**	179.2**	23.5*	2.6n.s.
	Phos.	5.7*	79.5**	10.0**	0.4n.s.	2.6n.s.	2.7n.s.	0.5n.s.
	TXP	14.2**	8.3**	5.4**	23.1**	3.3n.s.	1.7n.s.	1.1n.s.
%Ca	Temp.	5.5n.s.	7.0n.s.	3.7n.s.	26.5*	8.2n.s.	8.1n.s.	226.1**
	Phos.	1.0n.s.	24.1**	245.9**	15.5**	330.3**	1.4n.s.	6.8*
	TXP	2.7n.s.	3.4n.s.	2.9n.s.	1.5n.s.	7.9**	0.4n.s.	7.1**
%Mg	Temp.	15.7*	8.2n.s.	1.9n.s.	643.6**	100.4**	5.0n.s.	15.0*
	Phos.	5.1*	1.0n.s.	13.9**	2.2n.s.	27.3**	0.1n.s.	0.4n.s.
	TXP	0.4n.s.	0.8n.s.	0.8n.s.	7.6*	1.8n.s.	0.2n.s.	0.3n.s.

Table 5. F values and the significances of Main Effects at each growth stage, and of each plant tissue at crop maturity. Part II. Total mineral contents.

Variable	Treat. effects	Pre-fruiting stages			Crop maturity			
		6th node	10th node	Full bloom	Root	Vine	Pod	Pea
mg. N	Temp.	81.3**	13.2*	148.9**	1.4n.s.	30.3**	17.2*	85.8**
	Phos.	43.5**	89.3**	265.4**	66.5**	65.7**	3.9n.s.	133.1**
	TXP	13.2**	26.5**	20.9**	5.6*	7.5**	1.1n.s.	6.7**
mg. P	Temp.	79.6**	50.9**	44.4**	1.6n.s.	23.6*	7.3n.s.	179.0**
	Phos.	75.2**	125.1**	86.9**	101.2**	89.2**	17.4**	152.3**
	TXP	10.1**	4.6*	7.4**	9.7**	4.6*	1.6n.s.	4.2*
mg. K	Temp.	154.5**	26.2*	27.7*	25.9*	47.2**	306.5**	57.0**
	Phos.	33.8**	48.8**	244.4**	79.5**	112.2**	116.7**	105.8**
	TXP	20.8**	7.1**	25.4**	6.4**	6.1**	9.8**	5.4*
mg. Ca	Temp.	399.2**	35.7**	16.8*	1.3n.s.	57.8**	5.0n.s.	65.3**
	Phos.	6.7*	54.1**	93.1**	32.4**	66.9**	17.0**	55.7**
	TXP	5.6*	18.5**	13.0**	2.3n.s.	6.2**	0.9n.s.	3.5n.s.
mg. Mg	Temp.	168.1**	55.1**	26.7*	35.3**	43.8**	26.0*	43.6**
	Phos.	31.1**	29.7**	186.8**	38.0**	79.7**	55.2**	85.2**
	TXP	7.7**	7.6**	15.2**	1.6n.s.	5.2*	2.8n.s.	4.2*

Table 6. Effects of Air and Soil Temperatures on Growth Response of pea plants at 3 pre-fruiting stages to phosphorus fertilization.

Temp. Regime Day/Night/Soil °C	Lbs. P per acre	6th node			10th node			Full bloom		
		Plant height cm	Mean inter-node length cm	Vine dry weight g	Plant height cm	Mean inter-node length cm	Vine dry weight g	Plant height cm	Mean inter-node length cm	Vine dry weight g
21/13/10	0	11.5a*	1.89a	0.19a	18.8a	1.86b	0.30b	43.8a	3.40a	0.65b
	44	12.5a	2.08a	0.22a	21.0a	2.11a	0.38a	50.0a	3.40a	1.35a
	176	12.0a	2.00a	0.23a	20.0a	2.00a	0.39a	50.5a	3.29a	1.82a
21/13/18	0	14.3a	2.38a	0.31b	28.5a	2.85a	0.42b	49.3b	3.45b	0.95b
	44	14.0a	2.34a	0.33b	29.3a	2.93a	0.75a	69.0a	4.01a	3.45a
	176	15.5a	2.58a	0.43a	29.3a	2.93a	0.88a	66.0a	3.84a	3.23a
30/21/10	0	9.3a	1.58a	0.18a	18.8a	1.90a	0.32a	30.3b	2.15b	0.50b
	44	8.8a	1.37a	0.18a	19.8a	1.91a	0.44a	44.8a	2.79a	1.24a
	176	8.5a	1.42a	0.16a	17.3a	1.93a	0.43a	42.0a	2.56a	1.21a
30/21/18	0	9.5a	1.61a	0.22a	19.0a	1.90a	0.34b	31.5b	2.13a	0.53b
	44	9.5a	1.66a	0.25a	20.3a	2.03a	0.53a	44.8a	2.43a	1.43a
	176	10.5a	1.78a	0.28a	18.3a	1.83a	0.52a	45.3a	2.46a	1.46a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular temperature regime, growth stage and variable are not significantly different at P = 0.05 according to Duncan's multiple range test.

at 30/21°. P effects on dry weight at the 44 lb./A were not significantly different from those at the 176 lb./A rate at all temperature regimes. The magnitude of weight increases due to P were greater at full bloom than at the 10th node stage.

MINERAL UPTAKE

Mineral Concentrations. When expressed as percent of dry matter, N generally decreased with plant age from the 6th node, through the 10th node to the full bloom stages (Table 7). P and K remained relatively constant at all three stages. Ca and Mg increased at the 10th node stage, but decreased at full bloom to about the same levels as at the 6th node stage. There were however minor variations in trends due to P level and temperature regime.

At the 6th node stage, P at the 176 lb./A increased N concentration only at the cool air and high soil temperature regime (21/13/18°). P at both rates of application had no significant effects on K, Ca and Mg concentrations. Tissue levels of P generally increased with applied P, but the magnitude of increased P uptake depended on the temperature regime.

At the 10th node stage, applied P at the 176 lb./A rate, but generally not at the 44 lb./A rate, increased N concentration except at 30/21/10°. P had some influence on K and Ca concentrations, but had no significant effect on Mg concentration. Both rates of applied P increased the P concentration of plants at all temperature regimes.

At full bloom P decreased N concentration at 21/13/18° but had no significant effect at the other 3 temperature regimes. P generally had no influence on K and Mg concentrations while it decreased Ca at all temperature regimes except 21/13/18°.

Table 7. Effects of Air and Soil Temperatures and Phosphorus nutrition on the concentrations of 5 minerals in the pea vine at 3 pre-fruiting growth stages.

Growth Stage	Day/Night/Soil °C	Lbs. P per acre	Per cent of dry matter				
			N	P	K	Ca	Mg
6th node	21/13/10	0	4.13a*	0.17b	2.03a	1.80a	0.52a
		44	4.20a	0.24a	2.05a	2.03a	0.54a
		176	4.52a	0.26a	1.93a	1.92a	0.58a
	21/13/18	0	4.68b	0.18c	2.74a	2.19a	0.59a
		44	4.90b	0.28b	2.75a	2.42a	0.59a
		176	5.33a	0.37a	3.03a	2.28a	0.65a
	30/21/10	0	5.12a	0.21b	2.51a	2.19a	0.63a
		44	5.04a	0.33a	2.16a	2.25a	0.62a
		176	5.33a	0.39a	2.16a	2.24a	0.65a
	30/21/18	0	5.18a	0.24b	2.43a	2.59a	0.66a
		44	5.32a	0.31ab	2.67a	2.25a	0.68a
		176	5.67a	0.39a	3.01a	2.12a	0.75a
10th node	21/13/10	0	3.97b	0.12c	1.96a	2.97a	0.67a
		44	3.88b	0.23b	2.30a	3.02a	0.70a
		176	4.63a	0.34a	2.73a	2.69a	0.71a
	21/13/18	0	4.02b	0.10c	2.72b	3.24a	0.75a
		44	5.12a	0.29b	2.92ab	2.75ab	0.76a
		176	5.12a	0.50a	3.27a	2.52b	0.78a
	30/21/10	0	4.59a	0.10c	2.19a	3.15a	0.73a
		44	4.48a	0.24b	2.32a	3.13a	0.73a
		176	4.93a	0.39a	2.48a	3.08a	0.81a
	30/21/18	0	4.41b	0.10c	2.11b	3.18a	0.81a
		44	5.01a	0.31b	2.73ab	2.72ab	0.82a
		176	4.99a	0.45a	3.00a	2.42b	0.78a
Full bloom	21/13/10	0	3.22a	0.08b	1.97a	3.74a	0.71a
		44	3.09a	0.22a	1.87a	2.86b	0.65a
		176	3.11a	0.28a	1.88a	2.18b	0.64a
	21/13/18	0	2.99a	0.16a	1.39a	1.05a	0.35a
		44	2.56b	0.17a	1.75a	1.03a	0.66a
		176	2.53b	0.21a	1.77a	1.22a	0.66a
	30/21/10	0	3.77a	0.10c	2.20a	3.74a	0.69a
		44	3.11a	0.22b	2.03a	2.62b	0.57a
		176	3.44a	0.30a	2.21a	2.05c	0.57a
	30/21/18	0	3.54a	0.12c	2.07b	3.24a	0.74a
		44	3.72a	0.27b	2.27ab	2.20b	0.62a
		176	3.78a	0.33a	2.47a	2.11b	0.70a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular growth stage, temperature regime and nutrient are not significantly different at P = 0.05 according to Duncan's multiple range test.

Total Mineral Contents. At the 6th node stage, applied P at the 176 lb./A rate increased the total uptake of N and K at the high soil temperature of 18°, but not at the low soil temperature of 10° (Table 8). The 44 lb./A rate had no significant effect on the total uptake of N, K, Ca and Mg. Increase in P uptake due to the 176 lb./A applied P was greatest at 21/13/18°.

At the 10th node stage, applied P tended to increase the total uptake of N and K. At 21/13/18° the 176 lb./A rate resulted in decreases in N and K, compared to the 44 lb./A rate. P had no significant effect on Ca and Mg except at 21/13/18° where Ca and Mg increased with applied P, but only at the 44 lb./A rate. At the low soil temperature of 10° P uptake increased at both rates of applied P.

At full bloom the application of P increased the total content of each of the 5 minerals at all temperature regimes. The magnitude of increases in the uptake of the minerals, due to applied P, depended on the temperature regime. The magnitude was generally greater at the high soil temperature than at the low soil temperature. The magnitude of such an increase in mineral uptake due to P at the high soil temperature was reduced by the warm air temperature of 30/21° compared with that of 21/13°.

Table 8. Effects of Air and Soil Temperatures and Phosphorus nutrition on the total contents of 5 minerals in the pea vine at 3 pre-fruiting growth stages.

Growth Stage	Day/Night/Soil °C	Lbs. P per acre	Milligrams per plant				
			N	P	K	Ca	Mg
6th node	21/13/10	0	8a*	0.3b	4a	4a	1a
		44	9a	0.5a	4a	5a	1a
		176	10a	0.6a	4a	4a	1a
	21/13/18	0	15b	0.6b	8b	7a	2a
		44	16b	0.9b	9b	8a	2a
		176	23a	1.7a	12a	10a	3a
	30/21/10	0	9a	0.4b	5a	4a	1a
		44	9a	0.6ab	4ab	4a	1a
		176	8a	0.7a	3b	4a	1a
	30/21/18	0	11b	0.5b	5b	6a	2a
		44	13ab	0.8ab	6b	6a	2a
		176	16a	1.1a	8a	6a	2a
10th node	21/13/10	0	12b	0.4c	6b	9a	2a
		44	15ab	0.9b	9a	11a	3a
		176	18a	1.4a	11a	11a	3a
	21/13/18	0	17b	0.3b	7c	13b	3b
		44	39a	2.0a	21a	21a	6a
		176	19b	2.0a	14b	9b	3b
	30/21/10	0	14b	0.2c	7b	10a	2a
		44	20a	1.0b	10a	14a	3a
		176	21a	2.0a	10a	13a	3a
	30/21/18	0	15b	0.3b	7b	11a	3a
		44	26a	1.6a	14a	14a	4a
		176	26a	2.3a	14a	13a	4a
Full bloom	21/13/10	0	21c	0.5c	13b	25b	5c
		44	37b	2.9b	26ab	38a	8b
		176	53a	4.8a	33a	40a	11a
	21/13/18	0	28b	1.0b	19b	35b	7b
		44	86a	8.0a	69a	80a	18a
		176	100a	11.0a	70a	67a	19a
	30/21/10	0	18b	0.5b	11b	19b	3b
		44	38a	2.7a	25a	32a	7a
		176	41a	3.6a	26a	25a	7a
	30/21/18	0	18b	0.6b	11b	17b	4b
		44	53a	3.8a	32a	31a	9a
		176	55a	4.7a	36a	30a	10a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular growth stage, temperature regime and nutrient are not significantly different at P = 0.05 according to Duncan's multiple range test.

3.3 Effects of Phosphorus Nutrition on Yield Factors at Estimated Marketable Maturity of Peas as Influenced by Air and Soil Temperatures

GROWTH AND YIELD FACTORS

Applied P increased plant height at all temperature regimes except 30/21/18° (Fig. 7 and Table 9). P also increased the dry weights of all 4 tissues (root, vine, pod and pea seed) at all temperature regimes, but the magnitude of such increases depended on the tissue and temperature regime (Table 9).

The greater increases in root weight due to applied P particularly at the 44 lb./A rate were at the high soil temperature. There were large increases in vine weight due to P application but the effect of 176 lb./A were generally not different from those of 44 lb./A (Fig. 8 and Table 9). The smallest increase in vine weight due to applied P was accompanied by the largest increase in pea weight at the 30/21/10° regime. Increases in pea yield due to applied P resulted largely from increases in pea number and to a lesser extent on pea size and pod number (Fig. 9 and Table 9).

MINERAL UPTAKE

Mineral Distribution in Plant Tissues. Generally, N and P concentrations were highest in the pea seed, Ca in the vine, and Mg in the root (Table 10). The total contents of K, Ca and Mg were however greatest in the vine while P was greatest in the pea seed (Table 11). N was higher in vine and pea seed than in root or pod.

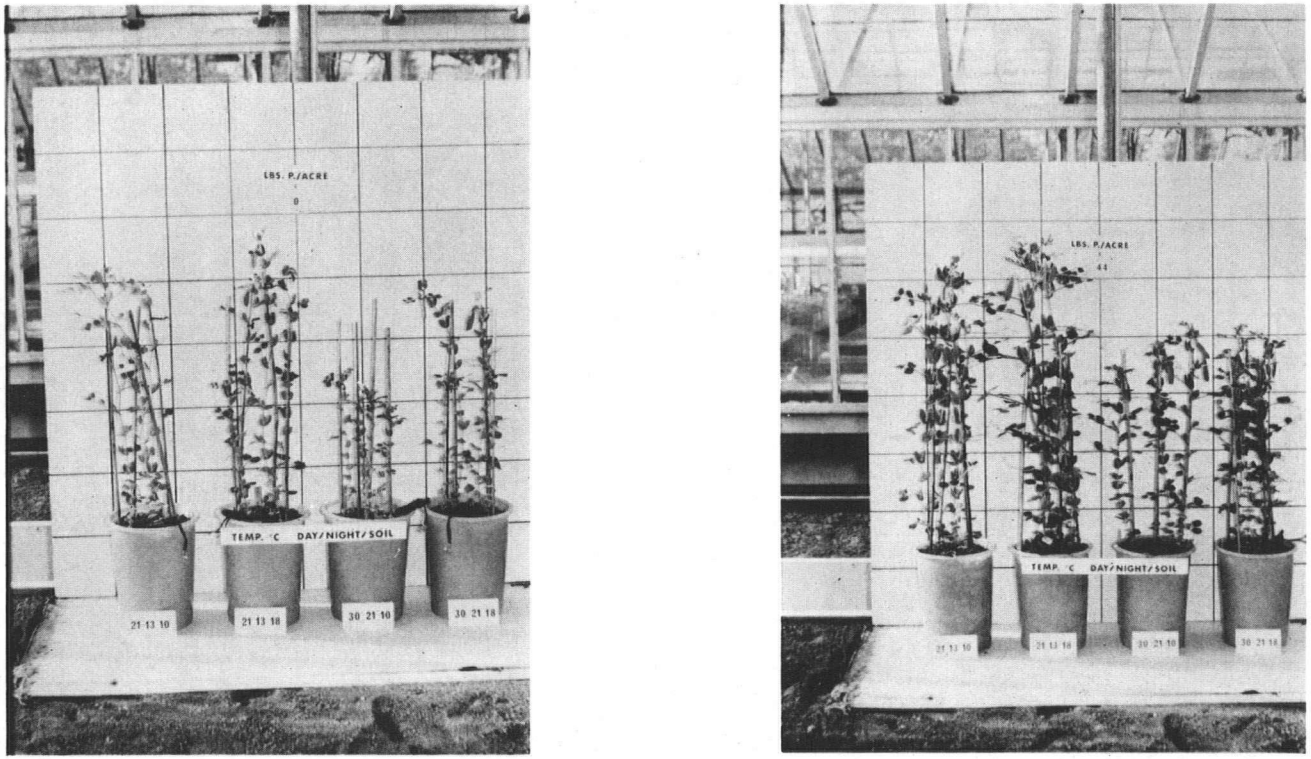


Fig. 7. Effects of air and soil temperatures on growth at 0 and 44 lb. P/A.

Table 9. Growth characteristics and yield factors in peas as influenced by Air and Soil temperatures and phosphorus.

Temp. Regime Day/Night/Soil °C	Lbs. P per acre	Plant height cm	Root dry weight g	Vine dry weight g	Pods		Peas			Total dry matter
					Mean No.	weight g	Mean No.	Fresh weight g	Dry weight g	
21/13/10	0	49b*	0.24b	0.71b	1.3b	0.24b	2.8b	0.96b	0.18b	1.37c
	44	66a	0.35ab	1.91a	2.5a	0.91a	10.0a	4.38a	0.93a	3.87b
	176	65a	0.42a	2.21a	2.8a	1.12a	10.8a	5.54a	1.31a	5.12a
21/13/18	0	55b	0.14b	0.75b	1.3b	0.31b	4.0b	1.73b	0.34b	1.54b
	44	74a	0.32a	2.78a	3.8a	1.28a	16.5a	8.84a	1.74a	6.12a
	176	71a	0.28a	2.94a	4.8a	1.46a	16.0a	7.60a	1.55a	6.23a
30/21/10	0	35b	0.22b	0.40b	1.0b	0.12b	1.8b	0.43b	0.08b	0.82b
	44	45a	0.33a	0.90a	2.0a	0.45a	7.0a	3.14a	0.78a	2.46a
	176	50a	0.38a	1.07a	1.8a	0.47a	7.8a	3.49a	0.76a	2.68a
30/21/18	0	38a	0.14c	0.43b	1.0b	0.12b	2.3b	0.80b	0.19b	0.88b
	44	47a	0.33a	1.17a	2.0a	0.41a	7.8a	3.68a	0.99a	2.90a
	176	48a	0.25b	1.26a	2.0a	0.47a	7.0a	3.49a	1.03a	3.01a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within a particular temperature regime and measurement are not significantly different at P = 0.05 according to Duncan's multiple range test.

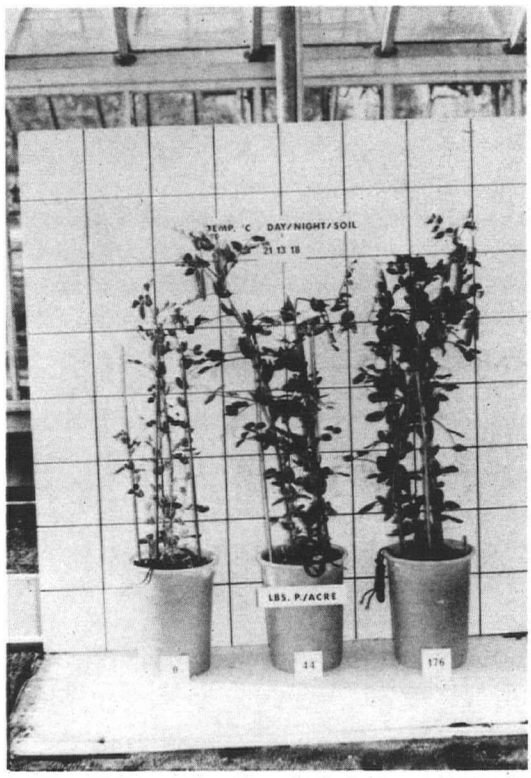


Fig. 8. Effect of phosphorus fertilization on the growth of the plant at the nearest-optimum air and soil temperature combination.

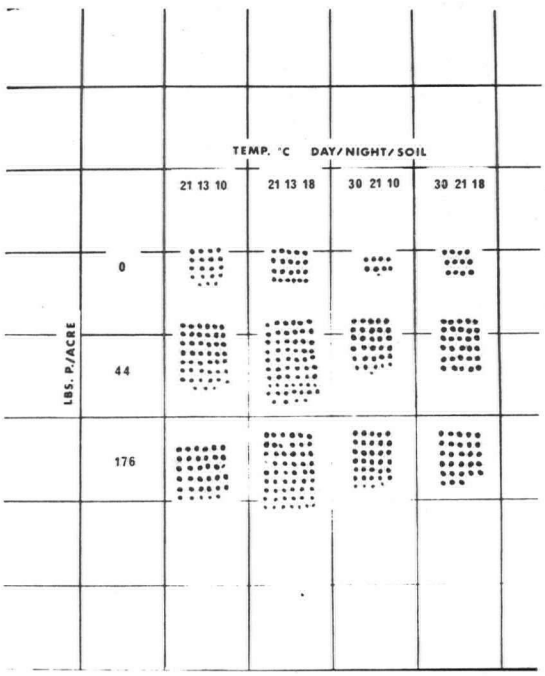


Fig. 9. Effect of air and soil temperatures and phosphorus on number and size of peas per pot (4 plants).

Table 10. Mineral uptake and distribution in the root, vine, pod and pea seed as influenced by Air and Soil temperatures and phosphorus. Mineral concentrations, percent of dry matter.

Temp. Regime Day/Night/Soil °C	Lbs. P per acre	Root					Vine				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg
21/13/10	0	2.72a*	0.07c	2.74a	0.69b	1.77a	2.40a	0.06b	1.95a	4.19a	0.80a
	44	2.28ab	0.13b	2.25b	0.90ab	1.56a	1.71b	0.08b	1.88a	3.73a	0.63b
	176	2.10b	0.21a	1.84b	1.26a	1.29a	1.54b	0.13a	1.73a	2.29b	0.52c
21/13/18	0	2.99a	0.16a	1.39a	1.05a	0.35b	2.67a	0.10c	2.00a	3.57a	0.88a
	44	2.56b	0.16a	1.75a	1.03a	0.66a	2.47a	0.13b	1.83a	3.14a	0.66a
	176	2.53b	0.21a	1.77a	1.22a	0.66a	1.92b	0.18a	1.93a	2.44b	0.67a
30/21/10	0	2.82a	0.10b	2.98a	0.86a	1.73a	2.96a	0.06b	2.21a	4.13a	1.00a
	44	2.34b	0.12b	2.75a	1.14a	1.61a	2.40b	0.17a	2.31a	3.44a	0.83a
	176	2.33b	0.29a	2.53a	1.19a	1.61a	2.21b	0.19a	2.23a	2.33b	0.75a
30/21/18	0	2.87a	0.14b	1.05b	1.07a	0.36c	2.66a	0.08b	2.44a	3.58a	0.99a
	44	2.54b	0.16b	1.65a	1.29a	0.68a	2.57a	0.11b	2.20a	3.35a	0.98a
	176	2.56b	0.23a	2.00a	1.37a	0.56b	2.54a	0.23a	2.48a	2.49b	0.89a
				Pod					Pea		
21/13/10	0	1.99a	0.06b	1.25a	1.65a	0.48a	4.46a	0.30b	1.43a	.14c	0.28a
	44	1.34b	0.15a	1.39a	1.57a	0.44a	4.12a	0.46a	1.66a	.12a	0.28a
	176	1.28b	0.18a	1.31a	1.40a	0.47a	4.01a	0.51a	1.64a	.11a	0.30a
21/13/18	0	1.90a	0.08c	1.22a	1.51a	0.49a	4.39a	0.34b	1.55a	.13a	0.30a
	44	1.56a	0.12b	1.41a	1.49a	0.47a	4.14a	0.39b	1.59a	.15a	0.31a
	176	1.90a	0.21a	1.57a	1.34a	0.47a	4.37a	0.49a	1.59a	.15a	0.31a
30/21/10	0	2.54a	0.12b	1.73a	1.76a	0.57a	4.68a	0.26c	1.67a	.19a	0.33a
	44	1.33b	0.10b	1.59a	1.99a	0.60a	4.33a	0.36b	1.66a	.15a	0.33a
	176	1.43b	0.19a	1.68a	1.68a	0.59a	4.21a	0.44a	1.68a	.14a	0.33a
30/21/18	0	2.26a	0.09b	1.61a	1.73a	0.70a	4.82a	0.28b	1.73a	.17a	0.35a
	44	1.36b	0.14a	1.57a	1.99a	0.67a	4.42a	0.37b	1.62a	.16a	0.32a
	176	1.54b	0.16a	1.78a	1.87a	0.66a	4.49a	0.65a	1.65a	.18a	0.34a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within each temperature regime, plant tissue and element are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 11. Mineral uptake and distribution in the root, vine, pod and pea seed as influenced by Air and Soil temperatures and phosphorus. Mineral contents, milligrams per plant.

Temp. Regime Day/Night/Soil °C	Lbs. P per acre	Root					Vine				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg
21/13/10	0	6.5a*	0.2c	6.6a	1.7b	4.1a	16.7a	0.4c	13.1b	29.6c	5.6b
	44	7.9a	0.4b	7.8a	3.1ab	5.4a	32.8a	1.6b	35.5a	68.7a	11.7a
	176	8.5a	0.9a	7.7a	5.6a	5.8a	34.5a	2.9a	38.0a	50.0b	11.5a
21/13/18	0	4.0b	0.3b	1.9b	1.5b	0.5b	19.8b	0.6c	14.9b	26.5c	6.4b
	44	8.3a	0.5a	5.8a	3.3a	2.1a	53.3a	3.4b	50.7a	87.3a	18.3a
	176	7.1a	0.6a	5.0a	3.4a	1.9a	72.9a	5.3a	56.0a	69.9b	19.4a
30/21/10	0	6.2b	0.2b	6.6b	2.0a	3.9b	11.3b	0.2b	9.0b	16.4b	3.9b
	44	7.8ab	0.4b	8.4ab	3.8a	5.5a	20.0a	1.5a	20.9a	30.7a	7.3a
	176	8.6a	1.1a	10.1a	4.8a	6.1a	25.4a	2.0a	23.9a	24.5a	7.9a
30/21/18	0	3.9b	0.2b	1.4b	1.5b	0.5b	11.4b	0.3c	10.6c	15.3c	4.1b
	44	8.3a	0.6a	5.8a	4.2a	2.1a	29.8a	1.3b	25.8b	39.3a	11.3a
	176	6.4a	0.6a	5.0a	3.4a	1.1b	31.9a	2.9a	31.2a	31.0b	11.1a
				Pod					Pea		
21/13/10	0	4.6b	0.2b	3.0b	3.8b	1.2b	7.9b	0.5c	2.6b	0.3b	0.5b
	44	12.8a	1.4a	12.1a	13.7a	4.0a	37.3a	4.3b	15.4a	1.1a	2.6c
	176	13.1a	1.9a	14.3a	16.9a	5.3a	52.5a	6.7a	21.5a	1.4a	3.9a
21/13/18	0	5.8b	0.4b	3.8b	4.6b	1.6b	14.9b	1.3b	5.3b	0.4b	1.0b
	44	19.7a	1.1b	17.8a	19.4a	5.9a	72.0a	5.9a	27.7a	2.6a	5.4a
	176	28.0a	3.1a	22.9a	19.3a	6.8a	67.7a	7.6a	24.6a	2.3a	4.8a
30/21/10	0	3.0b	0.2c	2.1b	2.0b	0.7b	3.7b	0.2b	1.3b	0.2b	0.3b
	44	5.9a	0.5b	7.1a	8.8a	2.7a	33.8a	2.8a	12.9a	1.2a	2.6a
	176	6.9a	0.9a	7.8a	7.5a	2.7a	32.0a	3.3a	12.8a	1.1a	2.5a
30/21/18	0	2.8b	0.2c	2.0c	2.1b	0.9b	9.1b	0.5c	3.3b	0.3b	0.7b
	44	5.5a	0.4b	6.4b	8.3a	7.8a	43.6a	3.7b	16.0a	1.6a	3.2a
	176	7.6a	0.8a	8.2a	8.8a	3.1a	45.6a	6.7a	17.0a	1.9a	3.5a

* Each figure is the mean of 4 replicates. Figures followed by the same letter within each temperature regime, plant tissue and element are not significantly different at P = 0.05 according to Duncan's multiple range test.

Mineral Concentrations. The influence of applied P on tissue levels of N, P, K, Ca and Mg depended on the tissue and the temperature regime. In the root, applied P at both rates generally decreased N concentration at all temperature regimes (Table 10). Root levels of P increased with the 176 lb./A rate of applied P except at 21/13/18°. Root K tended to increase with applied P at the high soil temperature, particularly when the air temperature was warm, and tended to decrease at the low soil temperature, particularly at the cool air temperature. Root Ca was affected by applied P at all temperature regimes but this was significant only at 21/13/10°. Root Mg was not influenced by P at the low soil temperature but was increased at the high soil temperature.

In the vine, applied P decreased N concentration at all temperature regimes except 30/21/18°. P at the higher rate increased the P concentration of the vine at all temperature regimes. Vine K and Mg were not significantly influenced by P at all temperature regimes except that both rates of P decreased Mg concentration at 21/13/10°. Ca was decreased by the higher rate of P at all temperature regimes.

In the pod and the pea seed, K, Ca and Mg concentrations were not influenced by P at all temperature regimes. N concentration of the pod decreased with applied P except at 21/13/18°. N concentration of the pea seed was not influenced by P at all temperature regimes. P concentration of the pod and the pea seed increased with applied P.

Total Mineral Contents. Applied P generally increased the total upake of N in all 4 tissues at all 4 temperature regimes (Table 11). P content of all tissues increased with applied P at all temperature regimes. K, Ca and Mg also generally increased with P application in all tissues.

3.4 Mineral Translocation Patterns

In addition to its effect on the total absorption of minerals, P also influenced the distribution patterns in the plant tissues. Mineral contents of the pea seed in relation to the contents of the entire plant varied with applied P. Pea seed content as percent of total plant content was employed as the indicator of translocation (Table 12). For example, while the amount of N translocated into the pea seed was 22% of the amount contained in the entire plant without applied P at 21/13/10°, applied P at the 44 lb./A rate increased the value to 41%. The distribution patterns of K and Mg were similar to those of N. Ca content of the pea seed was too low to permit definite interpretation of phosphorus and temperature effects. Increases in the translocation of minerals into the pea seed, due to applied P, were usually greater at the low soil temperature of 10° than at the high soil temperature of 18°.

3.5 Comparative Growth, Plastid Pigment and Mineral Responses of the Plant to Foliar Applications of Cycocel, Phosfon and B-Nine at Low Concentrations

The effects of the 3 growth retarding chemicals on growth characteristics, pigment contents, and mineral uptake

Table 12. Absorption and Translocation of N, P, K, Ca and Mg as Influenced by Phosphorus nutrition at 4 Air and Soil Temperature Regimes.

Temperature Regime Day/Night/Soil °C	Lbs. P per acre	Total uptake, mg/plant					Pea seed content as % plant content				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg
21/13/10	0	36	1.3	25	35	11	22	38	10	0.9	4
	44	91	7.7	71	87	24	41	56	22	1.3	11
	176	109	12.4	82	74	27	48	54	26	1.9	15
21/13/18	0	45	2.6	26	33	10	33	50	10	1.2	11
	44	153	10.9	102	113	32	47	54	27	2.3	16
	176	176	16.6	109	94	33	38	46	22	2.4	14
30/21/10	0	24	0.8	19	21	9	15	25	7	1.0	4
	44	68	5.2	49	45	18	39	54	27	2.7	15
	176	73	7.3	55	38	19	44	45	24	2.9	13
30/21/18	0	27	1.2	17	19	6	34	42	19	1.6	11
	44	87	6.0	54	53	19	50	62	29	3.0	17
	176	92	11.0	61	45	19	50	61	28	4.2	19

depended on the retarding chemical and its concentration (Tables 13, 14, and 15).

CYCOCEL at 1 ppm increased plant height (Fig. 10), internode length, total dry matter and pea yield but did not affect number of peas (Table 13). Chlorophyll a and the chlorophyll a:b ratio were significantly reduced while chlorophyll b was not influenced (Table 14). N, P and Mg concentrations were higher than in control plants (Table 15). Cycocel at 100 ppm caused a reduction in plant height, internode length and total dry matter production but did not affect pea yield. The reduction in total dry weight was largely a result of a decrease in vine weight. P, Mg and chlorophyll b concentrations were increased and K decreased by this treatment.

PHOSFON at 1 ppm increased internode length and the concentration of P but decreased K concentration. 100 ppm Phosfon caused a marked reduction in plant height, internode length, vine weight, pea weight and total dry matter, but increased the concentrations of chlorophylls a and b, and P and Ca.

B-NINE at 1 ppm did not significantly affect growth and yield or plastid pigment content. N, P and K concentrations were reduced and Mg increased. B-Nine at 100 ppm increased mean internode length as well as N and P contents. K was again less than in control plants.

The total number of peas, carotenoid concentration and chlorophyll:carotenoid ratio were not significantly influenced by any of the treatments (Tables 13, 14 and 15). The relative magnitude of treatment effects on mineral composition depended on

Table 13. Effects of Cycocel, Phosfon and B-Nine on growth and yield factors in peas.

	Plant height (cm)	Mean inter-node length (cm)	Vine dry weight (g)	Total number peas (no)	Pea fresh weight (g)	Pea dry weight (g)	Total dry matter (g)
Control	69.3b*	3.63b	4.57ab	28.7a	15.38b	3.27b	10.30b
Cycocel, 1 ppm	75.5a	4.15a	4.86a	27.8a	20.46a	4.45a	11.94a
Cycocel, 100 ppm	62.8c	3.33c	3.96b	24.8a	13.77b	2.97b	9.17c
Phosfon, 1 ppm	73.6ab	3.90a	4.49a	27.7a	16.86b	3.38b	10.17b
Phosfon, 100 ppm	38.3d	2.26d	2.94c	21.0a	9.75c	1.95c	6.66d
B-Nine, 1 ppm	69.96b	3.70b	4.16ab	26.5a	14.25b	3.15b	9.50b
B-Nine, 100 ppm	71.3ab	3.93a	3.97b	28.3a	14.71b	3.43b	9.53b

* Each figure is the mean of 12 plants. Figures followed by the same letter within a particular measurement are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 14. Effects of Cycocel, Phosfon and B-Nine on plastid pigment contents of pea plants.

	mg/g fresh leaf				Ratio	Ratio
	Chloro. a	Chloro. b	Total Chloro.	Carote-noids	Chloro. a:b	Chloro./ carot.
Control	1.90b*	0.78b	2.68b	0.28a	2.44a	9.6a
Cycocel, 1 ppm	1.67c	0.73b	2.40c	0.24a	2.29b	10.0a
Cycocel, 100 ppm	2.00b	0.90a	2.90ab	0.27a	2.22b	10.7a
Phosfon, 1 ppm	1.79b	0.74b	2.53b	0.27a	2.42a	9.4a
Phosfon, 100 ppm	2.14a	0.91a	3.05a	0.29a	2.35a	10.5a
B-Nine, 1 ppm	1.89b	0.78b	2.67b	0.28a	2.42a	9.5a
B-Nine, 100 ppm	1.88b	0.78b	2.66b	0.23a	2.42a	11.6a

* Each figure is the mean of 12 plants. Figures followed by the same letter within a particular measurement are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 15. Mineral composition and total contents in pea plants as influenced by two concentrations of Cycocel, Phosfon and B-Nine.

	Mineral composition (percent of dry matter)					Total mineral uptake (mg/plant vine portion)				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Control	3.80b*	0.08c	4.46a	2.94b	0.76b	174a	3.7b	204a	134a	35ab
Cycocel, 1 ppm	4.05a	0.10c	4.22ab	2.95b	0.79a	197a	4.9ab	205a	143a	38a
Cycocel, 100 ppm	4.05a	0.13b	2.64c	3.07ab	0.80a	160b	5.1a	105c	122b	32b
Phosfon, 1 ppm	3.86b	0.13b	3.59b	2.99b	0.75b	173a	5.8a	161a	134a	34b
Phosfon, 100 ppm	3.89b	0.16a	4.35a	3.23a	0.74b	114c	4.7ab	128b	95c	22c
B-Nine, 1 ppm	3.56c	0.06d	4.07b	2.88b	0.80a	148b	2.5c	169a	120b	33b
B-Nine, 100 ppm	3.95a	0.11c	3.99b	2.90b	0.78ab	157b	4.4ab	158a	115b	31b

* Each figure is the mean of 12 plants. Figures followed by the same letter within a particular element are not significantly different at P = 0.05 according to Duncan's multiple range test.

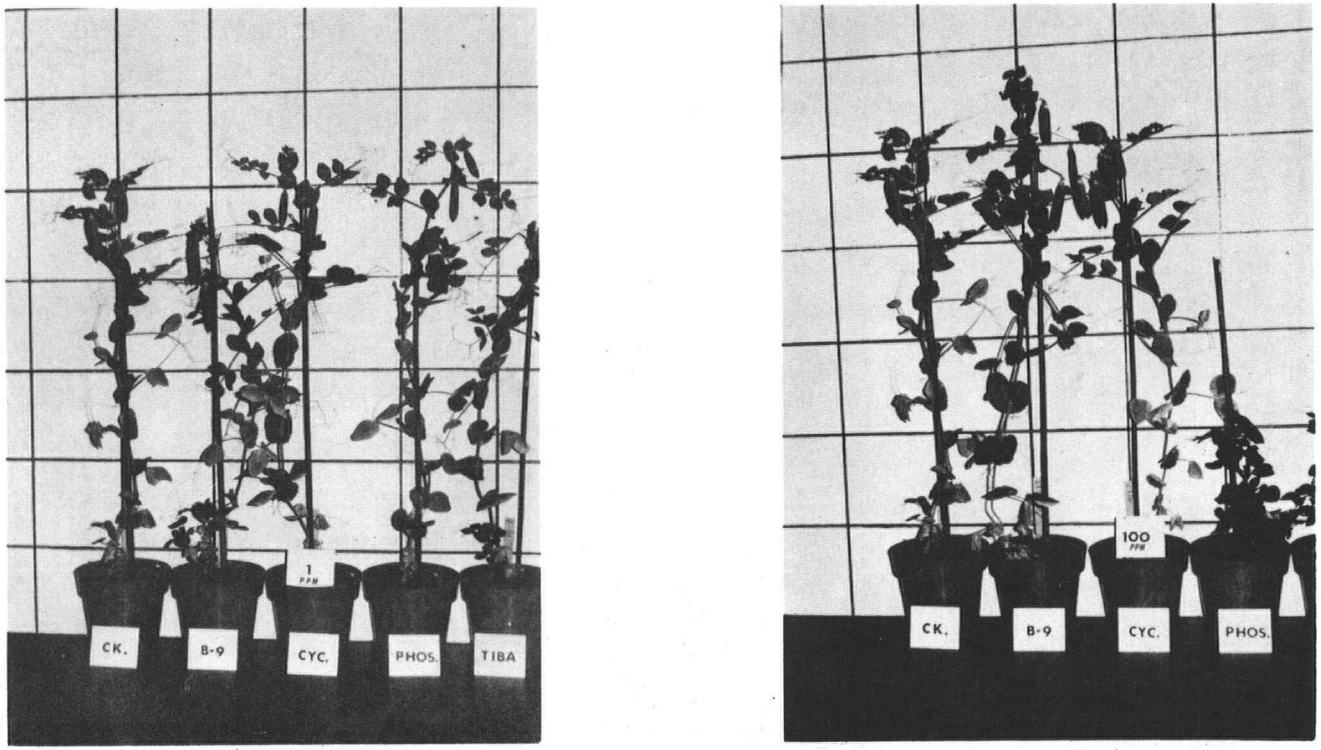


Fig. 10. Effects of B-Nine, Cycocel and Phosfon at 1 and 100 ppm on growth of the plant.

whether they were expressed on a percent of dry matter basis or expressed on a total uptake per plant (vine portion) basis (Table 15).

3.6 Effects of Temperature and Applied Phosphorus on Glucose, Hexose phosphates and Adenosine phosphates in Pea Tissues

Glucose and hexose phosphates had absorbance maxima at about 325 mμ, and adenosine phosphates at about 260 mμ (Fig. 11). ADP and ATP did not give peaks as sharp as those of glucose and hexose phosphates.

In 5-day old pea radicles, an increase in temperature from 20 to 25° led to a marked increase in the level of glucose, had no effect on G-1-P and G-6-P, but decreased F-6-P, F-D-P, ADP and ATP levels (Table 16). With an increase to 30°, glucose remained high while the levels of hexose phosphates increased, and those of ADP and ATP were virtually unchanged. Radicle weight increased at 25° but there was no significant difference between radicles at 20 and 30°.

In leaves and roots of 30-day old plants, there were significant interactions between temperature and P for the phosphorylated compounds except G-6-P in the leaf and ATP in the root. The simple effects of P at each temperature regime were therefore determined.

Applied P at 44 lb./A increased leaf weight at all temperature regimes, and increased root weight only at the cool

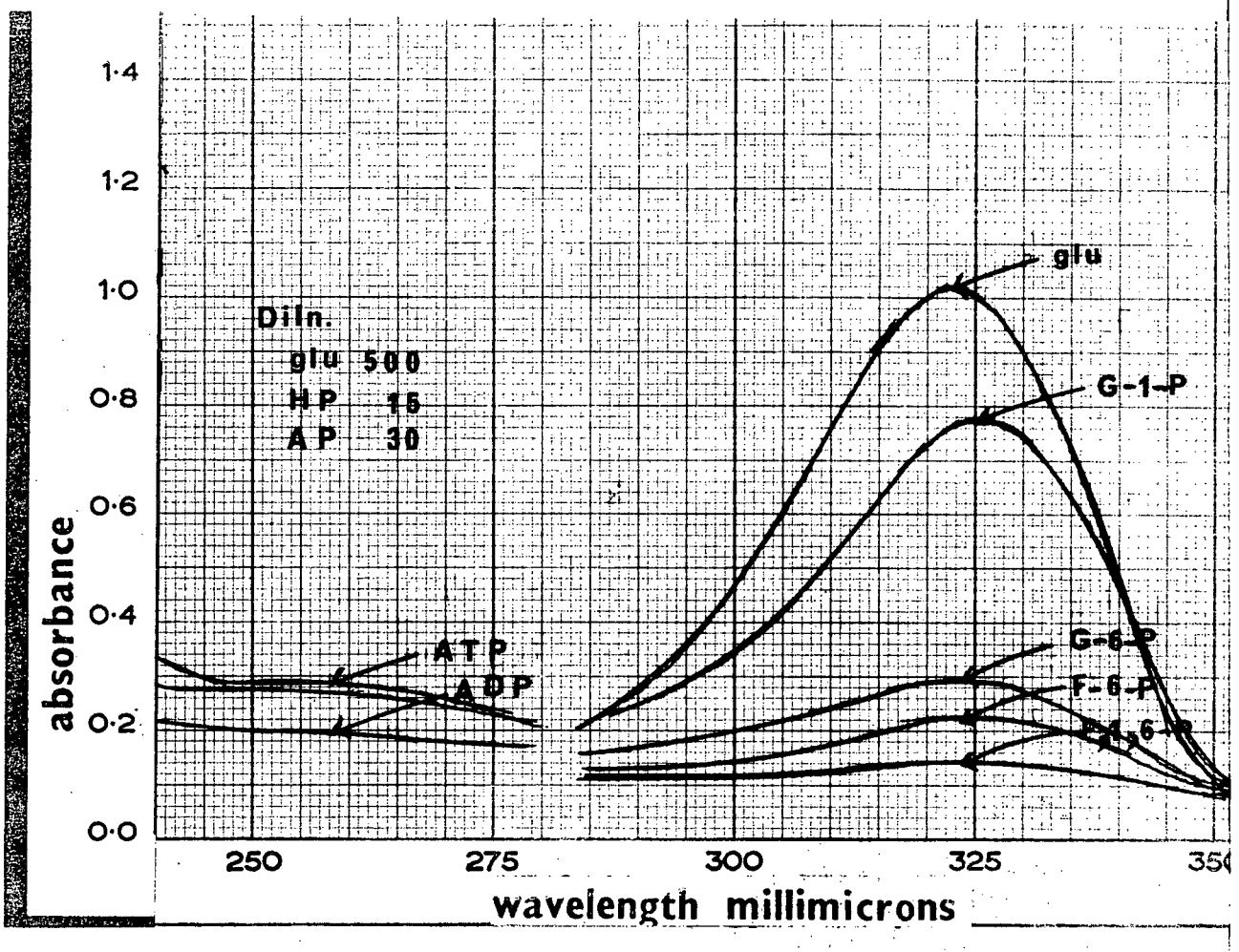


Fig. 11. Typical absorption spectra of glucose (glu), hexose phosphates (HP) and adenosine phosphates (AP) of 5-day old pea radicles grown at 25°. 1 g of fresh tissue was used.

Table 16. Effect of temperature on Glucose, Hexose phosphates, ADP and ATP levels of 65-day old pea radicles. Fresh weight (F.W.) in g; compounds in uM/g. F.W.

Temp. °C	F.W.	Glu.	G-1-P	G-6-P	F-6-P	F-D-P	ADP	ATP
20	1.86b*	76b	.54b	.19b	.15b	.23b	.21a	.17a
25	2.21a	150a	.46b	.20b	.10c	.12c	.17b	.13b
30	1.79b	149a	1.64a	.39a	.18a	.40a	.15c	.13b

* Each figure is the mean of 3 replicates. Figures followed by the same letter within each compound are not significantly different at P = 0.05 according to Duncan's multiple range test.

day/night air temperature of 21/13° (Table 17). P significantly decreased leaf glucose only at 30/21/18°. Leaf G-1-P decreased with applied P at the cool air temperature but not at the warm air temperature. P decreased G-6-P in the leaf at all temperature regimes. F-6-P was increased by P at all temperature regimes except 21/13/18°. F-D-P in the leaf decreased with applied P at 21/13/18°. At 21/13/10° there was a decrease in leaf ADP and an increase in ATP, due to applied P. At 21/13/18°, however, P had no significant effect on ADP but decreased ATP.

In the root, applied P significantly decreased the levels of G-1-P, G-6-P, F-6-P and ATP at 21/13/18°. At 30/21/18°, P increased glucose, G-1-P, G-6-P, F-D-P and ADP but decreased F-6-P.

3.7 Effects of Cycocel and Phosfon on Glucose, Hexose phosphates and Adenosine phosphates in 5-day old Pea Radicles

Cycocel at 1 ppm increased the levels of G-1-P and G-6-P (Table 18). F-6-P tended to increase with increasing concentration of Cycocel. F-D-P decreased at 1 ppm, was not influenced at 10 and 100 ppm, and increased at 1000 ppm. Cycocel at all concentrations decreased ADP. ATP decreased at 1 and 10 ppm, was not significantly affected at 100 ppm, but increased at 1000 ppm. Only 1000 ppm Cycocel decreased radicle weight.

Phosfon at up to 100 ppm increased glucose. At 1 and 10 ppm, Phosfon decreased G-6-P, F-D-P, ADP and ATP, but increased F-6-P. At 1000 ppm, Phosfon markedly decreased glucose

Table 17. Tissue levels of Glucose, Hexose phosphates, ADP and ATP as influenced by air and soil temperatures and applied phosphorus. Levels of compounds in μM/g fresh weight.

Temperature Regime Day/Night/Soil °C	Lbs. P per acre	Fresh wt. g	Glu.	G-1-P	G-6-P	F-6-P	F-D-P	ADP	ATP
LEAF									
21/13/10	0	5.1b*	47a	1.70a	.51a	.26b	.82a	.31a	.11b
	44	11.5a	52a	.53b	.33b	.46a	.70a	.24b	.14a
21/13/18	0	5.5b	58a	.71a	.43a	.26a	.66a	.27a	.14a
	44	17.5a	53a	.56b	.32b	.28a	.47b	.30a	.10b
30/21/10	0	4.9b	51a	.76a	.55a	.38b	.40b	.33a	.13a
	44	9.4a	54a	.65a	.33b	.48a	.53a	.32a	.14a
30/21/18	0	4.4b	120a	.71a	.52a	.32b	.44b	.31a	.14a
	44	9.3a	45b	.60a	.33b	.51a	.55a	.26a	.14a
ROOT									
21/13/10	0	11.9b	36a	.53a	.33a	.32a	.38b	.25a	.10a
	44	16.8a	21b	.46a	.26b	.32a	.43a	.22a	.10a
21/13/18	0	13.5b	17a	.47a	.43a	.42a	.50a	.20a	.08a
	44	17.6a	14a	.30b	.25b	.30b	.48a	.17a	.06b
30/21/10	0	11.4a	13a	.19b	.28a	.48a	.40a	.16a	.08a
	44	12.5a	11a	.32a	.31a	.32b	.37a	.17a	.09a
30/21/18	0	10.5a	11b	.47b	.37b	.50a	.43b	.15b	.09a
	44	10.6a	16a	.60a	.50a	.40b	.57a	.22a	.10a

* Each figure is the mean of 3 replicates. Means followed by the same letter within a tissue, temperature regime and compound are not significantly different at P = 0.05.

Table 18. Effects of Cycocel and Phosfon on the levels of Glucose, Hexose phosphates, ADP and ATP in 5-day old pea radicles. Fresh weight (F.W.) in g.; compounds in μM/g F.W.

Retardant	ppm	F.W.	Glu.	G-1-P	G-6-P	F-6-P	F-D-P	ADP	ATP
Cycocel	0	1.93a*	60d	.53b	.21b	.09c	.09b	.19a	.12b
	1	2.04a	70d	.93a	.49a	.09c	.07c	.15b	.10c
	10	1.98a	97c	.65b	.24b	.12b	.09b	.12b	.10c
	100	1.86a	123a	.55b	.23b	.31a	.10b	.13b	.13b
	1000	1.19b	110b	.45c	.23b	.29a	.15a	.13b	.18a
Phosfon	0	1.74a	71b	.51b	.22b	.11c	.12a	.21a	.13c
	1	1.61a	122a	.50b	.16c	.19a	.09b	.15b	.11b
	10	1.62a	118a	.43b	.14c	.14b	.05c	.12c	.10b
	100	1.22b	119a	.35c	.15c	.15b	.05c	.13c	.11b
	1000	0.48c	47c	1.31a	.35a	.15b	.05c	.16b	.20a

* Each figure is the mean of 3 replicates. Figures followed by the same letter within a growth retardant and variable are not significantly different at P = 0.05 according to Duncan's multiple range test.

but increased the hexose monophosphates and ATP. Radicle weight decreased at 100 and 1000 ppm Phosfon.

4. DISCUSSION

4.1 Growth Responses to Phosphorus Nutrition

Increased pea yields due to applied P were accompanied by relative decreases in vine weights, with no relative changes in pod weights. Increases in yield thus appeared to have occurred through the use of photosynthate that would otherwise have remained in leaves and/or stem when P was not added to a P-deficient soil. This may be interpreted as an enhanced translocation of photosynthate from the leaves, through the pod to the pea seed, since the phosphorylation of sugars has been implicated in translocation (Canny, 1962).

There was a deleterious effect of warm air temperature on growth, which increased with age of plants. Beyond the 10th node stage the optimum air temperature for pea plant growth was indicated to be lower than for preceding stage(s) of growth. This is consistent with the results of Wang and Bryson (1956), Went (1957) and Stanfield et al. (1966).

An increase in soil temperature from 10 to 18° generally increased plant weight at the 3 pre-fruiting growth stages. These observations are in general agreement with those of Apple and Butts (1953) for pole beans, Ketcheson (1957) and Mederski and Jones (1963) for corn, Lingle and Davis (1959) for tomato, Singh and Mack (1966) for beans, and Mack et al. (1964) for peas. However, the response to soil temperature was found in the present studies to also depend on air temperature. For example, although the high soil temperature of 18° increased

growth compared with the low soil temperature of 10°, the increases were greater at the cool day/night air temperature of 21/13° than at the warm air temperature of 30/21°.

Applied P did not offset the growth-limiting effects of warm air temperature or low soil temperature up till about 40 days after planting (full bloom stage). Growth promotion by applied P was always greater at the high soil temperature, particularly when the air temperature was warm. This differs from the results of Mack et al. (1964) for peas, and Apple and Butts (1953) for pole beans. Mack et al. (1964) found that the dry weight response of pea plants to P fertilizer at 70 lb./A was similar at the soil temperatures tested (13 to 25°). Failure to detect differences in the magnitude of growth increases by P at different soil temperatures as reported by Mack et al. (1964) may have resulted from the native P content of the experimental soil. They used a soil containing 15 to 25 ppm P while the soil used in the present experiments contained only 4 ppm P. The high soil P might have led to decreased response to P fertilizer. Apple and Butts (1953), however, stated that other factors not under their control such as light intensity might have affected their results. The basis of differences in these results and those of the present studies might therefore be due to several factors. The bean plant may be more sensitive to low soil temperature so that P would offset some of the deleterious effects of cold soil. Increased dry matter due to P is consistent with the role of P in protein synthesis and growth by cell wall extension (Marre, 1961).

At crop maturity the effects of air and soil temperatures and applied P on plant growth varied with the four tissues (root, vine, pod and pea seed). Applied P contributed mainly to the promotion of pea seed yield at all temperature regimes. The magnitude of such increases in pea yield however depended on both air and soil temperatures. The greatest increase due to P at the 44 lb./A rate was at the warm air and low soil temperature regime of 30/21/10°. Increases in yield due to P at the other three temperature regimes were practically of the same magnitude. The air temperature of 30/21° was supra-optimal and the soil temperature of 10° was sub-optimal for pea plant growth. At 30/21/10° the great increase might thus have been due to a lack of adequate utilization of growth factors at other than optimal air and soil temperature combination. Such deleterious effects might thus be expected to be minimized by P because of its role in growth processes.

The greatest vine and pod weight increases due to P were at the cool air and high soil temperature regime of 21/13/18° which appeared to have been the nearest optimal regime of those tested. This suggests that under optimal or close to optimal conditions applied P contributed more to the vine and the pod than to pea seed because translocation of photosynthate to the seed was not limiting.

The greatest increase in any tissue weight due to applied P was in pea seed at 30/21/10°. This was accompanied by the smallest increase in root weight. This indicates that there probably was a reduced translocation of photosynthate to the root due to a diversion to the pea seed at this temperature regime.

4.2 Mineral Uptake Responses to Phosphorus Nutrition

In greenhouse-grown plants, applied P led to absolute increases in N and P in the vine and the pod. There was thus a synergistic effect of P on N, similar to that reported for Japanese mint (Singh and Singh, 1968). The vine, and to a lesser degree the pod, reflected the P status of the plant better than did the pea seed, in accordance with observations made by Ulrich and Berry (1961) that different parts of a plant will reflect the P status of the plant to different degrees. P had no effect on K, Ca and Mg concentrations but increased their total contents. The application of P thus led to a multiple effect on the other plant minerals, such as was described by Smith and Reuther (1951). Relatively constant concentrations of K, Ca and Mg apparently were due to a dilution effect resulting from larger plants since the accumulation of dry weight dilutes the minerals (Smith, 1962).

Consideration of age effects are best confined to between seedling and bloom stages because subsequent pod and pea development would cause major redistribution of nutrients. Vine contents at crop maturity are thus not meaningfully comparable to vine contents at the purely vegetative growth stages or at the inception of reproductive development.

N, P and K concentrations in the pea plant generally decreased with physiological age, particularly after the 10th node stage. Ca and Mg tended to increase with plant age. These results generally agree with those of Bradley and Fleming (1960)

for vegetables (cucumbers, tomatoes and watermelons). The results are in partial agreement with those of MacLean and Byers (1968) who studied the mineral composition of 3 cultivars of peas, including Dark Skin Perfection, in field experiments. They found that P, Ca and Mg remained relatively constant with aging. This might have been due to the fact that sampling was done at 2-node intervals and stopped at the 12th node stage. Their results for this cultivar at the 6th and 10th node stages agree with the results of the present studies.

There were marked increases in the total contents of all five minerals as a result of P fertilization at all temperature regimes. Increased mineral uptake due to applied P is consistent with suggestions that phosphorylated compounds may be involved in the processes of ion uptake and transport (Marre, 1961; Sutcliffe, 1962).

The effect of applied P in increasing the percent of P in the plant was greater at the high soil temperature, in contrast to the reports for pole beans (Apple and Butts, 1953), tomato (Locascio and Warren, 1960), and corn (Ketcheson, 1957), that percent P was increased more at the lower soil temperatures.

At crop maturity N and P concentrations were highest in the pea seed, Ca in the vine, and K and Mg in the root. This indicates that N and P are mobile, although P has been found to circulate up and down in the pea plant (Biddulph, 1958). That K and Mg are immobile was indicated by the finding that they were more concentrated in the root than in any other tissue.

High root concentrations were also found by Bowling and Weatherley (1964) for K in Ricinus communis, and for Mg in apple (Rogers et al., 1953). Ca appears to be marginal but could be regarded as being mobile in the pea plant.

The high soil temperature increased N and P concentrations in all tissues except the pea seed. K was decreased in the root but was not influenced in other tissues. The high soil temperature also decreased the total contents of other minerals except P in the root. This shows that at the high soil temperature more of each mineral was transported out of the root into shoot tissues. Shtrausberg (1955) similarly found that cold roots diminished the movement of minerals to cucumber shoots. The warm air temperature, compared with the cool air temperature, reduced the magnitude of enhanced mineral transport by the high soil temperature.

Applied P increased the total contents of each of the five minerals, particularly in the vine. Increases in the mineral content of the shoot tissues were accompanied by their decreases in the root. For example N translocated into the pea seed was 22% of that in the entire plant at 21/13/10°. This value was increased to 41% with the application of P at 44 lb./A, which represents almost a 100% increase.

Analyses of plants at crop maturity indicated that the effects of temperature on the absorption of P were greater than those on its translocation. In control plants increases in P absorption due to the high soil temperature were 50 and 150% at the cool and warm air temperatures respectively while

increases in translocation were small and not significant. At the nearest optimal P rate of 44 lb./A high soil temperature increased P absorption more at the cool than at the warm air temperature. There was no soil temperature effect on translocation at the nearest optimal P level because P is normally mobile, and translocation is mainly increased under stress of deficiency.

Increased mineral uptake at the high soil temperature in the present experiments was not due to a larger root system. Apple and Butts (1953) and Power et al. (1964) attributed the increase of P in pole beans and barley respectively as a result of high soil temperature, to greater root weights. In the present experiments, the high soil temperature tended, in fact, to decrease root weight. Increased mineral uptake would therefore be due to increased mineral-absorbing capacity of the root, which may be a function of metabolism or anatomical features or both. It was shown to be related to increased utilization of ATP. This increased capacity of the root for mineral absorption was also accompanied by an efficient utilization of P for growth and yield.

Efficiency of fertilizer P in promoting actual pea yield was greatest at the cool air temperature but at high soil temperature (21/13/18°) where pea yield per unit (lb.) of applied P was 40 mg/plant at the 44 lb./A rate. At the low soil temperature (21/13/10°) it was only 21 mg/plant. Also if efficiency is expressed as lb. P required to give 50% of maximum yield, the conclusion is the same since 18 lb./A would be required at the high soil temperature while 29 lb./A would be required at the low soil temperature.

Increased uptake of minerals resulting from P application is consistent with the role of P in the active uptake of solutes (Laties, 1959). For prediction of ultimate pea yield, the following critical levels of minerals in the leaves of plants at the 10th node stage were established: N, 4.0; P, 0.25; K, 2.8; Ca, 2.8; Mg, 0.8 percent of dry matter. These are in general agreement with those tentatively established by MacLean and Byers (1968) for N, P and K in pea leaves of plants at the 5th to 8th node stages.

4.3 Comparative Growth, Plastid Pigment and Mineral Responses of the Plant to Foliar Applications of Cycocel, Phosfon and B-Nine at Low Concentrations

Cycocel at 1 ppm was found to stimulate growth and to increase yield, while it decreased chlorophyll concentration as earlier reported by Adedipe et al. (1968). Furthermore, Cycocel increased the levels of N, P and Mg but decreased K, which agrees with Knavel's (1968) results, except that Ca was not affected in the pea plant as it was in the tomato.

While relatively high concentrations of B-Nine have been reported to retard the growth of fruit trees (Batjer et al., 1964; Jaffe and Isenberg, 1965), to increase the amount of bloom the following spring (Batjer et al., 1964), and to reduce shoot length of pea plants by about 40% (Sprent, 1967), data obtained in the present studies show that the low concentrations of 1 and 100 ppm were ineffective in altering the growth pattern of pea plants. Concentrations of N and P were

found to be increased and K decreased by 100 ppm of B-Nine in agreement with Knavel's (1968) results for tomatoes but in contrast to the results of Southwick et al. (1968) for apples.

The influence of the retardants on mineral content depended on the unit of expression of nutrient amounts. For example, at 100 ppm Cycocel increased the concentration of N by about 6% when the level was expressed as percent of dry matter. However, when the level was expressed as total mineral in the vine there was a decrease of about 8%. The 6% increase was thus due to a "concentration effect" resulting from smaller plants. Similarly, Phosfon at 100 ppm increased P by 100% when it was expressed as percent of dry matter, but had much less effect on total P content of the vine. The use of both units of expression thus gives a better indication of net uptake while it also explains, if only partially, differences in mineral content of treated plants as compared to untreated plants.

On the bases of pronounced reduction in plant height and marked increase in chlorophyll concentration, Phosfon appears to be the most effective growth retardant, thus agreeing with the reports of Cathey and Stuart (1961), Majumder (1968) and Moore (1967). At 1 ppm however, Cycocel stimulated growth, increased yield and the levels of N, P and Mg while Phosfon at the same concentration did not have a significant effect on yield and increased the level of P only. Since 100 ppm Phosfon was perhaps too drastic, and 1 ppm had little effect, it may well be that growth stimulation may occur at less than 1 ppm or between 1 and 100 ppm.

It is concluded that B-Nine at a concentration of up to 100 ppm is ineffective in stimulating pea plant growth. Cycocel at 1 ppm is the most effective in terms of growth and yield stimulation. Phosfon at 100 ppm is the most effective in terms of growth retardation but decreases yield. Phosfon at concentrations lower than 100 ppm, perhaps less than 1 ppm, may stimulate pea growth and may be, like Cycocel, promising for use in increasing pea yield without deformative effects. This possibility should be investigated.

4.4 Phosphorus Metabolism as Influenced by Temperature, Phosphorus and Growth Retarding Chemicals

The levels of glucose, hexose phosphates, ADP and ATP in pea tissues varied with temperature, applied P and the 2 growth retarding chemicals (Cycocel and Phosfon). Glucose and glucose phosphates as well as ADP and ATP were usually higher in the leaf than in the root of 30-day old plants. Fructose phosphates were of the same magnitude in both the leaf and the root. For example, at the air and soil temperature regime that is nearest-optimum (21/13/18°), the leaf/root contents, in μM/g fresh weight were: glucose, 53/14; G-1-P, .56/.30; G-6-P, .32/.35; F-6-P, .28/.30; F-D-P, .47/.48; ADP, .27/.17; and ATP, .10/.06. These levels are within the ranges given by Marre (1961) for higher plants in general.

Hexose phosphates in 5-day old radicles were usually lower than in the leaf or root of 30-day old plants, depending on temperature and applied P level. Glucose content was higher

in the radicle than in the leaf or root, while ADP and ATP were about the same. Low concentrations of hexose phosphates in the radicles may be due to a slower rate of incorporation of phytin products into these compounds. This interpretation is supported by the work of Loughman and Russell (1957) which showed that the incorporation of absorbed P in barley seedlings into hexose phosphates was slower than into nucleotides. The higher levels of phosphorylated compounds in the leaf is understandably attributable to the additional contribution resulting from photosynthetic phosphorylation (Whatley and Losada, 1964).

At 25 and 30°, pea radicles contained twice as much glucose as at 20°. This could be due to increased activity of phosphorylase in the breakdown of seed starch. At 25°, however, the levels of hexose phosphates decreased sharply compared with those at 20 and 30°. The accumulation of these compounds at 30° implies either increased respiratory activity or an accumulation of glycolytic substrates due to reduced utilization. On the other hand, decreases in these compounds at 25° implies either a general decrease in respiratory activity or a rapid utilization of these glycolytic substrates and products along the cycle. ATP decreased by 22% at 25 and 30° compared to 20°, probably showing an increased utilization of ATP for other aspects of cell metabolism and growth. Decreased levels of hexose phosphates and ATP were accompanied by a 19% increase in radicle fresh weight at 25°. This shows that glycolytic activity might have been high, thus leading to a

rapid turnover of substrates and products in the glycolytic sequence. This is consistent with the observations of Hatch and Turner (1959) and Mossberry et al. (1964) that in higher plants, ATP controls glycolytic and oxidative metabolism.

In the radicles, the greatest change was in F-D-P, with the temperature of 30° leading to a 74% increase, while 25° led to a 48% decrease. It would therefore seem that the control of glycolysis is dependent on the level of this substrate as well as that of ATP, as suggested by Marre (1961).

In 30-day old plants, the changes in phosphorylated compounds brought about by air and soil temperatures were interdependent. There was a decreased utilization of ATP in the phosphorylation of glucose, due to the warm air temperature of 30/21° compared with 21/13°. The availability of glucose was not the limiting factor because it was twice as high. On the other hand there was an increased utilization of ATP at the high soil temperature of 18° than at the low soil temperature o of 10°, particularly at the cool air temperature. This pattern of changes was also the case in the root. This therefore suggests that increased mineral uptake (as earlier reported) may not be directly related to carbohydrate contents per se (Humphries, 1956) but to other compounds involving their utilization or interconversions, such as the phosphorylated sugars. The high soil temperature increased growth probably because sugars were rapidly and continuously phosphorylated in kinase reactions, and ATP was also utilized in other plant

growth processes (Marre, 1961). The reverse was probably the case with the warm air temperature which was earlier shown to decrease total contents of minerals in the pea plant tissues. Increased mineral uptake at the high soil temperature may therefore have resulted from energy derived from ATP as suggested by Sutcliffe (1962).

Applied P generally led to decreases in hexose phosphates except F-6-P. The greatest of such decreases were at the cool air and high soil temperature (21/13/18°) which has been shown to be the nearest optimal regime for plant growth, leaf growth and pea yield. At this temperature regime, applied P led to a decrease in ATP. ATP was thus being utilized more at this temperature regime than at any other. This may explain the large increase in plant growth at this temperature regime, and may be related to the suggestion by Power et al. (1964) that it is possible for low soil temperature to limit the utilization of certain metabolites. This limitation is greater at the warm air temperature of 30/21° than at 21/13°.

The stimulation of pea stem growth by adenine at high temperature (Galston and Hand, 1949) may therefore represent a lack of utilization of adenine for phosphoroclastic reactions involving ATP rather than an absolute deficiency of adenine at high temperature. The positive growth response of peas to sucrose at 23° (Ketellaper and Bonner, 1961) might have resulted from increased translocation of sugars to the root, which in the present experiments was shown to be low in glucose at high temperature.

Potts and Ormrod (1969) found no changes in the levels of organic, lipid and total phosphates when pea plants were abruptly transferred from 25/15 to 35/25° day/nigh temperatures and held for up to 6 days at the higher temperature. They suggested that their results did not preclude the possibility that the concentration of compounds within the organic phosphorus fraction did vary. This was in fact the case in the present experiments. Changes in phosphorylated compounds were not large enough in the Potts and Ormrod study to be detected by gross fractionation techniques. Also, the pretreatment of the plants at 25/15° in their work may have resulted in an equilibration of the fractions, or that more than 6 days were required to cause changes after such a relatively favourable pretreatment. The effect of temperature in influencing phosphorus metabolism appears to be controlled by glycolytic kinases or phosphoglucoisomerase or aldolase or all, depending on the type and age of tissue.

In interpreting the results of studies of the effects of Cycocel and Phosfon on glucose and phosphorylated compounds the cross-over theorem of Chance et al. (1958) may be used for locating the control site(s). In this technique it is necessary to compare the compounds that show reciprocal trends, that is, the compound(s) which decreased as the other(s) increased. At 1 ppm Cycocel, G-6-P increased while F-D-P and ATP decreased (Fig. 12). This indicates that at this concentration of Cycocel, there was an increased utilization of ATP for the phosphorylation of sugars (hexoses). Hexokinase activity might thus have been

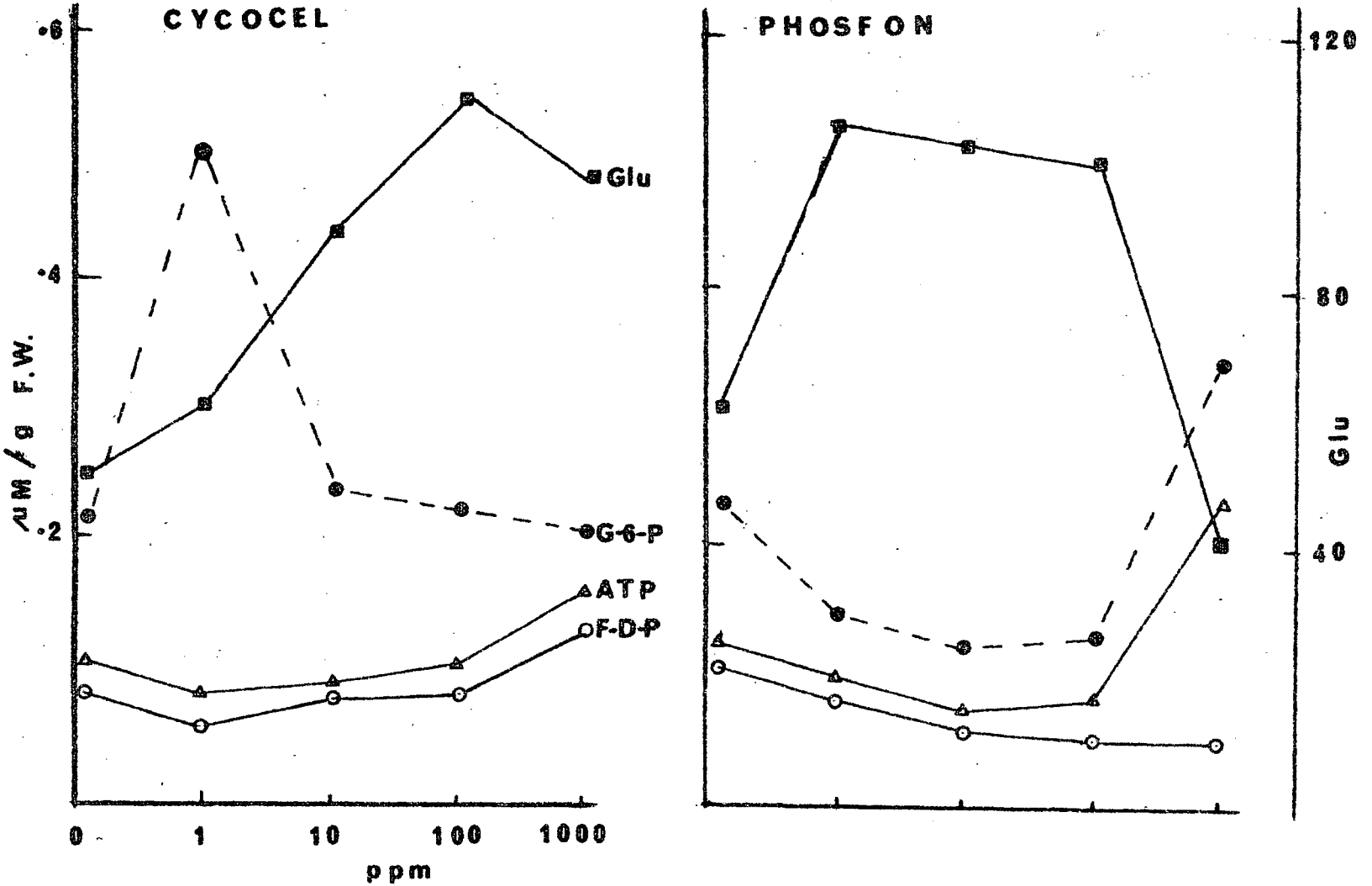


Fig. 12. Comparative Rate Effects of Cycocel and Phosfon on P utilization patterns of 5-day old pea radicles grown at 25°.

stimulated. A lack of accumulation of F-D-P might have been due to a possible rapid utilization of F-D-P by aldolase to form triosephosphates, but this possibility was not investigated.

Phosfon at concentrations of up to 100 ppm, on the other hand, decreased G-6-P and F-D-P, while at 1000 ppm it increased ATP. The general decreases in hexose phosphates was probably due to a lack of phosphorylation of sugars by ATP. The supply of glucose was however not the limiting factor. While Cycocel at 1 ppm may have stimulated hexokinase, Phosfon at the same concentration may have inhibited it, as reflected by the level of G-6-P. There is thus a differential concentration effect of these two growth retarding chemicals. This response is similar to growth response earlier reported in the present studies in which Cycocel at 1 ppm was found to stimulate growth while Phosfon at the same concentration had no significant effect on growth.

These responses support Cleland's (1965) view that the inhibitory effects of growth retarding chemicals are not necessarily limited to the hormonal level. Tanaka and Tolbert (1966) found that Cycocel stimualted choline kinase activity in spinach and pea leaves, but it is not known if other growth retarding chemicals, for example Phosfon and B-Nine, will also influence this kinase.

Tuli et al. (1964) showed N^6-benzyladenine to be an inhibitor of respiratory kinases. Ormrod and Williams (1960) found that 2,4-D and gibberellic acid caused a striking increase in acid-soluble organic phosphorus as quickly as 1 minute

after the treatment of Trifolium hirtum. It is clear that growth retarding chemicals may influence the endogenous levels of auxins (Kuraishi and Muir, 1963; Halevy, 1963; Reed et al., 1965), or gibberellins (Hinneman et al., 1964; Badlev et al., 1965; Tanaka and Tolbert, 1966), but they may also influence neither (Cleland, 1965). Auxins and gibberellins on the other hand depend on products of primary metabolites for thier biosynthesis. Brook et al. (1967) suggested that alterations in nucleic acid metabolism by Phosfon could in turn alter a wide variety of metabolic processes resulting in retarded growth. Alteration in glycolytic metabolism reported in the present experiments may be one of such processes. The more primary effect of growth retarding chemicals may in fact lie in changes in the levels of basic metabolites, such as glycolytic intermediates brought about by effects on glycolytic kinases, or indeed of other enzymes. This means that growth retarding chemicals should be considered more as antimetabolites (Lockhart, 1962) rather than as antigibberellins.

Alteration in the levels of basic metabolites may in turn influence the biosynthesis of growth regulatory substances, which could in turn modify growth. Ultimate morphological expression may in fact be controlled by different aspects of plant metabolism in different species, and with varying concentrations of different growth retaridng chemicals.

The dwarfing effects of high air temperature on pea plant growth were similar to those of relatively high concentrations of growth retarding chemicals, particularly Phosfon. Also,

the high temperature of 30° led to similar effects on phosphorylated compounds, as those brought about by 1000 ppm Cycocel and Phosfon. It thus appears that one of the ways by which high air temperature as well as high concentrations of growth retarding chemicals limit plant growth, is the decreased utilization of ATP in kinase reactions and in growth processes such as protein synthesis and cell wall extension.

The effects of growth retarding chemicals on respiratory and other kinases, auxins and gibberellins need to be studied in the same set of experiments to throw light on which aspect of metabolism is the most primarily influenced.

For uniformity in the nomenclature of plant growth regulators it is suggested that growth retarding chemicals be called "RETARDINS" in line with other groups such as auxins, gibberellins, kinins and morphactins.

5. SUMMARY AND CONCLUSIONS

In greenhouse and controlled environment experiments, the influences of temperature, phosphorus nutrition and growth retarding chemicals on growth and mineral composition of the pea plant were investigated. The utilization of P under 4 air and soil temperature regimes within the physiological range was also studied. The dwarfing effect of high temperature was related to that due to relatively high concentrations of growth retarding chemicals. From the results of the experiments the following conclusions can be drawn.

(i) P fertilizer applied at rates of up to 352 lb./A increased plant growth and yield of peas. Yield increases resulted from decreases in vine relative to total dry matter, with no relative effects on pod weight. Efficiency of P in promoting pea yield was highest at the 44 lb./A rate.

(ii) N and P concentrations were highest in the pea seed, while K, Ca and Mg concentrations were highest in the vine. The total content of P, in milligrams per plant, was highest in the pea seed, while those of N, K, Ca and Mg were highest in the vine. P tended to increase the total contents of all 5 minerals in all 3 tissues (vine, pod and pea seed).

(iii) Between 6th node and full bloom stages (about 20 and 40 days of growth), the warm air temperature of 30/21° (day/night) depressed vine growth and mineral uptake, compared with the cool air temperature of 21/13°. Depressions of growth and mineral uptake by the warm air temperature were greater at the high soil temperature of 18° than at 10°.

(iv) The high soil temperature increased vine weight and mineral uptake as compared to the low soil temperature. Increases in growth and mineral uptake were greater at the cool than at the warm air temperature.

(v) Increases in mineral concentrations at the warm air temperature were largely due to "concentration effects" resulting from smaller plants. Increases due to the high soil temperature were absolute because they occurred regardless of plant size.

(vi) The greatest increase in growth resulting from added P was at the cool air and high soil temperature regime of 21/13/18°. Increase in P rate beyond the 44 lb./A tended to decrease growth compared with that at the 44 lb./A rate.

(vii) Applied P did not offset the growth-limiting effects of either warm air or low soil temperature at the 6th and 10th node stages. At full bloom and at estimated marketable crop maturity, however, P became more limiting, and its application offset the deleterious effects of warm air and cold soil.

(viii) Increase in mineral uptake at the high soil temperature was not due to increased root growth, but was a result of increased metabolic activity of the root leading to increased capacity for mineral absorption. The effect of soil temperature on total absorption of P was greater than on translocation into the pea seed.

(ix) Of the 3 growth retarding chemicals investigated, Cycocel at 1 ppm was the most effective in terms of growth and yield stimulation. Phosfon at 100 ppm was the most effective in terms of growth retardation, but markedly decreased yield. B-Nine at concentrations of up to 100 ppm was ineffective in altering plant growth pattern. Cycocel and Phosfon applied at low concentrations appear to be promising for use in increasing pea yield without deformative effects. Effects of the growth retardants on mineral uptake largely reflected plant size differences, and were not absolute increases or decreases.

(x) The effects of relatively high concentrations of Cycocel and Phosfon were similar to those of high air temperature with respect to dwarfing of plants and changes in the levels of glucose, hexose phosphates, ADP and ATP. It appears that one of the ways by which high concentrations of growth retarding chemicals as well as high temperature depress plant growth is the decreased utilization of ATP in kinase reactions and growth processes.

(xi) The greater mineral uptake and translocation at the high soil temperature was due not to increase in glucose per se, but to decrease in its phosphorylation by ATP, and its interconversions to other hexose phosphates.

(xii) The most satisfactory or nearest-optimum air and soil temperature regime for plant growth and mineral uptake was the 21/13/18° day/night/soil temperatures.

(xiii) In soils that are cold in early spring, P may be used to give the seedlings a vigorous "start" and to compensate for loss in yield that might have resulted if the summer became warm.

(xiv) For prediction of ultimate pea yield, the following critical levels of minerals in the leaves of plants at the 10th node stage were established: N, 4.0; P, 0.25; K, 2.8; Ca, 2.8; Mg, 0.8 percent of dry matter.

(xv) For uniformity in the nomenclature of plant growth regulators it is suggested that growth retarding chemicals be called "RETARDINS" in line with other groups such as auxins, gibberellins, kinins and morphactins.

BIBLIOGRAPHY

1. ADEDIPE, N.O., D.P. ORMROD and A.R. MAURER. 1968. Response of pea plants to soil and foliar applications of Cycocel ((2-chloroethyl) trimethylammonium chloride). Can. J. Plant Sci. 48: 323-325.

2. ALBAUM, E.G. 1952. The metabolism of phosphorylated compounds in plants. Ann. Rev. Plant Physiol. 3: 35-38.

3. ANDERSON, D.T. and G.C. RUSSELL. 1964. Effects of various quantities of straw mulch on the growth and yield of spring and winter wheat. Can. J. Soil Sci. 44: 109-118.

4. A.O.A.C. (ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS). 1965. Atomic absorption spectrophotometry: methods of analysis. 10th ed., 13, 16, 23-24.

5. APPLE, S.B. and J.S. BUTTS. 1953. Soil temperature studies. 1. The effect of soil temperature and phosphcrus on growth and phosphorus uptake by pole beans. Proc. Amer. Soc. Hort. Sci. 61: 325-332.

6. ARAMBARRI, P. and O. TALIBUDEEN. 1959. Factors influencing the isotopically exchangeable phosphate in soils. III. The effect of temperature in some calcareous soils. Plant and Soil 11: 364-376.

7. ARNON, D.I., M.B. ALLEN and F.R. WHATLEY. 1954. Photo-synthesis by isolated chloroplasts. Nature 174: 394-396.

8. BADLEV, B., A. LANG and A.O. AGATEP. 1965. Gibberellin production in pea seeds developed in excised pods. Effect of growth retardant AMO-1618. Sci. 147: 155-157.

9. BATJER, L.P., M.W. WILLIAMS and G.C. MARTIN. 1964. Effects of N-dimethylamino succinamic acid (B-Nine) on vegetative and fruit characteristics of apples, pears, and sweet cherries. Proc. Amer. Soc. Hort. Sci. 85: 11-16.

10. BEATTIE, W.R., L.L. HARTER and B.L. WADE. 1942. Growing peas for canning and freezing. U.S.D.A. Farmers' Bulletin 1920, Wash., D.C.

11. BEEVERS, H. 1961 Respiratory metabolism in plants. Harper and Row, New York.

12. BEEVERS, L. and J.B. HANSON. 1964. Oxidative phosphorylation by root and shoot mitochondria from corn seedlings as affected by temperature. Crop Sci. 4: 549-550.

13. BIDDULPH, O., S. BIDDULPH, R. CORY and H. KOONTZ. 1958. Circulation patterns for phosphorus, sulfur and calcium in the bean plant. Plant Physiol. 33: 293-300.

14. BIELESKI, R.L. 1964. The problem of halting enzyme action when extracting plant tissues. Anal. Biochem. 9: 431-442.

15. BONNER, J. 1943. Effects of application of thiamine to Cosmos. Bot. Gaz. 104: 475-479.

16. BONNER, J. 1957. The chemical cure of climatic lesions. Engng. Sci. Mag. 20: 28-30.

17. BOWLING, D.J.F. and P.E. WEATHERLEY. 1964. Potassium uptake and transport in roots of Ricinus communis. J. Exptl. Bot. 15: 413-421.

18. BRADLEY, G.A. and J.W. FLEMING. 1960. The effects of position of leaf and time of sampling on the relationship of leaf phosphorus and potassium to yield of cucumbers, tomatoes and watermelons. Proc. Amer. Soc. Hort Sci. 75: 617-624.

19. BRENCHLEY, W.E. 1920. On the relation between growth and the environmental conditions of temperature and bright sunshine. Ann. Appl. Biol. 6: 211-244.

20. BROOK, J., S.H. WEST and D.S. ANTHONY. 1967. Effects of Phosfon on nucleic acid metabolism in Pisum sativum Alaska. Plant Physiol. 42: 785-790.

21. BROUWER, R. 1956. Investigations into the occurrence of active and passive components in the ion uptake of Vicia faba. Acta. Bot. Neerl. 5: 287-314.

22. BROUWER, R. 1959. The influence of the root temperature on the growth of peas. Jaarb IBS. 1959: 27-36. In Nielsen and Humphries, 1965.

23. BROUWER, R. 1965. Ion absorption and transport in plants. Ann. Rev. Plant Physiol. 16: 241-266.

24. BUCKMAN, H.O. and N.C. BRADY. 1960. The nature and property of soils. 6th ed., MacMillan, New York.

25. BURSTONE, M.S. 1962. Enzyme histochemistry and its application in the study of neoplasms. Acad. Press, New York.

26. BUTLER, G.W. 1953. Ion uptake by young wheat plants. I. Time course of the absorption of potassium ahd chloride ions. Physiol. Plant 6: 594-616.

27. CAMPBELL, W.A. and J.T. PRESLEY. 1945. Design for constant temperature tanks. Phytopathology 35: 213-216.

28. CANNON, W.A. 1925. Physiological features of roots with special reference to the relation of roots to the aeration of soil. Carnegie Inst. Wash. Pub. 368: 1-168.

29. CANNY, M.J. 1962. The mechanism of translocation. Ann. Bot. (N.S.) 26: 603-617.

30. CATHEY, H.M. 1964. Physiology of growth retarding chemicals. Ann. Rev. Plant Physiol. 15: 271-302.

31. CATHEY, H.M. and N.W. STUART. 1961. Comparative plant growth-retarding activity of AMO-1618, Phosfon and CCC. Bot. Gaz. 123: 51-57.

32. CHANCE, B., W. HOLMES and J. HIGGINS. 1958. Localization of interaction sites in multi-component transfer systems: Theorems derived from analogues. Nature 182: 1190-1193.

33. CHERRY, J.H. and R.H. HAGEMAN. 1960. Separation and identification of soluble nucleotides from etiolated corn seedlings as a function of growth. Plant Physiol. 35: 343-352.

34a. CLARKSON, D.T. 1966. Effect of aluminum on the uptake and metabolism of phosphorus in barley seedlings. Plant Physiol. 41: 165-172.

34b. CLARKSON, V.A. 1960. Effect of black polyethylene mulch on soil and microclimate temperature and nitrate level. Agron. J. 52: 307-309.

35. CLELAND, R. 1965. Evidence on the site of action of growth retardants. Plant Cell Physiol. 6: 7-15.

36. CLEMENTS, H.F. 1964. Interaction of factors affecting yield. Ann. Rev. Plant Physiol. 15: 409-442.

37. CONWAY, E.J. 1953. A redox pump for the biological performance of osmotic work, and its relation to the kinetics of free ion diffusion across membranes. Intnl. Rev. Cytol. 2: 419-445.

38. COOPER, D.J., K.F. NIELSEN, J.W. WHITE and W. KALBFLEISCH. 1960. Apparatus for controlling soil temperatures. Can. J. Soil Sci. 40: 105-107.

39. DAHLGREN, G. and N.L. SIMMERMAN. 1963. Intramolecular catalysis of the hydrolysis of N-dimethylamino maleamic acid (Co_{11}). Sci. 140: 485-486.

40. DORMAAR, J.F. and J.W. KETCHESON. 1960. The effect of nitrogen form and soil temperature on the growth and phosphorus uptake of corn grown in the greenhouse. Can. J. Soil Sci. 45: 177-184.

41. DOWNTON, W.J.S. and E.B. TREGUNNA. 1968. Carbon dioxide compensation -- its relation to photosynthetic carboxylation reactions, systematics of the Gramineae and leaf anatomy. Can. J. Bot. 46: 207-215.

42. DUNCAN, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

43. EL-SHARKAWY, M.A. and J.D. HESKETH. 1964. Effects of temperature and water deficit on leaf photosynthetic rates of different species. Crop Sci. 4: 514-518.

44. EMBLETON, T.W., M.J. GARBER, W.W. JONES and S.J. RICHARDS. 1958. Effects of irrigation treatments and rates of nitrogen fertilization on young Hass Avocado trees. IV. Micronutrient content of leaves. Proc. Amer. Soc. Hort. Sci. 71: 310-314.

45. EPSTEIN, E. 1960. Spaces, barriers, and ion carriers: ion absorption by plants. Amer. J. Bot. 47: 393-399.

46. EPSTEIN, E. and E.C. HAGEN. 1952. A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. 27: 457-474.

47. FLETCHER, H.F., D.P. ORMROD, A.R. MAURER and B. STANFIELD. 1966. Response of peas to environment. I. Planting date and location. Can. J. Plant Sci. 46: 77-85.

48. FRIED, M., C.E. HAGEN, J.F. SAIZ DEL RIO and E. LEGGETT. 1957. Kinetics of phosphate uptake in the soil-plant system. Soil Sci. 84: 427-437.

49. FRIED, M. and R.E. SHAPIRO. 1961. Soil-plant relationships in ion uptake. Ann. Rev. Plant Physiol. 12: 91-112.

50. GALSTON, A.W. Biochemistry. In Experimental control of plant growth, F.W. Went, ed., Chronica Botanica, Waltham, Massachusetts. 1957.

51. GALSTON, A.W. and M.E. HAND. 1949. Adenine as a growth factor for etiolated peas and its relation to thermal inactivation of growth. Arch. Biochem. 22: 434-443.

52. GAUCH, H.G. 1956. Mineral nutrition of plants. Ann. Rev. Plant Physiol. 31: 155-161.

53. GHOSH, A. and B. CHANCE. 1964. Oscillations in glycolytic intermediates in yeast cells. Biochem. Biophys. Res. Commun. 16: 174-181.

54. GOODMAN, M., A.A. BENSON and M. CALVIN. 1955. Fractionation of phosphates from Scenedesmus by anion exchange. J. Amer. Chem. Soc. 77: 4257-4261.

55. HALEVY, A.H. 1963. Interaction of growth retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. Plant Physiol. 38: 731-737.

56. HALEVY, A.H. and H.M. CATHEY. 1960. Effect of structure and concentration of some quaternary ammonium compounds on growth of cucumber seedlings. Bot. Gaz. 112: 151-154.

57. HATCH, M.D. and J.F. TURNER. 1958. Glycolysis by an extract from pea seeds. Biochem. J. 69: 495-501.

58. HESS, B. 1963. Control of metabolic rates. In Control mechanisms in respiration and fermentation. B. Wright, ed. Ronald Press, New York.

59. HIGHKIN, H.R. 1960. The effect of constant temperature environments and of continuous light on the growth and development of pea plants. Cold Spring Harbour Symposia on Quantitative Biology, vol. XXV: 231-238. New York.

60. HINMAN, W.C., J.D. BEATON and D.W.L. READ. 1962. Some effects of moisture and temperature on transformation of monocalcium phosphate in soil. Can. J. Soil Sci. 42: 229-239.

61. HINNEMANN, H., J.A.D. ZEEVART, H. KENDE and A. LANG. 1964. The plant growth retardant CCC as inhibitor of gibberellin biosynthesis in Fusarium moniliforme. Planta 61: 229-235.

62. HONERT, T.V. van den, J.J.M. HOOYMANS and W.S. VOLKERS. 1955. Experiments on the relation between water absorption and mineral uptake by plant roots. Acta Bot. Neerl. 4: 139-155.

63. HUMPHRIES, E.C. 1956. The relation between the rate of nutrient uptake by excised barley roots and their content of sucrose and reducing sugars. Ann. Bot. (N.S.) 20: 411-417.

64. HYLMO, B. 1958. Passive components in the ion absorption of the plant. II. The zonal water flow, ion passage and pore size in the roots of Vicia faba. Physiol. Plant 11: 382-400.

65. JACOBSON, L., R. OVERSTREET, R.M. CARLSON and J.A. CHASTAIN. 1957. The effect of pH and temperature on the absorption of potassium and bromide by barley roots. Plant Physiol. 32: 658-662.

66. JAFFE, M.J. and F.M. ISENBERG. 1965. Some effects of N-dimethylamino succinamic acid (B-nine) on the development of various plants with special reference to the cucumber, Cucumis sativus L. Proc. Amer. Soc. Hort Sci. 87: 420-428.

67. JUO, P. and G. STOTZKY. 1967. Interference by nitrate and nitrite in the determination of carbohydrates by anthrone. Anal. Biochem. 21: 149-151.

68. KAMIYA, N. 1960. Physics and chemistry of protoplasmic streaming. Ann. Rev. Plant Physiol. 11: 323-340.

69. KAUFMANN, S. and S.G.A. ALIVISATOS. 1955. Purification and properties of the phosphorylating enzyme from spinach. J. Biol. Chem. 216: 141-152.

70. KETCHESON, J.W. 1957. Some effects of soil temperature on phosphorus requirements of young corn plants in the greenhouse. Can. J. Soil Sci. 37: 41-47.

71. KETELLAPER, H.J. and J. BONNER. 1961. The chemical basis of temperature response in plants. Plant Physiol. 36:(suppl.) xxi.

72. KHYM, J.X. and W.E. COHN. 1953. The separation of sugar phosphates by ion-exchange with the use of the borate complex. J. Amer. Chem. Soc. 75: 1153-1156.

73. KNAVEL, D.E. 1968. Influence of growth retardants on growth, nutrient content and yields of tomato plants grown with various fertility levels. Hort. Sci. 3: 101.

74. KNOLL, H.A., N.C. BRADY and D.J. LATHWELL. 1964. Effect of soil temperature and phosphorus fertilization on the growth and phosphorus content of corn. Agron. J. 56: 145-147.

75. KRAMER, P.J. 1949. Plant and soil water relationships. 1st ed. McGraw-Hill, Toronto.

76. KRAMER, P.J. 1956. Physical and physiological aspects of water absorption. Encycl. Plant Physiol. 3: 124-159. Springer-Verlag, Berlin.

77. KURAISHI, S. and R.M. MUIR. 1963. Mode of action of growth retarding chemicals. Plant Physiol. 38: 19-24.

78. LANGRIDGE, J. 1963. Biochemical aspects of temperature response. Ann. Rev. Plant Physiol. 14: 441-462.

79. LANGRIDGE, J. and B. GRIFFING. 1959. A study of high temperature lesions in Arabidopsis thaliana. Australian J. Biol. Sci. 12: 117-135.

80. LARSON, W.E. and W.O. WILLIS. 1957. Light, soil temperature, soil moisture and alfalfa-red clover distribution between corn rows of varying spacing and row directions. Agron. J. 49: 422-426.

81 LATIES, G.G. 1959. Active transport of salt into plant tissues. Ann. Rev. Plant Physiol. 10: 87-112.

82. LETEY, J., L.H. STOLZY, G.B. BLANK and O.R. LUNT. 1961. Effect of temperature on oxygen diffusion rates and subsequent shoot growth, root growth, and mineral content of two plant species. Soil Sci. 92: 314-321.

83. LETEY, J., L.H. STOLZY, N. VALORAS and T.E. SZUSZKIEWICZ. 1962. Influence of oxygen diffusion rate on sunflower. Agron. J. 54: 316-319.

84. LINGLE, J.C. and R.M. DAVIS. 1959. The influence of soil temperature and phosphorus fertilization on the growth and mineral absorption of tomato seedlings. Proc. Amer. Soc. Hort. Sci. 73: 312-322.

85. LOCASCIO, S.J. and G.F. WARREN. 1960. Interaction of soil temperature and phosphorus on growth of tomatoes. Proc. Amer. Soc. Hort. Sci. 75: 601-610.

86. LOCKHART, J.A. 1962. Kinetic studies of certain anti-gibberellins. Plant Physiol. 37: 759-764.

87. LOUGHMAN, B.C. and R.S. RUSSELL. 1957. The absorption and utilization of phosphate by young barley plants. IV. The initial stages of phosphate metabolism in roots. J. Exptl. Bot. 8: 280-293.

88. LUNDEGARDH, H. 1947. Mineral nutrition of plants. Ann. Rev. Biochem. 16: 503-528.

89. LUNDEGARDH. H. 1955. Mechanisms of absorption, transport, accumulation, and secretion of ions. Ann. Rev. Plant Physiol. 6: 1-24.

90. MACK, A.R. 1965. Effect of soil temperature and moisture on yield and nutrient uptake by barley. Can. J. Soil Sci. 45: 337-346.

91. MACK, A.R. and W.A. EVANS. 1965. Soil temperature control system for field plots. Can. J. Soil Sci. 45: 105-107.

92. MACK, H.J., S.C. FANG and S.B. APPLE, Jr. 1964. Effects of soil temperature and phosphorus fertilization on snap-beans and peas. Proc. Amer. Soc. Hort. Sci. 84: 332-338.

93. MACKINNEY, G. 1940. Criteria for purity in chlorophyll preparations. J. Biol. Chem. 132: 91-109.

94. MACLEAN, K.S. and D.L. BYERS. 1968. Nutrient content of field-grown peas. Can. J. Plant Sci. 48: 155-160.

95. MAJUMDER, S.K. 1968. Morphogenetic responses of tomato plants to combined and individual applications of gibberellic acid, phosfon and B-Nine. Plant and Soil 28: 291-297.

96. MARRE, E. 1961. Phosphorylation in higher plants. Ann. Rev. Plant Physiol. 12: 195-218.

97. MAURER, A.R., D.E. JAFFRAY and H.F. FLETCHER 1966. Response of peas to environment. III. Assessment of the morphological development of peas. Can. J. Plant Sci. 46: 285-290.

98. MCKELL, C.M., A.M. WILSON and M.B. JONES. 1961. A flotation method for easy separation of roots from soil samples. Agron. J. 53: 56-57.

99. MCKELL, C.M., A.M. WILSON and W.A. WILLIAMS. 1962. Effect of temperature on phosphorus utilization by native and introduced legumes. Agron. J. 54: 109-113.

100. MEDERSKI, H.J. and J.B. JONES, Jr. 1963. Effect of soil temperature on corn plant development and yield. I. Studies with a corn hybrid. Soil Sci. Soc. Amer. Proc. 27: 186-189.

101. MES, M.G. 1959. Influence of temperature on the symbiotic fixation of legumes. Nature 184: 2032-2033.

102. MEYER, D.R. and A.J. ANDERSON. 1959. Temperature and symbiotic nitrogen fixation. Nature 183: 61.

103. MOORE, T.C. 1967. Kinetics of growth retardant and hormone interaction in affecting cucumber hypocotyl elongation. Plant Physiol. 42: 677-684.

104. MOORIS, D.L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Sci. 107: 254-255.

105. MOSSBERRY, Y., A.M. MAYER and L.W. MAPSON. 1964. Inhibition of glycolysis by adeonsine triphosphate. J. Exptl. Bot. 15: 29-34.

106. NEFF, M.S., H.L. BARROWS and C.B. SHEAR. 1958. Response of tung trees to applied nitrogen, potassium and magnesium in different soil types. Proc. Amer. Soc. Hort. Sci. 71: 237-240.

107. NELSON, W.L., B.A. KRANTZ, C.D. WELCH and N.S. HALL. 1949. Utilization of phosphorus as affected by placement. II. Cotton and corn in North Carolina. Soil Sci. 68: 137-144.

108. NIELSEN, K.F. and R.K. CUNNINGHAM. 1964. The effects of soil temperature, form and level of N on growth and chemical composition of Italian ryegrass. Soil Sci. Soc. Amer. Proc. 28: 213-218.

109. NIELSEN, K.F., R.L. HALSTEAD, A.J. MACLEAN, S.J. BOURGET and R.M. HOLMES. 1961. The influence of soil temperature on the growth and mineral composition of corn, bromegrass and potatoes. Soil Sci. Soc. Amer. Proc. 25: 369-372.

110. NIELSEN, K.F., R.L. HALSTEAD, A.J. MACLEAN, R.M. HOLMES and S.J. BOURGET. 1960. Effects of soil temperature on the growth and chemical composition of Lucerne. Proc. 8th Intnl. Grassland Congr., 287-292.

111. NIELSEN, K.F. and E.C. HUMPHRIES. 1965. Soil temperature and plant growth, an annotated bibliography (1951-1964). Can. Dept. Agric. Swift Current Pub. 108: 36-40.

112. NIELSEN, K.F. and E.C. HUMPHRIES. 1966. Effects of root temperature on plant growth. Soils and Fertilizers 29: 1-7.

113. OHLROGGE, A.J. 1962. Some soil-root-plant relationships. Soil Sci. 93: 30-38.

114. OLSEN, S.R., F.S. WATANABLE and R.E. DANIELSON. 1961. Phosphorus absorption by corn roots as affected by moisture and P concentration. Soil Sci. Soc. Amer. Proc. 25: 289-294.

115. ORMROD, D.P. 1962. Note on inexpensive multiple plant growth cabinets. Can. J. Plant Sci. 42: 742-745.

116. ORMROD, D.P. and W.A. BUNTER, Jr. 1961. Influence of temperature on the respiration of rice seedlings. Crop. Sci. 1: 353-354.

117. ORMROD, D.P. and W.A. WILLIAMS. 1960. Phosphorus metabolism of Trifolium hirtum All as influenced by 2,4-dichlorophenoxyacetic acid and gibberellic acid. Plant Physiol. 35: 81-87.

118. POTTS, J.R.M. and D.P. ORMROD. 1969. The effect of abrupt temperature increase on growth and phosphorus fractions of Pisum sativum. Can. J. Bot. 47. In Press.

119. POWER, J.F., D.L. GRUNES, G.A. REICHMAN and W.O. WILLIS. 1964. Soil temperature and phosphorus effects upon nutrient absorption by barley. Agron. J. 56: 355-359.

120. POWER, J.F., D.L. GRUNES, W.O. WILLIS and G.A. REICHMAN. 1963. Soil temperature and phosphorus effects upon barley growth. Agron. J. 55: 389-392.

121. PRESTON, W.H. Jr. and C.B. LINK. 1958. Use of 2,4-dichlorobenzyl tributylphosphonium chloride to dwarf plants. Plant Physiol. 33 (suppl.) xlix.

122. REED. D.J., T.C. MOORE and J.D. ANDERSON. 1965. Plant growth retardant B-995: a possible mode of action. Sci. 148: 1469-1471.

123. REITZ, H.J. and R.C.J. KOO, Jr. 1960. Effects of nitrogen and potassium fertilization on yield, fruit quality, and leaf analysis of Valencia orange. Proc. Amer. Soc. Hort. Sci. 75: 244-252.

124. RICHARDS, S.J., R.M. HAGAN and T.M. MCCALLA. 1952. Soil temperature and plant growth. In Soil physical conditions and plant growth. B.T. Shaw, ed. Acad. Press, New York.

125. RIDDELL, J.A., H.A. HAGEMAN, C.M. JANTHONY and W.L. HUBBARD. Retardation of plant growth by a new group of chemicals. Sci. 135: 391.

126. RINNE, R.W. and R.G. LANGSTON. 1960. Studies on lateral movement of phosphorus 32 in peppermint. Plant Physiol. 35: 216-219.

127. ROBINSON, R.R., V.G. SPRAGUE and C.F. CROSS. 1959. The relation of temperature and phosphate placement to growth of red clover. Soil Sci. Soc. Amer. Proc. 23: 225-228.

128. ROGERS, B.L., L.P. BATJER and A.H. THOMPSON. 1953. Seasonal trend of several nutrient elements in Delicious apple leaves expressed on a percent and unit area basis. Proc. Amer. Soc. Hort. Sci. 61: 1-5.

129. ROWAN, K.S. 1966. Phosphorus metabolism in plants. Intnl. Rev. Cytol. 19: 301-391.

130. SCOTT, R.W., W.E. MOORE, M.J. EFFLAND and M.A. MILLET. 1967. Ultraviolet spectrophotometric determination of hexoses, pentoses and uronic acids after their reactions with concentrated sulfuric acid. Anal. Biochem. 21: 68-80.

131. SEKIOKA, H. 1963. Effect of some environmental factors on the translocation and storage of carbohydrates. I. Relationship between the translocation of carbohydrates or radioisotopes and soil and air temperatures in sweet potato. Soils and Fertilizers 26: 31-59.

132. SHAW, M. 1968. Personal communication.

133. SHTRAUSBERG, D.V. 1955. The effect of soil temperature on the utilization of various nutrients by plants. Soils and Fertilizers 20: 2.

134. SIMPSON, K. 1960. Effect of soil temperature and moisture on the uptake of phosphorus by oats. J. Sci. Food Agric. 11: 449-456.

135. SINGH, H.N. and H.J. MACK. 1966. Effects of soil temperatures on growth, fruiting, and mineral composition of snapbeans. Proc. Amer. Soc. Hort. Sci. 88: 378-383.

136. SINGH, H.N. and D.P. SINGH. 1968. Studies in the mineral nutrition of Japanese mint. Plant and Soil 28: 363-371.

137. SMITH, P.F. 1962. Mineral analysis of plant tissues. Ann. Rev. Plant Physiol. 13: 81-108.

138. SMITH, P.F. and W. REUTHER. 1951. The response of young Valencia orange trees to different boron supply in sand culture. Plant Physiol. 26: 110-114.

139. SOUTHWICK, F.W., W.J. LORD and W.D. WEEKS. 1968. The influence of succinamic acid, 2,2-dimethyl hydrazine (Alar) on the growth, productivity, mineral nutrition and quality of apples. Proc. Amer. Soc. Hort. Sci. 92: 71-81.

140. SPRENT, J.I. 1967. Effects of nutrient factors, water supply, and growth-regulating substances on the vegetative growth pattern of peas and their relationship to the node of first flower. Ann. Bot (N.S.) 31: 607-618.

141. STANFIELD, B., D.P. ORMROD and H.F. FLETCHER. 1966. Response of peas to environment. II. Effects of temperature in controlled-environment cabinets. Can. J. Plant Sci. 46: 195-203.

142. STEWARD, F.C. and H.E. STREET. 1947. The nitrogenous constituents of plants. Ann. Rev. Biochem. 16: 471-502.

143. STUMPF, P.K. 1955. Fat metabolism in higher plants. III. Enzymic oxidation of glycerol. Plant Physiol. 30: 55-58.

144. SUTCLIFFE, J.F. 1962. Mineral salts absorption in plants. Pergamon Press. Oxford.

145. TANADA, T. 1956. Effect of ribonuclease on salt absorption by excised mung bean roots. Plant Physiol. 31: 251-253.

146. TANAKA, K. and N.E. TOLBERT. 1966. Effects of Cycocel derivatives and gibberellins on choline kinase and choline metabolism. Plant Physiol. 41: 313-318.

147. THOMAS, C.A., Jr. 1956. New scheme for performance of osmotic work by membranes. Sci. 123: 60-61

148. TOLBERT, N.E. 1960. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. II. Effects on growth of wheat. Plant Physiol. 35: 380-385.

149. TULI, V., D.R. DILLEY and S.H. WITTWER. 1964. N^6-benzyladenine: Inhibitor of respiratory kinases. Sci. 146: 1477-1479.

150. ULRICH, A. and W.L. BERRY. 1961. Critical phosphorus levels for lima bean growth. Plant Physiol. 36: 626-632.

151. WANG, J.W. and R.A. BRYSON. 1956. A study of phyto-meteorological effects on the growth and development of peas. Dept. Meteorol., Univ. of Wisconsin Pub.

152. WEISSMAN, G.S. 1964. Effect of ammonium and nitrate nutrition on protein level and exudate composition. Plant Physiol. 39: 947-952.

153. WELCH, F.L., D.L. MULVANEY, L.V. BOONE, G.E. MCKIBBEN and J.W. PEMBLETON. 1966. Relative efficiency of broadcast versus banded phosphorus for corn. Agron. J. 58: 283-287.

154. WENT, F.W. 1944. Plant growth under controlled conditions. III. Correlation between varying physiological processes and growth in the tomato plant. Amer. J. Bot. 31: 597-618.

155. WENT, F.W. 1957. The experimental control of plant growth. Chronica Botanica, Waltham, Massachusetts.

156. WETTSTEIN, D. von. 1957. Chlorophyll-letale und der submikropische formueschel der plastiden. Exp. Cell Res. 12: 427-506.

157. WHATLEY, F.R. and M. LOSADA. 1964. The photochemical reactions of photosynthesis. In Photophysiology I. A.C. Griese, ed. Acad. Press, New York.

158. WILLIAMS, R.F. 1955. Redistribution of mineral elements during development. Ann. Rev. Plant Physiol. 6: 25-42.

159. WILLIS, W.O., J.F. POWER, G.A. REICHMAN and D.L GRUNES. 1963. Constant temperature water baths for plant growth experiments. Agron. J. 55: 200.