SOIL FERTILITY EFFECTS ON GROWTH, NODULE PARAMETERS, AND NITROGENASE ACTIVITY (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA (<u>PISUM SATIVUM</u> SUBSPECIES <u>ARVENSE</u> (L.) POIR)

В**у**

ROBERT KEITH BERG, JR.

Bachelor of Science in Agriculture

Sec. Sales

Oklahoma State University

Stillwater, Oklahoma

1981

Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE May, 1982

Thesis 1982 84935 cop.2

ŝ

UNIVERSITY LIBRARY

SOIL FERTILITY EFFECTS ON GROWTH, NODULE PARAMETERS, AND NITROGENASE ACTIVITY (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA (<u>PISUM SATIVUM</u> SUBSPECIES <u>ARVENSE</u> (L.) POIR)

Thesis Approved:

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere appreciation and gratitude to my major adviser, Dr. J. Q. Lynd, for his advice, encouragement, and friendship throughout my studies at Oklahoma State University. To the other members of my committee, Dr. R. J. Crabtree and Dr. R. W. McNew, I am thankful for their comments and assistance in the preparation of this manuscript. Appreciation is expressed to the Oklahoma State University Soil and Plant Testing Laboratory for their help with the mineral composition analysis in these experiments as well as to the Department of Agronomy for the facilities and financial assistance provided during my research and study at this university.

I am especially grateful to Mrs. Fairy Lynd and Chaibundt Lurlarp for their aid in the laboratory work done in these studies. Of my lovely wife, Pam, I am proud, not only for her typing of this manuscript but also for her love, understanding, patience, and encouragement, throughout all of my university studies. To our wonderful children, Robin and Ryan, I am thankful and also to both of our parents, families, and friends for all their help and prayers.

iii

TABLE OF CONTENTS

Chapter	Page	
I.	INTRODUCTION	
II.	LITERATURE REVIEW	
	A. Origin and Distribution	
III.	MATERIALS AND METHODS	
IV.	DISCUSSION OF RESULTS	
	A. Series I 18 B. Series II 25 C. Series III 44	
V.	SUMMARY AND CONCLUSIONS	
LITERAT	URE CITED	

LIST OF TABLES

Table		Pa	ige
I.	The Taxonomy of the Austrian Winter Pea	•	4
II.	Some Known Varieties of Field Peas and the Area Some of Them Were Developed	•	8
III.	Recommended Seeding Rates and Row Spacings for Field Peas .	•	9
IV.	Epipedon (15 cm) Analysis of a Typical Eufaula Loamy Sand .	•	12
V.	Source and Nutrient Levels for Austrian Winter Pea, Series I, II, and III	•	13
VI.	Treatment Combinations for Austrian Winter Pea, Series I, II, and III	•	13
VII.	Age, Planting and Harvest Dates for Austrian Winter Pea	•	14
VIII.	Austrian Winter Pea Parameters Measured for Greenhouse Experiments With Eufaula Soil	•	17
IX.	Effects of K Levels, With and Without P and Ca, on Growth, Nodulation, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series I	•	19
Χ.	Effects of K Levels, With and Without P and Ca, on Whole Nodule Composition of Austrian Winter Pea, Series I	•	20
XI.	Effects of K Levels on Growth, Nodulation, and Nitrogenase (C2H2 Reduction) of Austrian Winter Pea, Series I	•	21
XII.	Effects of Ca and P Levels on Growth, Nodulation, and Nitrogenase (C_2H_2 Reduction) of Austrian Winter Pea, Series I	•	22
XIII.	Comparisons for Effects of K Levels, with P and Ca, on Growth, Nodulation, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series I	•	23
XIV.	Correlations Among Growth, Nodulation, Nitrogenase (C2H2 Reduction) and Whole Nodule Composition of Austrian Winter Pea, Series I	•	24

v

Table

Χ٧.	Effects of K Levels, With and Without P and Ca, on Growth, Nodulation, and Nitrogenase (C2H2 Reduction) of Austrian Winter Pea, Series II
XVI.	Effects of K Levels, With and Without P and Ca, on Whole Nodule Composition of Austrian Winter Pea, Series II 27
XVII.	Effects of K Levels, With and Without P and Ca, on Nodule Cytosol Composition of Austrian Winter Pea, Series II
XVIII.	Effects of K Levels, With and Without P and Ca, on Nodule Organelle Composition of Austrian Winter Pea, Series II 29
XIX.	Effects of K Levels on Growth, Nodulation, Nitrogenase (C ₂ H ₂ Reduction), and Nodule Composition of Austrian Winter Pea, Series II
XX.	Effects of Ca and P Levels on Growth, Nodulation, Nitrogenase (C ₂ H ₂ Reduction), and Whole Nodule Composition of Austrian Winter Pea, Series II
XXI.	Effects of Ca and P Levels on Nodule Cytosol and Organelle Compositions of Austrian Winter Pea, Series II
XXII.	Comparisons for Effects of K Levels, With P and Ca, on Growth, Nodulation, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series II
XXIII.	Comparisons for Effects of K Levels, With P and Ca, on Whole Nodule Composition of Austrian Winter Pea, Series II
XXIV.	Comparisons for Effects of K Levels, With P and Ca, on Nodule Cytosol Composition of Austrian Winter Pea, Series II
xxv.	Comparisons for Effects of K Levels, With P and Ca, on Nodule Organelle Composition of Austrian Winter Pea, Series II
XXVI.	Correlations Among Growth, Nodule Parameters, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series II
XXVII.	Correlations Among Growth, Nodule Parameters, Nitrogenase (C ₂ H ₂ Reduction), and Whole Nodule Composition of Austrian Winter Pea, Series II
XXVIII.	Correlations Among Nodule Parameters, Nitrogenase (C ₂ H ₂ Reduction), and Nodule Cytosol Composition of Austrian Winter Pea. Series II

Page

-

Table

XXIX.	Correlations Among Nodule Parameters, Nitrogenase (C ₂ H ₂ Reduction), and Nodule Organelle Composition of Austrian Winter Pea, Series II	
XXX.	Effects of K Levels, With and Without P and Ca, on Growth, Nodulation, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series III	
XXXI.	Effects of K Levels, With and Without P and Ca, on Whole Nodule Composition of Austrian Winter Pea, Series III 46	1
XXXII.	Effects of K Levels, With and Without P and Ca, on Nodule Cytosol Composition of Austrian Winter Pea, Series III 47	
XXXIII.	Effects of K Levels, With and Without P and Ca, on Nodule Organelle Composition of Austrian Winter Pea, Series III	,
XXXIV.	Effects of K Levels on Growth, Nodulation, Nitrogenase (C ₂ H ₂ Reduction), and Nodule Composition of Austrian Winter Pea, Series III	
XXXV.	Effects of Ca and P Levels on Growth, Nodulation, Nitrogenase (C ₂ H ₂ Reduction), and Whole Nodule Composition of Austrian Winter Pea, Series III	
XXXVI.	Effects of Ca and P Levels on Nodule Cytosol and Organelle Compositions of Austrian Winter Pea, Series III	
XXXVII.	Comparisons for Effects of K Levels, With and Without P and Ca, on Growth, Nodulation, and Nitrogenase (C ₂ H ₂ Reduction) of Austrian Winter Pea, Series III 53	
XXXVIII.	Comparisons for Effects of K Levels, With and Without P and Ca, on Whole Nodule Composition of Austrian Winter Pea, Series III	,
XXXIX.	Comparisons for Effects of K Levels, With and Without P and Ca, on Nodule Cytosol Composition of Austrian Winter Pea, Series III	
XL.	Comparisons for Effects of K Levels, With and Without P and Ca, on Nodule Organelle Compositions of Austrian Winter Pea, Series III	
XLI.	Correlations Among Growth and Nodule Parameters for Growth, Nodulation, and Whole Nodule Composition of Austrian Winter Pea, Series III	
XLII.	Correlations Among Growth and Nodule Parameters for Nodule Cytosol and Organelle Compositions of Austrian Winter Pea, Series III	

CHAPTER I

INTRODUCTION

Legumes contribute an important link in the earth's nitrogen cycle. By symbiotic association with specific Rhizobium, they convert nitrogen from the air into an organic form that becomes available to other plants. Thus they tap an abundant and otherwise unavailable source of nitrogen so vital for plant and animal growth. Legumes provide important sources of protein for the ever-increasing human and livestock populations. They can also be very useful in forage and soil management practices for efficient agricultural production. Field peas have been utilized extensively in the past. However, since World War II their use has declined. This is primarily the result of dramatic increases in farm expenses, especially for land, as well as the availability of relatively inexpensive nitrogen fertilizers in the more developed regions of the world. With the recent concern for energy supplies, as relates to the high manufacturing costs of nitrogen fertilizers plus the constant evaluation for improved agricultural practices throughout the world, there has been a renewed interest in using legumes to their fullest extent in modern agriculture. Although there has been extensive research with peas, especially in genetics (Smith, 1971), physiology (Bond, 1948; Pate et al., 1965; Cghoghorie and Pate, 1972; Pate and Flinn, 1973), and biochemistry (Raacke, 1957; Flinn and Pate. 1968; Smith. 1973). there has been little information available on the effects of plant nutrients on the nodule composition and nitrogenase

activity levels of field peas. The objective of this study was to determine the effects of plant nutrients P, K, and Ca in factorial combinations on the growth, nodulation, nitrogenase activity (C_2H_2 reduction), and nodule composition of Austrian Winter Pea with three greenhouse experiments.

CHAPTER II

LITERATURE REVIEW

A. Origin and Distribution

Cultivated and native peas are believed to have originated within the Mediterranean region that includes southern Europe and northern Africa (Piper, 1924; Bland, 1971). Worldwide, the leading countries in dry pea production include the Soviet Union, China, India, and the United States. In North America, field peas are grown in southern Canada, the Pacific Northwest, and in the southeastern United States. Within the United States, the leading dry pea producing states are Idaho, Oregon, Washington, Minnesota, and North Dakota (Martin et al., 1976).

B. Botany

The taxonomy of the field pea is presented in Table I. Field peas are generally referred to as <u>Pisum sativum</u> subspecies <u>arvense</u> (L.) Poir, and have red or purple colored flowers as opposed to garden peas, <u>P</u>. <u>sativum</u> subspecies <u>hortense</u>, which have white flowers. Pea species can also be divided according to three general colors of the seed. Winter peas have gray, brown, or mottled seed coats, Canadian types refer to those with yellow or white seed coats and yellow or orange cotyledons, while other types of peas have green seed coats and cotyledons.

According to Piper (1924), Bland (1976), and Martin et al. (1976) field peas are shallow-rooted, cool season annuals. They are generally

weak-stemmed herbs with hollow, succulent stems ranging from 0.5 to 3 m in length. They have 1-3 pairs of leaflets bearing pinnate leaves. Several pairs of tendrils and rachis allow them to vine in a fashion similar to the vetches (<u>Vicia spp</u>). Axillary peduncles support 1-3 red or purple flowers which later develop into greenish-colored pods, each having from 4 to 9 round, angular, or wrinkled seeds. They are self-fertile, have hypogeal germination, and the cotyledons are generally green or yellow. Field peas are diploid and have 14 chromosomes per cell.

TABLE I

THE TAXONOMY OF THE AUSTRIAN WINTER PEA¹

Kingdom.	Plantae		
Division:			
	Tracheophy ca		
Subdivision:	Spermopsida		
Class:	Angiospermae		
Subclass:	Dicotyledoneae		
Family:	Leguminosae		
Subfamily:	Papilionoidae		
Tribe:	Vicieae		
Genus:	Pisum		
Species:	sativum		
Subspecies:	arvense		
Cultivar: Austrian Winte			

1. Adapted from Bland (1971) and Keeton (1972).

C. Uses

Austrian winter peas have a variety of important and practical uses. Fresh and dry pea production can supply an inexpensive source of highquality protein (24%) for human and livestock dietary needs. Dry peas have been exported to several European and Asian countries. Murray and Slinkard (1973) have estimated that 80% of the Austrian winter peas raised in the United States are sold to Japan yearly and that their most important use is in making <u>An Paste</u>, a high-protein, sweet filling used in or on cookies, crackers, and pastries by the Japanese. Dry peas can also substitute for canned peas by soaking them in water. Peas do not cause intestinal problems sometimes associated with beans (Piper, 1924). Another important use is for the split-pea market. The exact procedure for the processing of split peas commercially is a trade secret but basically the seed coat is removed, the cotyledon divided, and most of the embryos are taken out. The seed coats and embryos are then used in the manufacture of livestock feed (Martin et al., 1976).

Winter peas can be used as a green manure crop to add nitrogen and organic matter to the soil. In rotations with cereal crops, winter peas can be used to help control weed and disease problems. They need less fertilizer than cereals, can be used as a cover crop to help prevent soil erosion, and may provide economic marketing advantages as a result of diversification. They may be grown alone or in association with other crops (such as oats or other small grains) for hay, pasture, or silage. The peas increase the protein content and feeding value of the mixture plus they have good potential for regrowth after grazing compared to other dicotyledons which have epigeal rather than hypogeal germination. The small grains provide support for the pea vines to reduce lodging and make harvesting easier as well as to help balance the nutrients in the livestock ration (Piper, 1924; Robinson, 1960; Carter and Larson, 1964; Klebesadel, 1969; Murray and Slinkard, 1973; Martin et al., 1976). Field peas may

also be used to provide feed or otherwise attract ducks (<u>Anas platyrhynchos</u>) to improve waterfowl hunting or to be used in other wildlife management programs (Shearer et al., 1971).

D. Cultivation

Field peas can withstand moderate to heavy frosts, except during anthesis, but they exhibit sensitivity to high temperatures especially when combined with high humidity. They are somewhat resistant to drought and are grown in semi-arid to humid regions. However, best results are obtained in cooler areas with moderate rainfall. Peas can grow in a wide range of soil types with the optimum proposed as a well-drained, calcareous loam (Piper, 1924).

In general, winter peas are planted in the fall and harvested the following spring as a winter annual. There are also spring pea varieties which are planted in the spring and harvested later as a summer annual. This distinction is similar to that between spring and winter varieties of wheat (<u>Triticum aestivum</u>), however, the flowering of most Austrian winter pea varieties is not dependent upon exposure to cold temperatures (Piper, 1924; Martin et al., 1976; Leffel, 1978). Murray and Auld (1980) have studied spring planting, as opposed to the normal fall planting, of Austrian winter peas and compared this to the planting of spring pea varieties in Idaho. They observed that fall planted winter peas flowered and matured sconer than spring planted winter peas. More importantly they found that spring planted winter peas took longer to mature and generally yielded less than spring pea varieties. Early planting consistantly gave greater seed yields than late planting, when sowing was done in the spring. The advantages of planting winter peas in the spring were reduced seeding expenses because of lower seeding rates, increased disease resistance, and higher market prices for winter peas compared to spring peas.

Breeding work has been done by plant scientists at the University of Idaho working with Michigan State, Washington State, and Oregon State Universities. Since 1965, much of their work has been sponsored by the Idaho Fea and Lentil Commission to improve the yield, winterhardiness, and pest resistance of the standard common variety of Austrian winter pea (Murray and Slinkard, 1973). Some distinction has also been noted between marrowfat and blue pea types (Bland, 1971). Marrowfat peas typically have large seeds, high quality, and high market prices but they give low yields and are prone to lodge. The blue types, however, have less problems with lodging and have high yields but this is offset by lower market prices. In England, most (80%) of the peas planted for dry pea production are marrowfat peas. Table II lists some of the known varieties of field peas.

Austrian winter peas should be sown from September to October, or the same time as small grains, using seed with at least 90% germination. The seeding rate and row spacing are shown in Table III and depend on such things as planting date, the condition of the seedbed, available moisture, the size and quality of the seed, as well as the variety used. The seeds should be inoculated with <u>Rhizobium leguminosarum</u> (Frank) and planted about 5 cm (2 in) deep. Piper (1924) noted that pea seeds have germinated from as deep as 20 cm (8 in). Within the row, spacings of about 14-17 seeds per m (5-6 seeds per ft) should give an established stand of 8 to 14 seedlings per m (3-5 seedlings per ft). Speed and uniformity of germination can be increased by not planting in heavy straw residue and by

TABLE II

SOME KNOWN VARIETIES OF FIELD PEAS AND THE AREA SOME OF THEM WERE DEVELOPED¹

Winter Peas	Released from
Small seeded Common	
Fenn	Idaho
Melrose	Idaho
Large seeded	
ID 2	Idaho
ID 89-1	Idaho
Romack	Georgia
Others	
Popago	Arizona
Dixie Wonder	
Spring Peas	
Alaska	
Garfield	
Tracer	
Latah	
First And Best	
Cther Field Peas	
Arthur	
Canadian Beauty	
Golden Vine	
Early Britain	
Marrowfat	
Blackeye Marrowfat	
Prussian Blue	
Wisconsin Blue	
Concordia	Sweden
Kaiser	Germany
Amraoti	India
Bangalia	India
Chancellor	
Stral	

 Adapted from Piper (1924), Carter and Larson (1964), Murray et al. (1978), Auld et al. (1979), and Murray and Auld (1980). rolling the soil before and after planting to get better contact between the soil and the seed. Heavy straw residues may interfere with seed placement, may increase some diseases of peas, or may release inhibitory toxins upon decomposition. Care should be exercised when rolling a clay soil to avoid overworking it or any other soil type where erosion is or may be a problem. Peas respond to applications of phosphorus (P), potassium (K), and small amounts of sulfur when grown on soils deficient in these nutrients according to Murray et al. (1978). Phosphorus can be banded at planting time, potassium may need to be added to some sandy soils, and caution should be used to avoid application of too much sulfur.

TABLE III

RECOMMENDED SEEDING RATES AND ROW SPACINGS FOR FIELD PEAS¹

Kind of Seed	Seeding Rate ² Kg/ha (lbs/acre)	Row Spacing cm (in)
Breeder, Foundation,		
and Registered	45-70 (40-60)	30-35 (12-14)
Certified and Common	70-100 (60-90)	15-18 (6-7)
Small-seeded Varieties ³		
9860 seeds/Kg (4400 seeds/lb)	100 (90)	
Large-seeded Varieties ³		
7920 seeds/Kg (3600 seeds/lb)	135 (120)	
Fall Planted Winter Varieties ³	85-100 (75-90)	
Spring Planted Winter Varieties3	135 (120)	
Spring Variaties	140-200 (125-125)	
Deag (ante Minture	70/h = (60/h = 0)	
reas/oats Mixture	(00/40)	

- 1. Adapted from Carter and Larson (1964), Murray and Slinkard (1973), and Murray et al. (1978).
- 2. Higher rates for rough seedbeds, heavy straw residue, and/or late planting dates. Lower rates for low moisture conditions and/or early planting dates.

3. Refer to Table II.

The most common weed problems in peas are caused by annual weeds such as wild oats (Avena fatua), lambsquarters (Chenopodium album), and several of the mustards (Sisybrium spp). Various insects feed on field peas including the pea weevil (Bruchus pisorum), pea aphid (Illinois pisi), pea moth (Laspeyresia nigricana), alfalfa looper (Colias eurytheme), celery looper (Noctuidae family), wireworms (Elateridae family), and root-knot nematodes. Disease problems are mainly the result of a root rot complex (Fusarium solani f. pisi and Pythium ultimum). However, bacterial blight (Pseudomonas pisi), Fusarium wilt (F. oxysporum f. pisi), Ascochyta blight (Ascochyta pisi, A. pinodella, and Mycosphaerella pinodes), powdery mildew (Erysipha polygoni), downy mildew (Peronspora pisi), white mold (Sclerotinia), anthracnose (Collectotrichum pisi), and Septoria pisi as well as a few viruses are known to have an effect on the growth of field peas (Martin et al., 1976). Ali-Khan and Zimmer (1972) recommend a three to five year rotation as a good control measure for pest problems. Other control methods may include crop residue management, cultivation, changing the time of planting or harvesting, biological control, the use of resistant varieties, treated seed, or pesticides.

Dry peas may be harvested with a combine. Peas may also be swathed, cured, and baled for later use as livestock feed. They may be grazed directly in the field (usually in combination with small grains) or they can be chopped and used to make silage. Yields are highly variable and depend not only on the management skill of the producer but also on the numerous environmental factors that influence the crop. According to Martin et al. (1976) approximately 9 million hectares (ha) are planted annually for dry pea production throughout the world, with an average yield of 1.1 metric tons/hectare (mt/ha). In the United States during the early 1970's about 80,000 ha were planted for dry pea production annually which yielded 1.8 mt/ha. Based on work done by Carter and Larson (1964) in North Dakota, dry forage estimates may range from 1.7 to 4.5 mt/ha (0.75 to 2 T/acre), with and without irrigation, respectively for Austrian winter peas. When planted with oats the yields ranged from 2.3 to 10.5 mt/ha (1.0 to 4.6 T/acre). Dry pea yields from Murray and Auld (1980) in Idaho ranged from 1.8 to 7.6 mt/ha (1.600 to 6.700 lbs/acre).

CHAPTER III

MATERIALS AND METHODS

These series were conducted in the greenhouse at Oklahoma State University, Stillwater, OK. The soil used was a Eufaula loamy sand (siliceous, thermic, Psammentic Paleustalf) with analysis presented in Table IV. In Series I three Kg of air dry soil were weighed and thoroughly mixed with the proper fertility treatment prior to being divided equally into 1 Kg among each of 3 replicate pots. Fertility treatments were applied to the first series with the second and third series raised in the same soil. The source and nutrient levels are given in Table V with treatment combinations shown in Table VI.

TABLE IV

EPIPEDON (1.5 CM) ANALYSIS OF A TYPICAL EUFAULA LOAMY SAND¹

pH Operation Matthew (27)	6.1
Urganic Matter (%)	1.4
Total N (%)	0.03
Available P (ppm)	30.5
CEC (meq/100 g soil)	2.88
Exchangeable cations (meg/100 g soil)	
Ca	1.40
Mg	0.60
X	0.11
Na	0.06
Prior use: Gropped to small grains for 14 years.	
1. Adapted from Gray and Roozitalab (1976).	

TABLE V	۷
---------	---

Nutrient	Source	Level
Ca.	CaCO ₃	6 meq/100 g soil
P	Ca(H2PO4)2	200 ppm
K ₁	KC1	200 ppm
K2	KC1	400 ppm
K3	KC1	600 ppm

SOURCE AND NUTRIENT LEVELS FOR AUSTRIAN WINTER PEA, SERIES I, II, AND III

TABLE VI

TREATMENT COMBINATIONS FOR AUSTRIAN WINTER PEA, SERIES I, II, AND III

Treatment	Symbol	Treatment	Symbol
1	0	9	Ca
2	K1	10	CaK1
3	K2	11	CaK2
4	K3	12	CaK3
5	P	13	CaP
6	PK1	14	CaPK1
7	PK2	15	CaPK2
8	PK3	16	CaPK3

The seeds were inoculated by soaking in a solution of distilled water and <u>Rhizobium leguminosarum</u> (Frank) (at least 10⁹ viable cells per ml) for one hour prior to planting. Age, as well as the planting and harvest dates of each series are presented in Table VII. Ten seeds per pot were planted approximately 5 cm (2 in) deep, watered thoroughly, and thinned to 5 plants per pot after establishment. The plants were grown in a short-day photoperiod and were tied to support stakes and sprayed for insect control throughout the experiment.

TABLE VII

Series	Age (days)	Planting Date	Harvest Date
I	61	17 Oct 1980	17 Dec 1980
II	84	18 Dec 1980	11 Mar 1981
III	61	18 Mar 1981	18 May 1981

AGE, PLANTING AND HARVEST DATES FOR AUSTRIAN WINTER PEA

These experiments were harvested as follows: Series I between 8:00 and 10:00 a.m., in Series II half of the pots (24) were also harvested between 1:00 and 3:00 p.m. and Series III was harvested from 8:00 a.m. to 12:30 p.m. This refers to the time period from the harvest of the first pot to the beginning of the first nitrogenase incubation. The plants were harvested, after measuring their maximum length (except for Series I), by clipping at the soil level to remove the top growth. The remaining contents of the pot were then emptied and shaken gently to remove the root-nodule system which was briefly rinsed and then blotted to remove excess water. The root-nodule system was then placed in a 70 ml clear glass serum bottle and fitted with a rubber stopper for nitrogenase (nitric-oxide reductase EC 1.7.99.2) activity determinations as described by Hardy et al. (1968).

Purified, laboratory grade acetylene gas (5 cc) from Union Carbide, Linde Division was injected to 0.1 atmosphere pressure at 27° C in each sample bottle with a 5 cc plastic syringe fitted with a needle. These samples were then incubated for 60 min except Series II which had incubations of 60 and 90 min. Reduction samples of 5 cc were collected after each incubation period with a syringe and after thorough mixing, was transferred to a smaller (15 ml) brown glass serum bottle which had been fitted with a rubber stopper. Acetylene reduction to ethylene was determined for each incubation period on a Perkin Elmer 3920 Gas Chromatograph with a 1.83 m x 3.2 m Poropak N 80/100 column (Waters Assoc.). The ethylene standard used for calibrating and monitoring gas chromatograph analyses was a Scott Ev. Tech. 1090 ppm + 5% C₂H₄/N₂ (Supelco, Inc.). After collecting the reduction samples from the final incubation period, the stoppers were removed from the 70 ml sample bottles to allow the gases to escape (approximately 5-15 min). The stoppers were then replaced and these root-nodule samples were stored at 0-5° C until the nodules could be removed later that day and/or the following day.

The nodules were removed from the root system and counted after obtaining the root and nodule fresh weight on a Mettler balance (Scientific Products, Division of American Hospital Supply Corp.). The fresh nodules were then weighed to 0.1 mg on a Mettler H18 balance (Mettler Instrument Corp.). The whole excised nodules were stored at $0-5^{\circ}$ C in a test tube fitted with a rubber stopper which had a CHCl₃ cotton pad to retard the growth and development of other microorganisms. The tops and roots were bagged separately and identified according to treatment and replication.

then were dried at 40° C for 24 hrs and weighed.

The nodules were later removed from storage and weighed into two 1 g samples, each of which was placed in separate digestion tubes for nitricperchloric digestion. The first 1 g sample was digested simply as whole nodules, but the second sample was divided into its cell-free extract (cytosol) and organelle extract components. The nodules in the second 1 g sample were crushed in separate test tubes with a glass rod and 10 ml of distilled water were added for each gram of nodule weight. This homogenate was then ultrasonified at 7.3 K pulse frequency for 30 sec in an ice bath with a PT 105T Williams Polytron (Brinkman Instruments, Inc.). Next these preparations were separated into cytosol and organelles by refrigerated centrifugation for 10 min at $4x10^3$ g. The cytosol and organelles were then each transferred to separate digestion tubes for nitric-perchloric digestion.

The digestion was done by adding 5 ml of concentrated (70%) nitric acid and 2 ml of perchloric acid (72%), mixing thoroughly, and then allowing the sample to sit overnight. The following morning the digestion samples were placed in a 40-sample capacity digestion block and heated to 126° C for 1 hr, then to 151° C for 1/2 hr, and finally to 260° C for 2 hr. Temperature and timing were controlled by a micro processor (Tecator 1008 heating block control unit) and all digestions were carried out under a ventilated hood. After the digestions were completed the tubes were volumetrically brought up to 35 ml with 0.1N HCl, placed in a 50 ml erlenmeyer flask, and stoppered prior to being analyzed for P, K, Ca, Mg, Na, and Fe by atomic absorption techniques.¹ K. Ca, and Mg were

¹Laboratory procedures Oklahoma State University Agronomic Services Laboratory for Soil, Plant, and Water Analysis (revised 1980).

determined in lanthanum chloride solution.

These greenhouse experiments were carried out as a randomized complete block design with each treatment replicated 3 times. The 3 factors studied were: 2 levels of phosphorus, 2 levels of calcium, and 4 levels of potassium. Spatial randomization was accomplished, for each series after treatment application, by assigning a number from 1 to 48 for each treatment, then arranging the location of the pots according to the sequence determined from a table of random numbers. The resulting data were analyzed with the Statistical Analysis System (SAS, 1979). PROCEDURE ANOVA (Series I and III) and PROCEDURE GLM (Series II) were used to compare the fertility treatments (nutrient main effects) on each variable with Duncan's test for significant differences. PROCEDURE CORR was used to check for possible relationships among the variables that were measured. The parameters measured in these studies are listed in Table VIII.

TABLE VIII

AUSTRIAN WINTER PEA PARAMETERS MEASURED FOR GHEENHOUSE EXPERIMENTS WITH EUFAULA SOIL

Parameter	Units
Top Weight Top Length Root Weight Nodule Weight Nodule Number Nitrogenase Nodule Mineral Composition Whole Nodule Nodule Cytosol Nodule Organelles	g (dry) cm g (dry) g (fresh) nodules/culture µmol C ₂ H4/culture/hr % (P, K, Ca, Mg, Na, Fe)

CHAPTER IV

DISCUSSION OF RESULTS

A. Series I

In Series I the pots were first watered in a cement bench partly filled with sand by means of a soaker hose buried beneath the sand. Even though germination was rapid, growth proceeded slowly. The pots were moved to the top of a bench and watered in saucers. Partly due to a slow start, coupled with an early harvest, there was a shortage of nodule material for nodule composition analysis. The nodules of the three replicates were composited for each treatment and only the whole nodules were analyzed. As a result only significant correlations are discussed for whole nodule composition. The Fe content in this series was expressed in ppm ($\mu g/g$) rather than %.

The results of Series I are presented in Tables IX to XIV. The K level effects, with and without P and Ca, are given in Tables IX and X. K caused significant decreases in every parameter measured except top weight as shown in Table XI when Ca and P levels were pooled. The effects of Ca and P, with K levels pooled, are given in Table XII. Here Ca caused a significant increase in root weight but gave significant decreases in top weight, nodule number, and nodule weight. P gave significant decreases in every parameter measured except nitrogenase where it resulted in a nonsignificant increase. The comparisons for K level responses, with and without P and Ca, are presented in Table XIII along with significant

Treatment ¹	Parameter ²						
	Top Wt	Rt Wt	Nod Wt	Nod No	Nase		
0	2.23 a	0.40 abcd	0.696 a	158 a	46.0 a		
K ₁	1.87 ab	0.37 bcde	0.544 ab	73 b	2.7 b		
K ₂	1.73 abed	0.33 bcde	0.539 ab	59 Ъ	11.0 ab		
K3	1.80 abc	0.37 bcde	0.313 bc	40 Ъ	4.7 Ъ		
P	1.47 bcde	0.30 cde	0.529 ab	41 Ъ	46.7 a		
PK1	1.03 de	0.20 e	0.065 cd	5Ъ	6.7 ab		
PK2	1.60 abcd	0.30 cde	0.081 cd	22 Ъ	13.3 ab		
PK3	1.13 cde	0.20 e	0.141 cd	18 Ъ	11.3 ab		
Ca	1.47 bcde	0.57 a	0.120 cd	27 Ъ	12.7 ab		
CaK1	1.40 bcde	0.40 abcd	0.149 cd	23 Ъ	20.7 ab		
CaK2	1.33 bcde	0.50 ab	0.090 cd	17 b	4.3ъ		
Cakz	0.87 •	0.27 de	0.011 d	3 Ъ	2.3 Ъ		
CaP	1.37 bcde	0.47 abc	0.227 cd	18 Ъ	28.0 ab		
CaPK1	1.43 bcde	0.57 a	0.214 cd	26 Ъ	15.7 ab		
CaPK2	1.26 bcde	0.30 cde	0.177 cd	26 ъ	8.7 ab		
CaPK3	1.43 bcde	0.30 cde	0.074 cd	11 b	2.7 b		

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C2H2 REDUCTION) OF AUSTRIAN WINTER PEA, SERIES I

TABLE IX

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of three reps with 5 plants/culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Rt Wt = g dry, Nod Wt = g fresh, Nod No = Nodules/culture, Nase = μ mol C₂H₄/culture/hr.

Treatment ¹		Whol	e Nodule C.	omposition	2	
	P	К	Ca	Mg	Na.	Fe
0	0.28	1.81	0.30	0.33	0.80	84
K ₁	0.23	3.10	0.32	0.22	0.58	85
K2	0.28	3.60	0.73	0.15	0.27	100
Кз	0.04	0.63	0.05	0.02	0.04	16
P	0.97	2.30	0.33	0.34	0.89	111
PK1	0.96	4.05	0.83	0.26	0.58	1012
PK2	0.80	4.04	0.55	0.24	0.43	240
PKa	0.37	1.55	0.16	0.76	0.13	58
Ca	0.18	0.86	0.31	0.12	0.40	62
CaK1	0.13	1.24	0.20	0.08	0.13	43
CaK2	0.22	1.95	0.52	0.09	0.43	141
CaKa	0.25	1.57	1.10	0.16	1.07	110
CaP	0.09	0.46	0.15	0.06	0.15	31
CaPK+	0.08	0.78	0.13	0.03	0.08	32
CaPKo	0.11	1.18	1.90	0.04	0.13	65
CaPK3	0.41	3.51	0.81	0.14	0.47	1380

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES I

TABLE X

1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.

2. Figures are from analysis of composites for 3 treatment reps expressed as %, except Fe which is $\mu g/g$ nodule fresh weight with 5 plants/culture.

Treatment ¹	Parameter ²					
	Top Wt	Rt Wt	Nod Wt	Nod No	Nase	
0 K ₁ K ₂ K ₃	1.63 a 1.43 a 1.48 a 1.31 a	0.43 a 0.38 a 0.36 ab 0.28 b	0.393 a 0.243 b 0.222 b 0.135 b	61 a 32 ab 31 ab 18 b	33.3 a 11.4 b 9.3 b 5.3 b	

EFFECTS OF K LEVELS ON GROWTH, NODULATION, AND NITROGENASE (C2H2 REDUCTION) OF AUSTRIAN WINTER PEA, SERIES I

TABLE XI

1. Treatment levels as mg K/Kg soil; $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl. Ca and P levels pooled.

2. Figures are means of 12 observations with 5 plants/culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at 0.05 level.

Refer to Table IX for description of variables.

TABLE XII

EFFECTS OF CA AND P LEVELS ON GROWTH, NODULATION, AND NITROGENASE (C2H2 REDUCTION) OF AUSTRIAN WINTER PEA, SERIES I

Parameter		Trea	Treatment ¹		
		Ca		P	
Top Wt	with	1.32	b	1.34	b
(g dry)	without	1.61	a	1.59	a
Rt Wt	with	0.42	а.	0.33	Ъ
(g dry)	without	0.31	Ъ	0.40	а
Nod Wt	with	0.133	b	0.189	b
(g fresh)	without	0.364	a	0.308	a
Nod No	with	19	b	21	b
(nodules/culture)	without	52	a	50	a
Nase	with	11.9	a	16.6	a
(µmol C ₂ H4/culture/hr)	without	17.8	a	13.0	a

1. Treatment levels: $P = 200 \text{ mg } P/\text{Kg soil as } Ca(H_2PO_4)_2$; Ca = 6 meq/100 g soil as CaCC₃. K levels pooled.

Figures are means of 24 observations with 5 plants/culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

TABLE XIII

COMPARISONS FOR EFFECTS OF K LEVELS, WITH P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA, SERIES I

Parameters	Combination Treatment ¹	K Levels ¹				Treatment Effect ²	
		0	K1	K2	Кз		
Top Wt (g dry)	0 P Ca CaP	2.23 1.47 1.47 1.37	1.87 1.03 1.40 1.43	1.73 1.60 1.33 1.27	1.80 1.13 0.87 1.43	Ca ** P * CaxP **	
Rt Wt (g dry)	0 P Ca CaP	0.40 0.30 0.57 0.47	0.37 0.20 0.40 0.57	0.33 0.30 0.50 0.30	0.37 0.20 0.27 0.30	Ca *** P * K ** CaxPxK *	
Nod Wt (g fresh)	0 P Ca CaP	0.696 0.529 0.120 0.227	0.544 0.065 0.149 0.214	0.539 0.081 0.090 0.177	0.313 0.141 0.011 0.074	Ca *** P ** K *** CaxP ***	
Nod No (nodules/ culture)	0 P Ca CaP	158 41 27 18	73 5 23 26	59 22 17 26	40 18 3 11	CaxK * Ca ** P * CaxP **	
Nase (µmol C ₂ H4/ culture/hr)	0 P Ca CaP	46.0 46.7 12.7 28.0	2.7 6.7 20.7 15.7	11.0 13.3 4.3 8.7	4.7 11.3 2.3 2.7	K **	

1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl: P = 200 as $Ca(H_2PO_4)_2$; with Ca = 6 meq/100 g soil as CaCO₃.

Figures are means of three reps with 5 plants/culture.

2. *, **, *** Level of significance at P< 0.05, 0.01, and 0.001 respectively.

TABLE XIV

CORRELATIONS AMONG GROWTH, NODULATION, NITROGENASE $(C_2H_2 \text{ REDUCTION})$, AND WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES 1¹



1. Figures are correlation coefficients based on 48 observations, except whole nodule composition which has 16 observations, with 5 plants/culture.

*, **, *** Level of significance at P $\langle 0.05, 0.01$, and 0.001 respectively.

Cations as % except Fe which is $\mu g/g$ nodule fresh weight.

Treatment levels and variable descriptions are given in Table IX.

interactions. K was shown to have a significant effect on root weight, nodule weight, and nitrogenase. The P and Ca effects were significant for every parameter except nitrogenase. The most important interaction appeared to be CaxP which was observed for every parameter except root weight and nitrogenase. In addition root weight also had significant CaxPxK interaction and nodule weight gave a CaxK interaction. Correlations are given in Table XIV with significant correlations noted among all growth, nodulation, and nitrogenase parameters except for root weight x nodule weight, nodule number, and nitrogenase. The only whole nodule cation that was significantly correlated with growth, nodulation, or nitrogenase was Mg x root weight (-). In addition P x K, P x Na, and K x Fe were the only other whole nodule correlations observed.

B. Series II

The plants in Series II attained very favorable growth following germination within 3 days after planting. They grew rapidly with most treatments growing from 1.5 to 1.8 m in length similar to results of Auld et al. (1979). The plants in this series provided ample nodule material for analysis of 2 nodule composition replicates. Some treatment reps were combined to obtain 2 g of nodule material for the second nodule composition rep. The results of Series II are presented in Tables XV to XXIX.

Significant differences among the treatments for K level effects, with and without P and Ca, are given in Tables XV to XVIII. Ca treatments apparently increased the Ca levels of the nodule cytosol. In the nodule organelle fraction, the P treatment was significantly higher (P < 0.05) for Fe than all other treatments. There were no significant differences

TABLE XV

Treat-	Freat-					
ment ¹	ment ¹ Parameter ²					
	Top Wt	Top Ln	Rt Wt	Nod Wt	Nod No	Nase
0	6.47 d	164.3 ab	1.17 abcd	2.167 abcd	307 bcd	31.7 b
K ₁	6.73 abcd	164.2 ab	1.63 a	1.291 cd	298 bcd	21.7 b
K ₂	6.03 de	159.1 ab	1.37 abc	1.938 bcd	248 cd	188.0 ab
K ₃	6.13 de	168.5 ab	1.40 ab	1.539 bcd	327 bcd	74.0 b
P	7.77 abc	170.2 ab	1.10 bcd	2.834 abc	513 a	137.7 ab
PK1	6.50 d	161.7 ab	0.80 d	2.277 abcd	354 bcd	270.7 ab
PK2	7.83 ab	157.5 ab	0.93 bcd	3.685 a	373 bc	404.3 ab
PK3	7.97 a	171.0 ab	1.07 bcd	2.316 abcd	413 ab	330.7 ab
Ca	5.57 de	163.4 ab	1.00 bcd	2.141 abcd	278 cd	86.0 ab
CaK1	6.00 de	165.1 ab	1.10 bcd	1.195 d	258 cd	407.3 ab
CaK2	6.30 de	154.1 ab	0.90 bcd	1.802 bcd	235 d	250.7 ab
CaK3	5.13 e	149.9 b	0.67 d	2.872 ab	285 bed	547.7 a
CaP	6.30 de	177.8 a	0.87 cd	3.077 ab	242 ed	299.0 ab
CaPK1	6.57 cd	159.2 ab	1.00 bcd	1.887 bcd	237 d	179.7 ab
CaPK2	6.47 d	155.0 ab	0.87 cd	2.301 abcd	229 d	244.7 ab
CaPK3	6.63 bcd	171.9 ab	0.93 bcd	1.911 bcd	316 bed	229.3 ab

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA, SERIES II

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of three reps with 5 plants/culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Top Ln = cm, Rt Wt = g dry, Nod Wt = g fresh, Nod No = nodules/culture, Nase = μ mol C₂H₄/culture/hr.

Treat- ment ¹	Whole Nodule Composition ²								
	P	ĸ	Ca	Mg	Na	Fə			
0 K ₁ K ₂ K ₃ P PK ₁ PK ₂ PK ₃ Ca CaK ₁ CaK ₂ CaK ₃ CaP CaPK ₁	0.58 abc 0.51 bc 0.53 bc 0.48 c 0.62 abc 0.65 ab 0.71 a 0.67 ab 0.51 bc 0.61 abc 0.60 abc 0.58 abc 0.60 abc 0.62 abc	1.16 fg 1.46 efg 2.22 cdefg 3.22 bc 1.03 g 2.48 bcde 2.87 bcd 3.15 bc 1.05 g 1.92 defg 3.50 ab 4.43 a 1.40 efg 2.33 bcdef	0.62 bcd 0.51 cd 0.63 bcd 0.53 cd 0.68 abcd 0.52 cd 0.48 d 0.57 cd 0.78 abc 0.63 bcd 0.90 ab 0.97 a 0.73 abcd 0.72 abcd	0.70 a 0.68 ab 0.62 abcd 0.58 abcd 0.62 abcd 0.50 d 0.48 d 0.52 d 0.67 abc 0.72 a 0.55 bcd 0.48 d 0.58 abcd 0.58 abcd	1.99 a 1.40 bcd 0.98 e 0.95 e 1.93 a 1.38 bcd 1.44 bc 1.08 cde 1.61 ab 1.52 b 0.83 e 0.68 e 1.64 ab 1.53 b	0.41 a 0.25 a 0.39 a 0.39 a 0.71 a 0.41 a 0.51 a 0.63 a 0.49 a 0.58 a 0.64 a 0.63 a 0.63 a 0.63 a			
CaPK ₁ CaPK ₂ CaPK ₃	0.60 abc 0.62 abc 0.61 abc 0.62 abc	1.40 efg 2.33 bcdef 3.42 abc 3.40 abc	0.73 abcd 0.72 abcd 0.70 abcd 0.70 abcd	0.58 abcd 0.62 abcd 0.53 cd 0.58 abcd	1.64 ab 1.53 b 1.02 de 0.80 e	0.63 0.49 0.41 0.36			

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

TABLE XVI

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of three reps with 5 plants/culture. Cations are given as %.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Treatment ¹		Nodul	e Cytosol	Composit	ion ²	
	P	К	Ca	Mg	Na	Fe
0 K ₁ K ₂ K ₃ P PK ₁ PK ₂ PK ₃ Ca CaK ₁ CaK ₂ CaK ₃ CaP CaPK ₁ CaPK ₂	0.29 a 0.29 a 0.23 a 0.29 a 0.28 a 0.29 a 0.35 a 0.24 a 0.20 a 0.31 a 0.31 a 0.31 a 0.31 a	0.70 ef 1.13 def 1.58 bed 2.23 a 0.55 f 1.08 def 1.33 cde 1.55 bed 0.50 f 1.42 bede 1.97 abe 2.58 a 0.60 f 1.50 bed 2.13 ab	0.06 c 0.06 c 0.08 c 0.08 c 0.03 c 0.06 c 0.08 c 0.18 abc 0.17 abc 0.32 ab 0.33 a 0.15 abc 0.20 abc 0.13 bc	0.30 a 0.38 a 0.18 a 0.29 a 0.25 a 0.23 a 0.23 a 0.20 a 0.30 a 0.33 a 0.32 a 0.25 a 0.23 a 0.25 a 0.23 a	1.16 a 1.09 abc 0.49 d 0.66 cd 1.12 ab 0.88 abcd 1.00 abc 0.70 cd 1.03 abc 0.98 abcd 0.74 abcd 0.56 d 1.00 abc 1.00 abc 1.00 abc	0.16 ab 0.14 b 0.26 ab 0.15 ab 0.15 ab 0.17 ab 0.13 b 0.13 b 0.30 a 0.14 b 0.24 ab 0.14 b 0.14 b 0.13 ab 0.13 b 0.13 b 0.13 b 0.13 b 0.13 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.15 ab 0.15 ab 0.17 ab 0.17 ab 0.13 b 0.14 b 0.12 ab 0.13 b 0.14 b 0.14 b 0.15 ab 0.15 ab 0.17 ab 0.13 b 0.14 b 0.14 b 0.15 ab 0.15 ab 0.17 ab 0.13 b 0.14 b 0.14 b 0.15 ab 0.15 ab 0.13 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.14 b 0.15 ab 0.14 b 0.15 ab 0.14 b 0.15 ab 0.14 b 0.15 ab 0.14 b 0.15 ab 0.14 b 0.15 ab 0.15 ab 0.14 b 0.15 ab 0.15 ab 0.14 b 0.15 ab 0.15 ab 0.14 b 0.15 ab 0.15 ab 0.15 ab 0.16 ab 0.17 ab 0.17 ab 0.17 ab 0.18 ab 0.19 b 0.19 b 0.11 b 0.11 b 0.11 b 0.11 b

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON NODULE CYTOSOL COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

TABLE XVII

1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.

2. Figures are means of two reps, except K_1 and CaK_1 which represent one rep, with 5 plants/culture. Cations are given as %.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.
TABLE XVIII

Treatment ¹	Nodule Organelle Composition ²									
	P	K	ũa	Mg	Na	Fe				
0 K ₁ K ₂ K ₃ P PK ₁ PK ₂ PK ₃ Ca CaK ₁ CaK ₂ CaK ₃ CaP CaPK ₁ CaPK ₁	0.33 al 0.26 al 0.31 al 0.25 b 0.33 al 0.29 al 0.26 b 0.26 b 0.35 al 0.35 al 0.35 al 0.39 a 0.39 a	0 0.53 d 0 0.70 abcd 0 0.72 abcd 1.30 abc 0 0.69 bcd 0 0.98 abcd 0.90 abcd 1.11 abcd 0 0.55 d 1.10 abcd 0 1.45 a 1.43 ab 0.59 cd 1.19 abcd	0.25 c 0.21 c 0.73 a 0.24 c 0.33 bc 0.22 c 0.23 c 0.19 c 0.51 b 0.28 bc 0.42 bc 0.42 bc 0.43 bc 0.43 bc	0.33 bcde 0.34 abcde 0.34 abcde 0.31 cde 0.35 abcd 0.32 bcde 0.26 de 0.24 e 0.42 ab 0.32 bcde 0.33 bcde 0.33 bcde 0.30 cde 0.43 a 0.36 abc	0.75 a 0.54 abcd 0.31 d 0.36 d 0.65 ab 0.64 abc 0.52 abcd 0.41 cd 0.65 ab 0.48 abcd 0.42 bcd 0.42 bcd 0.26 d 0.64 abc	0.11 b 0.10 b 0.15 b 0.11 b 0.23 a 0.11 b 0.12 a 0.13 b 0.12 b 0.13 b 0.12 b 0.13 b 0.12 b 0.13 b 0.12 b				

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON NODULE ORGANELLE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of two reps, except K_1 and CaK_1 , (and $CaPK_1$ for K) which represent one rep, with 5 plants/culture. Cations are given as %.

observed among the treatments for whole nodule Fe or for cytosol P and Mg contents.

The effects of K levels (P and Ca levels pooled) are shown in Table XIX. K caused significant decreases in top length, nodule weight, nodule number, whole nodule and organelle Mg, and Na in the whole nodule, cytosol, and organelle compositions. The only increases caused by K appeared to be with the K levels in all 3 nodule compositions measured. The effects of Ca and P (K levels pooled) are shown in Tables XX and XXI. Ca caused only increases in the nodule compositions with the exception of a decrease in whole nodule Na. It caused increases in the Ca content of all nodule compositions and cytosol K, as well as P and Mg contents of the nodule organelles. In addition Ca caused decreases in top and root weights and in nodule number. The P treatment caused significant increases in top and nodule weights and nodule number, but decreased root weight. In the nodule compositions P increased whole nodule P and decreased whole nodule Mg and organelle Ca.

Comparisons of the K level responses, with and without P and Ca, plus significant interactions are given in Tables XXII to XXV. Here K effects were shown to be significant for nodule weight and number, K and Na content of all nodule compositions, as well as organelle Ca and Mg contents. Ca effects were significant for top and root weights, nodule number, whole nodule Na, cytosol K and Ca, and organelle P, Ca, and Mg. P effects are presented as being significant for top, root, and nodule weights, nodule number, whole nodule P and Mg, and organelle Ca. No significant P effect was noted for any cytosol parameter measured. No significant K, P, or Ca effect was observed for top length, nitrogenase, whole nodule Fe, cytosol P, Mg or Fe, or organelle Fe contents. Although

Parameters ¹			К	Trea	tment ²			
· · ·	0		K ₁		K2		Кз	
Top Wt	6.53	а.	6.45	a	6.66	а.	6.47	2
Top Ln	168.9	a	162.6	ab	156.4	Ъ	165.3	ab
RtWt	1.03	a	1.13	a	1.02	a	1.02	a
Nod Wt	2.555	a	1.662	Ъ	2.431	a	2.160	ab
Nod No	335	a	286	ab	272	Ъ	335	8
Nase	138.6	a	158.3	a	271.9	a	293.6	a
Whole nodule								
P (%)	0.58	2	0.60	a	0.61	a	0.59	a
K (%)	1.16	đ	2.05	C	3.00	Ъ	3.55	a
Ca (%)	0.70	a	0.59	a	0.68	a	0.69	a
Mg (%)	0.64	a	0.63	a	0.55	ъ	0.54	Ъ
Na (76)	1.79	8.	1.46	ъ	1.07	C	0.88	d
Fe (%)	0.56	a	0.35	a	0.47	a	0.50	a
Nodule cytosol								
P (%) ·	0.23	8	0.30	a	0.30	a	0.28	a
К (Ж)	0.59	đ	1.28	C	1.75	b	2.23	2
Ca (%)	0.11	a	0.12	8.	0.14	8.	0.16	a
Mg (%)	0.27	a	0.31	a	0.25	a.	0.27	a
Na (%)	1.08	a	0.97	a	0.74	Ъ	0.63	ъ
Fe (%)	0.15	a	0.17	a	0.16	a	0.19	a
Nodule organelles								
P (%)	0.35	a	0.31	a	0.31	a	0.31	8
К (%)	0.59	Ъ	0.99	a	1.12	a	1.21	a
Ca (%)	0.38	ab	0.28	Ъ	0.43	a.	0.30	ъ
Mg (%)	0.39	a	0.34	ab	0.31	ъ	0.29	ъ
Na (%)	0.67	a	0.60	a	0.41	Ъ	0.33	Ъ
Fe (%)	0.14	a	0.11	a	0.12	a	0.12	a.

EFFECTS OF K LEVELS ON GROWTH, NODULATION, NITROGENASE (C2H2 REDUCTION), AND NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

TABLE XIX

1. Refer to Table XV for description of variables.

2. Treatment levels as mg K/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl. Ca and P levels pooled. Figures are means of 12 observations, except nodule cytosol and organelle compositions = 8 observations ($K_1 = 6$ observations and in nodule organelle K, $K_1 = 5$ observations), with 5 plants/culture. Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Parameter		Treat	ment ¹
		Ca	Р
Top Wt	with	6.12 b	7.00 a
(g dry)	without	6.93 a	6.05 ъ
Top In	with	162.0 a	165.5 a
(cm)	without	164.6 a	161.1 a
Rt Wt	with	0.92 b	0.95 b
(g dry)	without	1.18 a	1.15 a
Nod Wt	with	2.148 a	2.536 a
(g fresh)	without	2.256 a	1.868 b
Nod No	with	260 ъ	335 a
(nodules/culture)	without	354 a	279 Ъ
Nase (umol	with	248.9 a	261.1 a
$C_2H_4/culture/hr)$	without	182.3 a	170.1 a
Whole Nodule			
P (%)	with	0.60 a	0.64 a
· · /	without	0.59 a	0.55 b
K (%)	with	2.68 a	2.51 a
	without	2.20 b	2.37 a
Ca (%)	with	0.77 a	0.64 a
	without	0.57 b	0.70 a
Mg (%)	with	0.59 a	0.55 b
	without	0.59 a	0.63 a
Na (%)	with	1.20 b	1.35 a
	without	1.39 a	1.24 a
Fe (%)	with	0.48 a	0.52 a
	without	0.46 a	0.43 a

EFFECTS OF CA AND P LEVELS ON GROWTH, NODULATION, NITROGENASE (C₂H₂ REDUCTION), AND WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

TABLE XX

 Treatment levels: P = 200 mg P/Kg soil as Ca(H2PO4)2; Ca = 6 meq/100 g soil as CaCO3. K levels pooled.

Figures are means of 24 observations with 5 plants/culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Refer to Table XV for description of variables.

TABLE XXI

EFFECTS OF CA AND P LEVELS ON NODULE CYTOSOL AND ORGANELLE COMPOSITIONS OF AUSTRIAN WINTER PEA, SERIES II

Composition		Treatment ¹	
		Ca	P
Nodule Cytosol			
P (%)	with	0.27 a	0.29 a
	without	0.28 a	0.26 a
K (%)	with	1.67 a	1.41 a
	without	1.28 b	1.55 a
Ca (%)	with	0.20 a	0.11 a
	without	0.06 ъ	0.16 a
Mg (%)	with	0.29 a	0.26 a
	without	0.25 a	0.28 a
Na (%)	with	0.82 a	0.88 a
	without	0.87 a	0.81 a
Fe (%)	with	0.16 a	0.16 a
	without	0.18 a	0.17 a
Nodule Organelles			
P (%)	with	0.36 a	0.32 a
	without	0.28 b	0.32 a
K (%)	with	1.08 a	0.97 a
	without	0.88 a	0.98 a
Ca (%)	with	0.40 a	0.30 b
	without	0.30 b	0.41 a
Mg (%)	with	0.35 a	0.32 a
	without	0.31 b	0.33 a
Na (%)	with	0.48 a	0.53 a
	without	0.52 a	0.46 a
Fe (%)	with	0.11 a	0.12 a
	without	0.13 a	0.12 a

 Treatment levels: P = 200 mg P/Kg soil as Ca(H₂PO₄)₂; Ca = 6 meq/100 g soil as CaCO₃. K levels pooled.

Figures are means of 15 observations for Ca treatment. P has 16 observations with treatment and 14 observations without treatment except organelle K which has 15 observations. Each culture contained 5 plants.

TABLE XXII

COMPARISONS FOR EFFECTS OF K LEVELS, WITH P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA, SERIES II

Parameter	Combination Treatment ¹		K Le	vels ¹		Treatment Effect ²
		0	K ₁	K2	Кз	
Top Wt (g dry)	0 P Ca CaP	6.47 7.77 5.57 6.30	6.73 6.50 6.00 6.57	6.03 7.83 6.30 6.47	6.13 7.97 5.13 6.63	Ca *** P ***
Top Ln (cm)	0 P Ca CaP	164.3 170.2 163.4 177.8	164.2 161.7 165.1 159.2	159.1 157.5 154.1 155.0	168.5 171.0 149.9 171.9	
Rt Wt (g dry)	0 P Ca CaP	1.17 1.10 1.00 0.87	1.63 0.80 1.10 1.00	1.37 0.93 0.90 0.87	1.40 1.07 0.67 0.93	Ca ** P ** CaxP **
Nod Wt (g fresh)	0 P Ca CaP	2.167 2.834 2.141 3.077	1.291 2.277 1.195 1.887	1.938 3.685 1.802 2.301	1.539 2.316 2.872 1.911	P ** K *
Nod No (nodules/ culture)	0 P Ca CaP	307 513 278 242	298 354 256 237	248 373 235 229	327 413 285 316	P ** K * Ca *** CaxP **
Nase (umol C ₂ H4/culture/ hr)	0 P Ca CaP	31.7 137.7 86.0 299.0	21.7 270.7 161.3 179.7	188.0 404.3 250.7 244.7	74.0 330.7 547.7 222.0	

Refer to Table XV for treatment levels and description of variables.
 Figures are means of three reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P<0.05, 0.01 and 0.001 respectively.

TABLE XXIII

Whole Nodule Composition	Combination Treatment ¹		Treatment Effects ²			
		0	K ₁	K2	Кз	
P (%)	0 P Ca CaP	0.58 0.62 0.51 0.60	0.51 0.65 0.61 0.62	0.53 0.71 0.60 0.61	0.48 0.67 0.58 0.62	P *** CaxP *
K (%)	0 P Ca CaP	1.16 1.03 1.05 1.40	1.46 2.48 1.92 2.33	2.22 2.87 3.50 3.42	3.22 3.15 4.43 3.40	Ca ** K ***
Ca (%)	0 P C a CaP	0.61 0.68 0.78 0.73	0.51 0.52 0.63 0.72	0.63 0.48 0.90 0.70	0.53 0.57 0.97 0.70	Ca ***
Mg (%)	0 P Ca CaP	0.70 0.62 0.67 0.58	0.68 0.50 0.72 0.62	0.62 0.48 0.55 0.53	0.58 0.52 0.48 0.58	P ** K ** CaxP *
Na (%)	0 P Ca CaP	1.99 1.93 1.61 1.64	1.40 1.38 1.52 1.53	0.98 1.44 0.83 1.02	0.95 1.08 0.68 0.80	Ca ** K *** Caxk *
Fe (%)	0 P Ca CaP	0.41 0.71 0.49 0.63	0.25 0.41 0.27 0.49	0.39 0.51 0.58 0.41	0.39 0.63 0.64 0.36	

COMPARISONS FOR EFFECTS OF K LEVELS, WITH P AND CA, ON WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

1. Refer to Table XVI for treatment levels. Figures are means of three reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P < 0.05, 0.01 and 0.001 respectively.

TABLE XXIV

Nodule Cytosol Composition	Combination .on Treatment ¹ K Levels ¹					Treatment Effects ²
		0	К ₁	K2	Кз	
	0	0.29	0.29	0.23	0.29	
P (%)	P	0.28	0.29	0.35	0.24	
	Ca	0.20	0.32	0.31	0.22	
	CaP	0.17	0.31	0.31	0.35	
	0	0.70	1.13	1.58	2.23	Ca. ***
К (%)	P	0.55	1.08	1.33	1.55	K ***
	Ca	0.50	1.42	1.97	2.58	
	CaP	0.60	1.50	2.13	2.55	
	0	0.06	0.06	0.08	0.08	Ca. ***
Ca (%)	P	0.08	0.03	0.06	0.08	
	Ca	0.18	0.17	0.32	0.33	
	CaP	0.15	0.20	0.13	0.15	
	0	0.30	0.38	0.18	0.29	
Mg (%)	P	0.25	0.23	0.23	0.20	
0.01	Ca	0.30	0.33	0.32	0.25	
	CaP	0.24	0.35	0.28	0.33	
	0	1.16	1.09	0.49	0.66	¥ ***
Na. (%)	P	1.12	0.88	1.00	0.70	
	Ca	1.03	0.98	0.74	0.56	
	CaP	1.00	1.00	0.73	0.61	
	0	0.16	0.14	0.26	0.15	
Fe (%)	P	0.17	0.11	0.13	0.30	
	Ca	0.14	0.24	0.14	0.18	
	CaP	0.13	0.22	0.11	0.13	

COMPARISONS FOR EFFECTS OF K LEVELS, WITH P AND CA, ON NODULE CYTOSOL COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

- Refer to Table XVII for treatment levels. Figures are means of two reps, except K₁ and CaK₁ which represent one rep, with 5 plants/culture.
- 2. *, **, *** Level of significant differences at P < 0.05, 0.01 and 0.001 respectively.

TABLE XXV

Nodule Organelle Composition	Combination Treatment ¹ K Levels ¹					Treatment Effects ²
		0	K1	K2	Кз	
P (%)	0 P Ca CaP	0.33 0.33 0.35 0.39	0.26 0.29 0.35 0.35	0.31 0.26 0.32 0.38	0.25 0.26 0.39 0.34	Ca ***
K (%)	0 P Ca CaP	0.53 0.70 0.55 0.59	0.70 0.98 1.10 1.19	0.72 0.90 1.45 1.41	1.30 1.11 1.43 1.02	<u>K</u> **
Ca (%)	0 P Ca CaP	0.25 0.33 0.51 0.43	0.21 0.22 0.28 0.38	0.73 0.23 0.42 0.33	0.24 0.19 0.49	Ca * P ** K * Caxk * P*K *
Mg (%)	0 P Ca.	0.33 0.35 0.42	0.34 0.32 0.32	0.34 0.26 0.33	0.31 0.24 0.30	CaxPxK * Ca * K **
Na (%)	O P Ca CaP	0.75 0.65 0.65 0.64	0.54 0.64 0.48 0.64	0.31 0.52 0.42 0.43	0.36 0.41 0.26 0.31	K ***
Fe (%)	0 P Ca CaP	0.11 0.23 0.12 0.10	0.10 0.11 0.13 0.11	0.15 0.10 0.12 0.11	0.11 0.13 0.13 0.09	CaxP * CaxK * PxK * CaxPxK *

COMPARISONS FOR EFFECTS OF K LEVELS, WITH P AND CA, ON NODULE ORGANELLE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

1. Refer to Table XVIII for treatment levels. Figures are means of two reps, except for K₁, CaK₁, and CaPK₁ which represent one rep, with 5 plants/culture.

2. *, **, *** Level of significant differences at P \langle 0.05, 0.01 and 0.001 respectively.

no significant nutrient effect was shown for Fe in any of the nodule compositions, organelle Fe gave all possible significant interaction combinations. CaxP was the dominant interaction in Series II and it occurred for root weight, nodule number, whole nodule P and Mg, and organelle Fe. CaxK, PxK, and CaxPxK interactions occurred for organelle Ca and Fe. In addition a CaxK interaction was observed for whole nodule Na. No interactions were significant for any cytosol parameters.

Significant correlations are presented in Tables XXVI to XXIX, and only the significant correlations are discussed. Top weight was observed to be correlated with root weight and nodule number, but not with top length. Root weight was negatively correlated with nitrogenase. All top and root growth parameters were significantly correlated only with whole nodule composition. Top weight was positively correlated with P and negatively with Ca. Top length and root weight were positively correlated with Mg but negatively with X. Nodule number x top weight were correlated as were nodule weight x nitrogenase. Nodule weight was correlated positively with whole nodule Fe and negatively with Mg. Nodule number was correlated with cytosol Ca (-) and with the Fe content of nodule organelles. In this series nitrogenase was positively correlated with nodule weight and negatively with root weight. It also was observed to be correlated only with whole nodule composition, positively with K and negatively with Mg and Na.

In the whole nodule composition P was correlated with top weight and whole nodule Fe. K showed positive correlations with nitrogenase as well as cytosol and organelle K and gave negative correlations for top length and root weight and organelle Mg as well as whole nodule, cytosol, and organelle Na. Ca was positively correlated with whole nodule Fe,

cytosol Ca, and organelle P and negatively with top weight, nodule number, and organelle Na. Mg was positively correlated with top length, root weight, whole nodule Na, and cytosol Mg. Negatively Mg was correlated with nodule weight, nitrogenase, and whole nodule K. Na was positively correlated with whole nodule Mg and cytosol and nodule organelle Na contents. It was negatively correlated with nitrogenase, and whole nodule, cytosol, and organelle K (r = -0.66 to -0.81, P< 0.001). Fe was observed to be correlated with nodule weight, whole nodule P and Ca, cytosol P (-) and Mg (-), and organelle P. The high Fe content of the nitrogenase enzyme and ferridoxin components of <u>Rhizobium</u> cells, cell membrane, and leghemoglobin components may contribute to Fe being positively correlated with whole nodule and organelle P while negative correlations were demonstrated for cytosol P.

In the nodule cytosol fraction P was positively correlated only with cytosol Mg but was negatively correlated with whole nodule Fe, and organelle P and Mg. K was significantly correlated with nitrogenase, whole nodule and organelle K, cytosol Ca, organelle Mg (-), and whole nodule, cytosol, and organelle Na (-). Ca was correlated with nodule number (-), whole nodule and organelle Ca, plus cytosol K and Mg. Mg was shown to be positively correlated with whole nodule Mg and cytosol P, Ca, and Na. Negatively it was correlated with nitrogenase and whole nodule and cytosol Fe. Na was apparently correlated with nitrogenase (-), whole nodule, cytosol, and organelle K (-), cytosol Mg, including whole nodule and organelle Na. Fe was positively correlated with nitrogenase and negatively with cytosol Mg.

In the nodule organelle fraction P was correlated positively with whole nodule Ca and Fe, and organelle Mg while being negatively correlated

TABLE XXVI

	Top ¥t	Top Ln	Rt Wt	Nod Wt	Nod No	Nase
Top Wt			•33		• 56	
Rt Wt						49
Nod Wt		h				*** •43 **
Whole Nodule						
P	.41					
K	**	36 **	41 **			•47 ***
Ca	30 *				31 *	
Mg		.28	•39	34		50
Na		*	**	*		*** 34 *
Fe				.46		
Nodule Cytosol				***		
Ca					43 *	
Nodule Organelles					40	
					**	

CORRELATIONS AMONG GROWTH, NODULE PARAMETERS, AND NITROGENASE (C2H2 REDUCTION) OF AUSTRIAN WINTER PEA, SERIES 11¹

1. Figures are correlation coefficients based on 48 observations, except for nodule cytosol and organelle composition which are based on 30 observations, with 5 plants/culture.

*, **, *** Level of significance for P < 0.05, 0.01, and 0.001 respectively.

Refer to Tables XV to XVIII for treatment levels and description of variables.

TABLE XXVII

CORRELATIONS AMONG GROWTH, NODULE PARAMETERS, NITROGENASE (C2H2 REDUCTION), AND WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II¹

Whole Nodule Composition							
P	x	Ca	Mg	Xa	Fa		
•41 **		30 *					
•	36		.28		•		
	41		•39 **		-		
	-		34		.46		
		31 *			•••		
	•47 ***		50 ***	34 *	.32		
			64	81	-		
					•37		
,	5		\geq	•53 ***			
-	•74 ***			83	*		
	•	.63					
			•48 **		37		
	66 ***	40		•79 ***	ha		
		**			•41		
	.81 *** 41			66 ***			
	68	37		•86			
	P .41 **	P K -41 -41 -41 41 41 41 66 66 68	P K Ca -4130 	P K Ca Ng P K Ca Ng -4130 ++36 .28 41 .39 ++31 31 31 64 ++ 66 ++ 66 ++ 6837	P K Ca Ng Na .41 30 36 .28 31		

 Figures are correlation coefficients based on 48 observations, except nodule cytosol and organella composition which are based on 30 observations (nodule organelle K = 29 observations), with 5 plants/ sulture.

*, **, *** Level of significance for P \langle 0.05, 0.01, and 0.001 respectively.

Refer to Tables XV to XVI for treatment levels and description of variables.

TABLE XXVIII

CORRELATIONS AMONG NODULE PARAMETERS, NITROGENASE (C2H2 REDUCTION), AND NODULE CYTOSOL COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II¹

		Nodu	le Cytoso	ol Composi	ltion	
	Р	К	Ca	Mg	Na	Fe
Nod No			43 *			
Nase Whole Nodule K		•36 * •74 ***		39 *	38 * 66	•36 *
Ca			•63 ***			
Mg				•49 **		
Na		83 ***			•79 ***	
Fe Nodule Cytosol P	41 *			37 * .65		
K			•38		65 ***	
Ca				.44		
Mg Nodule Organelles P	43 *				•50 **	40 *
K		•65 ***			54 **	
Ca			•43			
Mg	48 **	48 **				
Na		78 ***			•86 ***	

 Figures are correlation coefficients based on 30 observations (29 for nodule organelle K), except Nod No and Nase which have 48 observations, with 5 plants/culture.
 *, **, *** Level of significance for P < 0.05, 0.01, and 0.001 respectively. Refer to Tables XV to XVIII for treatment levels and description of variables.

TABLE XXIX

CORRELATIONS AMONG NODULE PARAMETERS, NITROGENASE (C₂H₂ REDUCTION), AND NODULE ORGANELLE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES II

		Nodule Organelle Composition ¹ P K Ca Mg Na .44 ** .44 .81 41 68 *** * *** .49 37 * *** .86 *** .43 48								
	P	K	Ca	Mg	Na	Fe				
Nod No						•49 **				
Nase		•44								
Whole Medule		**								
MUOTE NOGRIE		0.4		4.4	40					
ĸ		•01		41	00					
A	40	***		*	***					
Ca	•49				37					
	**				*					
Na		00			.86					
_		***			***					
Fe	•41 *									
Nodule Cytosol										
P	- 43			- 48						
•	*			**						
К		.65		- 48	78					
		***		**	***					
Ca			•43							
No		F / 1	*		01					
Na					•80					
Nedula Omena allea		**			***					
Nodure Organeries	~			40						
P				•43						
V				*	1:0					
Α					49					
Na					**					
128				\sim	•45					
					**					

 Figures are correlation coefficients based on 30 observations (29 for nodule organelle K), except Nod No and Nase which have 48 observations, with 5 plants/culture.

*, **, *** Level of significance for P < 0.05, 0.01, and 0.001 respectively.

Refer to Tables XV to XVIII for treatment levels and description of variables.

with cytosol P. K in this fraction was correlated with nitrogenase, whole nodule and cytosol K, and whole nodule, cytosol, and organelle Na (-). Mg was correlated positively with organelle P and Na while negative correlations were shown with whole nodule and cytosol K as well as cytosol P. Na was observed to have positive correlations with organelle Mg and whole nodule and cytosol Na, with negative correlations for whole nodule Ca plus the K content of all nodule compositions. Fe and Ca in this fraction apparently had one correlation each and these were with nodule number and cytosol Ca respectively.

C. Series III

The plant growth in Series III was similar to that of Series II with an abundance of nodule material available for chemical analysis. The results of this series are presented in Tables XXX to XLII.

Tables XXX to XXXIII show the differences among the treatments for K level effects, with and without P and Ca. Treatments containing P appeared to increase top growth, compared to the check, especially when combined with K. Ca generally decreased top growth, however, with increasing K levels an apparent increase occurred. The P treatment reduced root growth significantly compared to the check, but increased nodule weight. Both nodulation parameters increased with P applications, especially when K was present, however, Ca decreased nodule weight and apparently increased nodule number. In the nodule cytosol composition, the P treatment resulted in the highest Na content and Ca applications appeared to decrease Fe. Increasing K levels increased the K and decreased the Na contents of all three nodule compositions measured. No significant treatment differences were noted for cytosol Mg content in this series.

TABLE XXX

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA, SERIES III

Treat- ment ¹			Parameter ²					
-	Top Wt	Top Ln	Rt Wt	Nod Wt	Nod No	Nase		
0 K1 K2 K3 P PK1 PK2 PK3 Ca CaK1 CaK2 CaK3 CaP CaPK1 CaPK2 CaPK3	4.60 bc 4.87 bc 4.53 bc 5.00 b 4.63 bc 6.13 a 6.20 a 6.30 a 3.90 cd 3.93 cd 3.93 cd 3.50 d 4.67 bc 4.67 bc 4.67 bc 4.67 bc 4.63 bc 5.20 b	117.7 abc 119.4 ab 124.9 ab 124.9 ab 127.0 ab 124.5 ab 128.3 a 129.1 a 125.7 ab 110.5 bc 117.3 abc 102.7 c 115.6 abc 113.9 abc 113.9 abc 113.9 abc 123.2 ab	1.27 ab 1.53 a 1.50 a 1.13 ab 0.80 b 1.23 ab 1.30 a 1.40 a 1.40 a 1.43 a 1.27 ab 1.27 ab 1.27 ab 1.27 ab 1.27 ab 1.27 ab 1.23 ab 1.23 ab 1.37 a	1.285 e 1.326 e 1.398 e 1.873 bcd 2.236 ab 2.449 a 2.322 ab 2.198 ab 1.564 de 1.523 de 1.521 de 1.974 bcd 1.713 cde 1.948 bcd 1.963 bcd 2.110 abc	309 bed 339 bed 248 d 311 bed 345 bed 384 bed 488 b 650 a 378 bed 303 ed 365 bed 397 bed 397 bed 397 bed 397 bed 397 bed 398 bed 449 be	7.3 a 6.3 a 52.0 a 8.7 a 48.0 a 11.7 a 33.3 a 38.3 a 38.3 a 31.7 a 33.7 a 34.7 a 37.7 a 37.7 a 33.0 a		

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of three reps with 5 plants per culture.

Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Top Wt = g dry, Top Ln = cm, Rt Wt = g dry, Nod Wt = g fresh, Nod No = nodules/culture, Nase = μ mol C₂H₄/culture/hr.

Abbreviations are Wt = Weight, Ln = Length, Rt = Root, Nod = Nodule, No = Number, Nase = Nitrogenase.

TABLE XXXI

Treat- ment ¹	- Whole Nodule Composition ²									
	Р	K	Ca	Mg	Na	Fe				
0 K ₁ K ₂ K ₃ P PK ₁ PK ₂ PK ₃ Ca CaK ₁ CaK ₂ CaK ₃ CaP CaPK ₁ CaPK ₂ CaPK ₃	0.52 abc 0.28 d 0.44 abcd 0.57 abc 0.52 abc 0.59 ab 0.52 abc 0.55 abc 0.44 abcd 0.44 abcd 0.44 abcd 0.36 cd 0.41 bcd 0.53 abc 0.47 abcd 0.46 abcd 0.64 a	0.89 de 0.82 de 1.31 abcd 1.97 a 0.82 de 0.94 cde 1.12 bcde 1.69 abc 0.52 e 0.83 de 1.40 abcd 1.81 ab 0.80 de 0.78 de 1.45 abcd 1.75 ab	0.55 abc 0.47 c 0.46 c 0.48 c 0.45 c 0.45 c 0.45 c 0.42 c 0.43 c 0.74 a 0.61 abc 0.56 abc 0.56 abc 0.57 abc 0.57 abc 0.55 abc 0.52 bc	0.69 a 0.59 abc 0.52 cd 0.46 d 0.65 ab 0.48 cd 0.48 cd 0.32 e 0.54 bcd 0.53 bcd 0.53 bcd 0.48 cd 0.53 bcd 0.58 abcd 0.54 bcd 0.50 cd 0.52 bcd	1.77 bcd 1.98 abc 1.42 ef 1.32 fg 2.04 ab 1.90 bcd 1.77 bcd 0.81 i 1.72 cd 1.63 de 1.01 hi 1.05 ghi 2.21 a 1.87 bcd 1.25 fgh 1.26 fgh	0.14 abcde 0.12 cde 0.11 e 0.16 a 0.14 abcde 0.15 abcd 0.13 abcde 0.13 abcde 0.13 abcde 0.13 abcde 0.15 abc 0.13 abcde 0.12 bcde 0.12 bcde 0.12 bcde 0.13 abcde				

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

- 1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of two reps with 5 plants/culture.

Cations are given as %.

TABLE XXXII

effects	OF	ΚI	LEVELS,	WITH	AND	WITHOUT	P	AND	CA,	ON	NODULE
(CYT)SOI	L COMPC	SITIO	I OF	AUSTRIAN	I I	INTI	ER I	PEA,	
				SEI	RIES	III					

Treat- ment ¹	Nodule Cytosol Composition ²									
	Р	K	Ca	Mg	Na	Fe				
0	0.18 def	0.28 ab	0.11 ab	0.26 a	0.09 cd	0.07 ab				
K1	0.18 def	0.20 b	0.29 ab	0.26 a	0.10 bed	0.07 a				
<u>^2</u> Ko	0.20 bodef	0.50 ab	0.45 a	0.30 a.	0.10 bcd	0.07 a.D				
P	0.23 abcd	0.44 ab	0.08 ab	0.29 a	0.14 a	0.06 bcd				
PK4	0.26 a	0.48 ab	0.27 ab	0.25 a	0.12 abc	0.06 bcd				
PK2	0.24 abc	0.92 ab	0.02 b	0.24 a	0.11 abc	0.05 bcd				
PK3	0.22 abcde	0.75 ab	0.02 Ъ	0.28 a	0.06 •	0.06 bed				
Ca	0.18 def	0.29 ab	0.07 ab	0.34 a	0.11 abc	0.07 ab				
CaK1	0.19 cdef	0.70 ab	0.03 Ъ	0.29 a	0.11 abc	0.05 cd				
CaK ₂	0.15 f	0.76 ab	0.04 ab	0.24 a	0.08 de	0.05 cd				
Cak3	0.17 ef	0.92 ab	0.06 ab	0.21 a	0.05 e	0.06 bcd				
CaP	0.22 abcde	0.40 ab	0.08 ab	0.32 a	0.13 ab	0.06 bcd				
CaPK1	0.25 ab	0.52 ab	0.05 ab	0.29 a	0.12 abc	0.05 d				
CaPK2	0.25 ab	1.05 a	0.11 ab	0.25 a	0.10 bcd	0.05 cd				
Cark3	V.20 DCLEI	0.94 80	0.10 ab	0.24 a	0.07 de	0.05 bc				

1. Treatment levels as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.

2. Figures are means of two reps with 5 plants/culture.

Cations are given as %.

TABLE XXXIII

EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON NODULE ORGANELLE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

Treat- ment ¹	Nodule Organelle Composition ²											
	Р		K		Ca		Mg		Na.		Fe	
0 K1 K2 K3 P PK1 PK2 PK3 Ca CaK1 CaK2 CaK3 CaP CaPK1 CaPK2 CaPK3	0.26 d 0.24 f 0.27 c 0.29 c 0.29 c 0.33 a 0.30 t 0.28 c 0.28 c 0.28 c 0.28 c 0.28 c 0.24 f 0.38 a 0.35 a 0.32 t	lef def def def def def def def def def d	0.01 0.10 0.16 0.37 0.01 0.06 0.14 0.59 0.01 0.03 0.41 0.03 0.41 0.08 0.35 0.54	c bc ab c bc a c c a a c c a a c c a a c c a b c a b c a b c c b c b	0.20 0.28 0.39 0.21 0.17 0.18 0.18 0.21 0.33 0.22 0.30 0.32 0.21 0.23 0.24 0.25	ab ab ab ab ab ab ab ab ab ab ab ab ab a	0.37 0.35 0.39 0.32 0.40 0.33 0.32 0.26 0.35 0.35 0.35 0.30 0.35 0.30 0.33	ab abc a bcde a abcd bcde e a bcde e a bc de cde abc cde abcc	0.61 0.57 0.56 0.53 0.67 0.70 0.61 0.31 0.67 0.55 0.44 0.28 0.58 0.70 0.49 0.45	abc abcde abcde ab ab abc fg ab bcde ef g abcd a cde de	0.07 0.09 0.08 0.08 0.08 0.09 0.08 0.06 0.08 0.06 0.08 0.06 0.07 0.08 0.04 0.08 0.04 0.08	abc abc abc abc abc abc abc d abc d abc abc abc abc abc

- 1. Treatments as mg/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl; P = 200 as Ca(H₂PO₄)₂; with Ca = 6 meq/100 g soil as CaCO₃.
- 2. Figures are means of two reps with 5 plants/culture.

Cations are given as %.

The effects of K levels (Ca and P levels pooled) are presented in Table XXXIV. K applications significantly increased top and nodule weights, the K content of all nodule compositions, plus the P content of nodule organelles. The effects of Ca and P (K levels pooled) are given in Tables XXXV and XXXVI. P significantly increased top weight and nodulation plus the P content of all three nodule compositions, as well as whole nodule and cytosol Na contents. Significant decreases resulted for cytosol Fe and nodule organelle Ca and Mg contents. In this series Ca generally decreased top growth, whole nodule and organelle Na content, and cytosol Fe, with the only increase shown for whole nodule Ca.

Comparisons for the K level responses, with and without P and Ca. are shown in Tables XXXVII to XL, including significant interactions. K treatments significantly affected top weight, nodulation, whole nodule K, Mg, Na, and Fe content, nodule cytosol K and Na, plus nodule organelle P, K, Mg, and Na compositions. P treatments significantly affected top weight, nodulation, whole nodule P, Ca, and Na content, nodule cytosol P, Na, and Fe, plus nodule organelle P, Ca, and Mg compositions. Ca treatments significantly affected top growth, whole nodule Ca and Na content, cytosol Fe, as well as the Na composition of nodule organelles. The CaxP interaction was significantly observed in nodulation and whole nodule Mg and Na. CaxK also significantly affected whole nodule Mg and Na as well as cytosol Fe and organelle P and Fe contents. PxK affected nodule weight, whole nodule and organelle Na, and organelle P compositions. Apparent CaxPxK interactions were significant for Na content of all nodule compositions, whole nodule and organelle Fe, plus nodule organelle Mg content. It was observed that whole nodule Na was significantly affected not only by the addition of all three plant nutrients used in this

TABLE XXXIV

Parameters ¹		2		
	0	K1	K2	K3
Top Wt	4.45 c	4.92 ab	4.76 bc	5.28 a
Top Ln	116 .6 a	119 .1 a	117.7 a	122.9 a
Rt Wt	1.17 a	1.37 a	1.33 a	1.29 a
Nod Wt	1.699 b	1.811 b	1.801 b	2.039 a
Nod No	365 ъ	340 Ъ	371 Ъ	452 a
Nase	32.1 ab	16.5 b	61.2 a	21.1 b
Whole Nodule				
P	0.50 a	0.44 a	0.44 a	0.54 a
К	0.75 c	0.84 c	1.32 Ъ	1.80 a
Ca	0.58 a	0.52 a	0.50 a	0.53 a
Mg	0.61 a	0.53 Ъ	0.49 bc	0.44 c
Na	1.93 a	1.84 a	1.36 ъ	1.11 c
Fe	0.14 a	0.13 ab	0.12 b	0.14 a
Nodule Cytosol				
P	0.20 a	0.22 a	0.21 a	0.20 a
K	0.35 b	0.47 ab	0.82 a	0.82 a
Ca	0.08 a	0.16 a	0.15 a	0.07 a
Mg	0.30 a	0.27 a	0.26 a	0.24 a
Na	0.12 a	0.11 a	0.09 Ъ	0.07 c
Fe	0.06 a	0.06 a	0.05 ъ	0.06 a
Nodule Organelles				
P	0.26 ъ	0.30 a	0.30 a	0.29 a
K	0.01 c	0.06 c	0.26 ъ	0.49 a
Ca	0.22 a	0.23 a	0.28 a	0.24 a
Mg	0.36 a	0.34 a.	0.34 a	0.29 b
Na	0.63 a	0.63 a	0.52 b	0.39 c
Fe	0.07 a	0.08 a	0.08 a	0.08 a

EFFECTS OF K LEVELS ON GROWTH, NODULATION, NITROGENASE (C2H2 REDUCTION), AND NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

1. Refer to Tables XXX to XXXIII for description of variables.

^{2.} Treatment levels as mg K/Kg soil: $K_1 = 200$, $K_2 = 400$, $K_3 = 600$ as KCl. Ca and P levels pooled. Figures are means of 12 observations, except nodule compositions = 8 observations, with 5 plants/culture. Means followed by the same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

CaTop Wtwith 4.41 b(g dry)without 5.28 aTop Lnwith 113.5 b(cm)without 124.6 aRt Wtwith 1.30 a(g dry)without 1.27 aNod Wtwith 1.789 a(g fresh)without 1.886 aNod Nowith 379 a(nodules/plant)without 384 aNase (µmolwith 39.6 a $C_2H4/culture/hr)$ without 25.8 a	Treatment ¹					
Top Wtwith 4.41 b(g dry)without 5.28 aTop Lnwith 113.5 b(cm)with 113.5 b(cm)without 124.6 aRt Wtwith 1.30 a(g dry)without 1.27 aNod Wtwith 1.789 a(g fresh)without 1.886 aNod Nowith 379 a(nodules/plant)without 384 aNase (µmolwith 39.6 a $C_2H4/culture/hr)$ without 25.8 a	Р					
without 5.28 a Top Ln with 113.5 b (cm) without 124.6 a Rt Wt with 1.30 a (g dry) without 1.27 a Nod Wt with 1.789 a (g fresh) without 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	5.33 a					
Top In with 113.5 b (cm) without 124.6 a Rt Wt with 1.30 a (g dry) without 1.27 a Nod Wt with 1.789 a (g fresh) without 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	4.37 b					
without 124.6 a Rt Wt with 1.30 a (g dry) without 1.27 a Nod Wt with 1.789 a (g fresh) without 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	121.2 a					
Rt Wt with 1.30 a (g dry) without 1.27 a Nod Wt with 1.789 a (g fresh) with 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	116.9 a					
(g dry) without 1.27 a Nod Wt with 1.789 a (g fresh) with 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	1.26 a					
Nod Wt with 1.789 a (g fresh) without 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	1.35 a					
without 1.886 a Nod No with 379 a (nodules/plant) without 384 a Nase (µmol with 39.6 a C2H4/culture/hr) without 25.8 a	2.117 2					
Nod Nowith379 a(nodules/plant)without384 aNase (µmolwith39.6 aC2H4/culture/hr)without25.8 a	1.558 b					
(nodules/plant)without384 aNase (µmolwith39.6 aC2H4/culture/hr)without25.8 a	432 a					
Nase (µmol with 39.6 a C ₂ H ₄ /culture/hr) without 25.8 a	331 b					
C2H4/culture/hr) without 25.8 a	33.1 a					
Whole Nodule	32.3 a					
P(%) with 0.47 a	0.53 a					
without 0.50 a	0.43 h					
$K(\pi)$ with $1.17a$	1,17 #					
without 1.19 a	1.19 a					
Ca(%) with $0.60a$	0.50 h					
without 0.46 b	0.57 a					
Mg (π) with 0.52 a	0.51 a					
without 0.52 a	0.53 a					
Na (\overline{x}) with 1.50 b	1 63 0					
Nithout 1.63 a	1 40 h					
$Fe(f_{0}) \qquad \qquad \text{with} \qquad 0.13 \text{ a}$	0.13.0					
without 0.13 a						

TABLE XXXV

EFFECTS OF CA AND P LEVELS ON GROWTH, NODULATION, NITROGENASE (C₂H₂ REDUCTION), AND WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

1. Treatment levels: $P = 200 \text{ mg P/Kg soil as } Ca(H_2PO_4)_2$; Ca = 6 meq/100 g soil as CaCO₃. K levels pooled.

Figures are means of 24 observations, except nodule compositions = 16 observations, with 5 plants/culture.

Means followed by same letter are not significantly different according to Duncan's Multiple Range analysis at the 0.05 level.

Abbreviations are Wt = Weight, Ln = Length, Ht = Root, Nod = Nodule, No = Number, Nase = Nitrogenase.

Composition		Treatment ¹	
		Ca	Р
Nodule Cytosol		- <u></u>	
P (%)	with	0.20 a	0.23 a
	without	0.21 a	0.18 b
K (%)	with	0.70 a	0.69 a
	without	0.54 a	0.55 a
Ca (%)	with	0.07 a	0.09 a
	without	0.17 a	0.14 a
Mg (%)	with	0.27 a	0.27 a
,	without	0.26 a	0.26 a
Na. (%)	with	0.09 a	0.10 a
	without	0.10 a	0.09 b
Fe (%)	with	0.05 b	0.05 b
	without	0.06 a	0.06 a
Nodule Organelles			
P (%)	with	0.29 a	0.31 a
	without	0.28 a	0.27 b
K (%)	with	0.23 a	0.22 a
	without	0.18 a	0.19 a
Ca (%)	with	0.26 a	0.21 b
	without	0.23 a	0.28 a
Mg (%)	with	0.33 a	0.32 h
	Without	0.34 a	0.35 8
Na (%)	with	0.52 b	0.56 a
	without	0.52 8	0.52 a
Fe (%)	with	0.07 a	0.08 2
	without	0.08 -	0.00 a
	HT GILOUP	0.00 &	0.00 a

TABLE XXXVI

EFFECTS OF CA AND P LEVELS ON NODULE CYTOSOL AND ORGANELLE COMPOSITIONS OF AUSTRIAN WINTER PEA, SERIES III

1. Treatment levels: $P = 200 \text{ mg P/Kg soil as } Ca(H_2PO_4)_2$; Ca = 6 meq/100 g soil as CaCO₃. K levels pooled.

Figures are means of 16 observations with 5 plants/culture.

TABLE XXXVII

Parameter	Combination Treatment ¹			Treatment Effect ²		
		0	к ₁	K ₂	ĸ3	
Top Wt (g dry)	0 P Ca CaP	4.60 4.63 3.90 4.67	4.87 6.13 3.93 4.73	4.53 6.20 3.50 4.80	5.00 6.30 4.60 5.20	Ca *** P *** K **
Top Ln (cm)	0 P Ca CaP	117.7 124.5 110.5 113.9	119.4 128.3 117.3 111.3	124.9 129.1 102.7 113.9	127.0 125.7 115.6 123.2	Ca. ***
Rt Wt (g dry)	0 P Ca CaP	1.27 0.80 1.40 1.20	1.53 1.23 1.43 1.27	1.50 1.30 1.27 1.23	1.13 1.40 1.27 1.37	
Nod Wt (g fresh)	0 P Ca CaP	1.285 2.236 1.564 1.713	1.326 2.449 1.523 1.948	1.398 2.322 1.521 1.963	1.872 2.198 1.974 2.110	P *** K ** CaxP *** KxP *
Nod No (nodules/culture)	0 P Ca CaP	309 345 378 427	339 384 303 334	248 488 365 383	311 650 397 449	P *** K * CaxP *
Nase (µmol/ culture/hr)	O P Ca CaP	7.3 48.0 38.3 34.7	6.3 11.7 20.3 27.7	52.0 63.3 91.7 37.7	8.7 9.0 33.7 33.0	

COMPARISONS FOR EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON GROWTH, NODULATION, AND NITROGENASE (C₂H₂ REDUCTION) OF AUSTRIAN WINTER PEA, SERIES III

1. Refer to Tables XXX to XXXIII for treatment levels and description of variables.

Figures are means of three reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P < 0.05, 0.01 and 0.001 respectively.

TABLE XXXVIII

COMPARISONS FOR EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

Whole Nodule Composition	Combination Treatment ¹		K Lev	Treatment Effect ²		
		0	K1	K2	Кз	
P (%)	0 P Ca CaP	0.52 0.52 0.44 0.53	0.28 0.59 0.44 0.47	0.44 0.52 0.36 0.46	0.57 0.55 0.41 0.64	P **
K (%)	0 P Ca CaP	0.89 0.82 0.52 0.80	0.82 0.94 0.83 0.78	1.31 1.12 1.40 1.45	1.97 1.69 1.81 1.75	K ***
Ca (%)	0 P Ca CaP	0.55 0.45 0.74 0.60	0.47 0.45 0.61 0.57	0.46 0.42 0.56 0.55	0.48 0.43 0.70 0.52	Ca *** P *
Mg (%)	0 P Ca CaP	0.69 0.65 0.54 0.58	0•59 0•48 0•53 0•54	0.52 0.48 0.48 0.50	0.46 0.32 0.46 0.52	K *** CaxP ** CaxK **
Na. (%)	0 P Ca CaP	1.77 2.04 1.72 2.21	1.98 1.90 1.63 1.87	1.42 1.77 1.01 1.25	1.32 0.81 1.05 1.26	Ca ** P ** K *** CaxP ** CaxK ** PxK **
Fe (%)	0 P Ca CaP	0.14 0.14 0.14 0.13	0.12 0.15 0.13 0.12	0.11 0.13 0.12 0.11	0.16 0.11 0.15 0.15	CaxPxK * K * CaxPxK *

1. Refer to Tables XXX to XXXIII for treatment levels.

Figures are means of two reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P \langle 0.05, 0.01 and 0.001 respectively.

ON NODULE CYTOSOL COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III Nodule Cytosol Combination Treatment¹ K Levels¹ Effect²

		0	K ₁	K ₂	Кз	
P (%)	0 P Ca CaP	0.18 0.23 0.18 0.22	0.18 0.26 0.19 0.25	0.20 0.24 0.15 0.25	0.20 0.22 0.17 0.20	P ***
K (%)	0 P Ca CaP	0.28 0.44 0.29 0.40	0.20 0.48 0.70 0.52	0.56 0.92 0.76 1.05	0.66 0.75 0.92 0.94	K *
Ca (%)	0 P Ca CaP	0.11 0.08 0.07 0.08	0.29 0.27 0.03 0.05	0.45 0.02 0.04 0.11	0.12 0.02 0.06 0.10	
Mg (%)	O P Ca CaP	0.26 0.29 0.34 0.32	0.26 0.25 0.29 0.29	0.30 0.24 0.24 0.25	0.23 0.28 0.21 0.24	
Na (%)	O P C a CaP	0.09 0.14 0.11 0.13	0.10 0.12 0.11 0.12	0.10 0.11 0.08 0.10	0.09 0.06 0.05 0.07	P ** K *** CaxPxK *
Fe (%)	O P Ca CaP	0.07 0.06 0.07 0.06	0.07 0.06 0.05 0.05	0.07 0.05 0.05 0.05	0.06 0.06 0.06 0.06	Ca ** P ** Caxk *

1. Refer to Tables XXX to XXXIII for treatment levels.

Figures are means of two reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P < 0.05, 0.01 and 0.001 respectively.

TABLE XXXIX

COMPARISONS FOR EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA,

Nodule Organelle Composition	dule ganelle Combination mposition Treatment ¹			K Levels ¹				
		0	K1	K ₂	Кз			
P (%)	0 P Ca CaP	0.26 0.29 0.25 0.24	0.24 0.33 0.28 0.38	0.27 0.30 0.30 0.35	0.29 0.28 0.27 0.32	P *** K ** CaxK * PxK *		
K (%)	0 P Ca CaP	0.01 0.01 0.01 0.01	0.10 0.06 0.03 0.08	0.16 0.14 0.41 0.35	0.37 0.59 0.46 0.54	K ***		
Ca (%)	0 P Ca CaP	0.20 0.17 0.33 0.21	0.28 0.18 0.22 0.23	0.39 0.18 0.30 0.24	0.21 0.21 0.32 0.25	P *		
Mg (%)	0 P Ca CaP	0.37 0.40 0.39 0.30	0.35 0.33 0.35 0.35	0.39 0.32 0.35 0.30	0.32 0.26 0.27 0.33	P * K ** CaxPxK **		
Na (%)	0 P Ca CaP	0.61 0.67 0.67 0.58	0.57 0.70 0.55 0.70	0.56 0.61 0.44 0.49	0.53 0.31 0.28 0.45	Ca * K *** PxK * CaxPxK **		
Fe (%)	0 P Ca CaP	0.07 0.08 0.08 0.04	0.09 0.09 0.06 0.08	0.08 0.08 0.07 0.09	0.08 0.06 0.08 0.09	CaxK * CaxPxK **		

COMPARISONS FOR EFFECTS OF K LEVELS, WITH AND WITHOUT P AND CA, ON NODULE ORGANELLE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III

1. Refer to Tables XXX to XXXIII for treatment levels.

Figures are means of two reps with 5 plants/culture.

2. *, **, *** Level of significant differences at P < 0.05, 0.01 and 0.001 respectively.

TABLE XL

study but also that every interaction combination possible was significantly apparent with this parameter.

The significant correlations for Series III are shown in Tables XLI and XLII, and only the significant correlations are discussed. Top weight gave significant and positive correlations with top length and nodulation parameters, but not with root growth or nitrogenase. Both top growth parameters were positively correlated with cytosol P, but negatively with nodule organelle Ca. In addition, top weight was negatively correlated with Mg in the nodule organelles. Nodule weight was correlated positively with top growth, nodule number, and the P content of both nodule cytosol and organelle compositions. Negative correlations were given for nodule weight with cytosol Fe and nodule organelle Ca. Both nodulation parameters were negatively correlated with Mg content of the nodule organelles. Additionally, nodule number was positively correlated with the K content of both the cytosol and organelle nodule compositions. No significant correlations were demonstrated for root weight, nitrogenase, or cytosol Ca with any of the variables measured in this series.

The correlation between whole nodule P with top and nodule weights can be broken down by comparison with the cytosol and organelle fractions to indicate that top weight appeared to be related to P in the cytosol, while nodule weight appeared to be related to both the cytosol and organelle fractions. Also the negative whole nodule Ca correlations with top growth can be shown to be related to the nodule organelles as was the relationship between nodulation and Mg content. Except for Fe and Ca, a cation in one nodule fraction was generally correlated with the same cation in the other nodule compositions. In all three nodule fractions Mg was positively correlated with Na, but K was negatively correlated with

CORRELATIONS AMONG GROWTH AND NODULE PARAMETERS FOR GROWTH, NODULATION, AND WHOLE NODULE COMPOSITION OF AUSTRIAN WINTER PEA, SERIES III¹

	Тор	Тор	Nod	Nod	Whole Nodule Composition						
	Wt	In	Wt	No	P	K	Ca	Mg	Na	Fe	
Top Wt		.69	.68	• 50	•41		37				
Top Ln		~~~	•39	***	*		35 *				
Nod Wt				•58	•46 **			40 *			
Nod No Whole Nodule								44 **		56	
P K								53	75	• 50 ***	
Mg								**	***		
P P	•59 ***	•39	•58 ***		•41 *		36 *		***		
К				•42 *		•50 **		37 *	-•53 **		
Mg						-•47 **					
Na.						71 ***		•52 **	•80 ***		
Fe Nodule Organelles P			38 * .47								
К				•38 *		•84 ***		 65	85 ***		
Ca	46 **	38 *	44 **		38 *						
Mg	38 *		41	42 *		-•48 **		.63 ***	•38 *		
N a .						72 ***		•56 ***	•79 ***		

1. Figures are correlation coefficients based on 48 observations, except nodule compositions = 32 observations, with 5 plants/culture. *, **, *** Level of significance for P < 0.05, 0.01 and 0.001 respectively. Refer to Tables XXX to XXXIII for treatment levels and description of variables.

TABLE XLII

CORRELATIONS AMONG GROWTH AND NODULE PARAMETERS FOR NODULE CYTOSOL AND ORGANELLE COMPOSITIONS OF AUSTRIAN WINTER PEA, SERIES III¹

	Nodule Cytosol				Nodule Organelles						
	P	K	Mg	Na	Fe	P	K	Ca	Mg	Na	Fe
Top Wt	•59 ***							46 **	38 *		*******
Top Ln	•39 *							38 *			
Nod Wt	•58 ***				38 *	•47 **		44 **	41 *		
Nod No		.42					•38		42		
Whole Nodule		¥					*		*		
P	.41 *							38 *			
К		•50 **	47 **	71 ***			•84 ***		48 **	72 ***	
Ca	36 *										
Mg		37 *		• 52 **			65 ***		•63 ***	•56 ***	
Na		53		.80			85		.38	.79	
Nodule Cytosol		**		***			***		*	***	
P	\backslash			•38 *		•53 **		41 *			
K					45 **		•50 **			40 *	
Ca			\setminus								•38 *
Mg				$\overline{\ }$			34 *		•38 *		
Na							85 ***		•42 *	•88 ***	
Fe						55					
Nodule Organelles						***	•				
K									51 **	83 ***	
Mg	-								\geq	.61	

except nodule compositions = 32 observations, with 5 plants/culture.
*, **, *** Level of significance for P < 0.05, 0.01, and 0.001
respectively.
Refer to Tables XXX to XXXIII for treatment levels and description
of variables.</pre>

Mg and Na. In addition whole nodule P was positively correlated with whole nodule Fe and cytosol P, and negatively with Ca content of the nodule organelles. Cytosol P was positively correlated with both top growth parameters, nodule weight, and cytosol Na, while cytosol K content was positively correlated with nodule number and negatively with cytosol Fe. Cytosol Ca was correlated significantly only with Fe content of the nodule organelles, and cytosol Na was positively correlated with cytosol P. Cytosol Fe gave only significant negative correlations and these were with nodule weight, cytosol K, and organelle P contents. In the nodule organelle fraction P correlated positively with nodule weight, but negatively with cytosol Fe. Organelle K content was positively correlated with nodule number. Ca gave negative correlations with top growth, nodule weight, and cytosol P content. Mg was somewhat similar here in that it was also negatively correlated with top weight and nodulation. Mg was similar to the K content in that it too was correlated with the Mg and Na nodule compositions but positively rather than negatively. Organelle Fe content was only correlated significantly with Ca in the cytosol.

CHAPTER V

SUMMARY AND CONCLUSIONS

Austrian winter peas, in symblotic association with <u>Rhizoblum legumin-osarum</u> (Frank), can utilize atmospheric nitrogen to supply important and needed sources of protein for human and livestock nutrition. This crop may provide significant contributions to various forage and soil management programs for efficient agricultural production in many areas throughout the world. Field peas can be grown with small grains or other crops for hay, pasture, or silage. They can be planted for dry pea production and commercially used in the split-pea market or be exported to other countries. <u>An Paste</u> is a sweet, high-protein filling used by the Japanese with cookies, crackers, and other pastries which has provided a major market for nearly 80% of the dry Austrian winter peas grown in the United States. Field peas can also be used as a cover crop to reduce soil erosion, as a green manure crop to add nitrogen and organic matter to the soil, and be utilized in other crop rotations for soil improvement and pest control.

These experiments were designed to determine the effects of K levels, with and without P and Ca, in factorial combinations on various aspects of the legume, Austrian winter pea. The plants in these studies were harvested by first removing the top growth. The root-nodule systems were then gently and quickly rinsed and blotted to remove the soil and excess water prior to being placed in clear glass serum bottles. Samples of

5 cc were collected after 60 and 90 min to be analyzed for acetylene reduction to ethylene by gas chromatography in order to estimate the activity of the nitrogenase enzyme. The nodules were then removed from the root system, counted, weighed, and analyzed for P, K, Ca, Mg, Na, and Fe composition in the whole nodule, nodule cell-free extract (cytosol), and nodule organelle extract for each treatment by nitric-perchloric digestion. The cytosol and organelle compositions were obtained by refrigerated sonification and centrifugation prior to the digestion procedures. The results of these experiments were analyzed with the Statistical Analysis System to determine significant treatment effects, interactions, and correlations among the variables measured in this study.

Top growth in these experiments was consistently reduced by applications of Ca in all three series. Application of P increased top growth significantly except in Series I. K applications did not generally affect top growth significantly except for an increase in Series III. Root growth was significantly reduced by applied P in Series I and II. Ca applications increased root growth in Series I, but was decreased in Series II, while K reduced root growth only in Series I. Nodulation was reduced by both Ca and K applications except for an increase due to applied K in Series III. The application of P increased nodulation in Series II and III, however, it was reduced in Series I. A significant CaxP interaction was apparent for both nodule weight and number. Nitrogenase activity was not significantly enhanced by the addition of plant nutrients in this study, but rather was inhibited by K treatments in Series I.

In the nodule composition analysis, the addition of a plant nutrient generally increased the nodule content of that ion throughout these experiments. It was observed that the analysis of the whole nodule composition

often reflected the significant effects that occurred in the nodule cytosol or organelle compositions or both. The addition of Ca and K significantly increased the P content of nodule organelles in Series II and III respectively. When Ca was applied, the K content of the cytosol increased in Series II. The addition of P decreased the Ca content of nodule organelles and a similar response was noted for K applications in Series II. Ca additions increased the Mg content of the organelles in Series II. while P reduced the Mg content of this fraction in Series III, and K reduced it in both Series II and III. No significant effects were observed with cytosol Mg for any of the plant nutrients in this study. Ca applications reduced the Na content of the nodule organelles in Series III and applied K levels significantly reduced the Na content of all nodule compositions consistently. Also, applied P increased the Na content of the cytosol in Series III. Applied Ca and K significantly decreased the Fe content of the cytosol and whole nodule fractions respectively in Series III. Every possible interaction combination was significantly observed for nodule organelle Fe in Series II and for whole nodule Na in Series III.

Significant correlations were used for verification of trends that were observed in these studies. Top growth was significantly correlated with root growth except in Series III. Nitrogenase was positively correlated with top growth in Series I. In the nodule compositions, top growth was shown to be positively correlated with the Mg content of the nodules in Series II. This may possibly reflect the role of Mg either in the chlorophyll molecule or in nodule enzymatic reactions involving ATP (adenosine triphosphate) (Salisbury and Ross, 1978). However, in Series III, a negative correlation was observed between top weight and Mg in the nodule organelles. The P and Ca contents of the nodules were positively

and negatively correlated with top growth respectively. Series III suggested that top weight may be related to nodule cytosol P and organelle Ca. Top growth was also significantly correlated with nodulation. Series II indicated that root growth was positively correlated with Mg, but negative correlations were given for Series I. Negative correlations were also apparent for organelle Mg with whole nodule and cytosol K content in Series II. Nodulation was positively correlated with the P and K contents of the nodule cytosol and organelles in Series II. and was negatively correlated with Ca and Mg content of the nodules, however, positive and negative correlations were shown with nodule Fe content in Series II and III respectively. Nitrogenase was negatively correlated with root growth in Series II. Both Series I and II gave positive correlations for nitrogenase by nodulation. Positive correlations were also noted for nitrogenase x cytosol Fe and nitrogenase x K content of the nodule compositions in Series II along with negative correlations for Mg and Na. Correlations among the nodule cations themselves indicated that a cation in one fraction was usually significantly correlated with the same cation in the other nodule fractions. Positive relationships were observed not only for P x K but also for Mg x Na whereas, negative correlations were given for K x Mg and a consistent, highly negative inverse relationship was demonstrated for K x Na as has been observed in other legumes (Lynd et al., 1981).

The general requirements for the nutrients K, P, and Ca applied in these greenhouse studies for legumes are well recognized. However, it is also known that young seedlings depend on nutrients stored in the seed for survival until they can become established and are mature enough to depend on their own photosynthetic capabilities. The field pea responses
to these soil fertility treatments may be explained in that these treatments could have disrupted or caused an unfavorable imbalance in the normal seedling metabolism. Cotyledon weight decreases with age and when their reserves are exhausted, the seedlings are then more dependent on the fertility of their edaphic environment. It is logical that the limiting influence of any essential nutrients that are deficient becomes apparent at that time. It is presumptive that incorporation of high levels of fertilizer prior to sowing may sometimes result in less than optimum plant growth for field peas grown under conditions similar to these experiments. Other fertilization methods, such as topdressing after stand establishment, may result in improved growth and yield of field peas without inhibition during critical stages of germination and vigorous stand establishment.

65

LITERATURE CITED

- Ali-Khan, S. T. and R. C. Zimmer. 1972. Growing field peas. Can. Dept. Agric. Publ. 1493. Ottawa, Canada.
- Auld, D. L., G. A. Murray, and L. E. O'Keeffe. 1979. Melrose Austrian winter pea. Idaho Current Info. Series 497. Univ. of Idaho: Moscow, Idaho.
- Bland, B. F. 1971. Peas. In Crop Production: Cereals and Legumes. Academic Press: New York.
- Bond, L. 1948. Origin and developmental biology of root nodules of Pisum sativum. Bot. Gaz. 109:411-433.
- Carter, J. F. and K. L. Larson. 1964. Oats, peas, and vetch for hay and silage in North Dakota. N. Dakota Expt. Statn. Bull. 447.
- Flinn, A. M. and J. S. Pate. 1968. Biochemical and physiological changes during maturation of fruit of the field pea (<u>Pisum arvense</u> L.). Ann. Bot. 32:479-495.
- Gray, F. and M. H. Roozitalab. 1976. Benchmark and key soils of Oklahoma: a modern classification system. USDA Expt. Sta. Bull. MP 97. Oklahoma State Univ.: Stillwater, OK.
- Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N₂ fixation: laboratory and field evaluation. Plant Physiol. 43:1185-1207.
- Keeton, W. T. 1972. Biological Science. 2nd Edition. W. W. Norton & Co., Inc.: New York.
- Klebesadel, L. J. 1969. Chemical composition and yield of oats and peas separated from a forage mixture at successive stages of growth. Agron. J. 61:713-716.
- Leffel, R. C. 1978. Other Legumes. In Forages. M. E. Heath, D. S. Metcalfe, and R. F. Barnes (Editors). Iowa State Univ. Press: Ames, Iowa.
- Lynd, J. Q., E. A. Hanlon, Jr., and G. V. Odell, Jr., 1981. Potassium effects on improved growth, nodulation, and nitrogen fixation of hairy vetch. Soil Sci. Soc. Am. J. 45:302-306.

- Martin, J. H., W. H. Leonard, and D. L. Stamp. 1976. Miscellaneous Legumes. In Principles of Field Crop Production. 3rd Edition. Macmillan Publ. Co., Inc.: New York.
- Murray, G. A. and A. E. Slinkard. 1973. Fenn Austrian winter pea. Idaho Current Information Series 209. Univ. of Idaho: Moscow, Idaho.
- Murray, G. A., D. L. Auld, J. M. Kroft, G. A. Lee, F. L. Muehlbauer, and L. E. O'Keeffe. 1978. Dry pea and lentil production in the Pacific Northwest. Idaho Agric. Expt. Statn. Bull. 578. Univ. of Idaho: Moscow. Idaho.
- Murray, G. A. and D. L. Auld. 1980. Spring-planted Austrian winter peas. Idaho Current Information Series 526. Univ. of Idaho: Moscow, Idaho.
- Oghoghorie, C. G. O., and J. S. Pate. 1972. Exploration of the nitrogen transport system of a nodulated legume using ¹⁵N. Planta (Berl.) 104:35-49.
- Pate, J. S., J. Walker, and W. Wallace. 1965. Nitrogen-containing compounds in the shoot system of <u>Pisum arvense</u> L. II. The significance of amino acids and amides released from nodulated roots. Ann. Bot. N. S. 29(115):475-493.
- Pate, J. S. and A. M. Flinn. 1973. Carbon and nitrogen transfer from vegetative organs to ripening seeds of field pea (<u>Pisum arvense</u> L.). J. Expt. Bot. 24(83):1090-1099.
- Piper, C. V. 1924. Peas and pea-like plants. In Forage Plants and Their Culture. 2nd Edition. Macmillan: New York.
- Raacke, I. D. 1957. Protein synthesis in ripening pea seeds. Biochem. J. 66:101-116.
- Robinson, R. G. 1960. Oat-pea or oat-vetch mixtures for forage or seed. Agron. J. 52:546-549.
- Salisbury, F. B. and C. W. Ross. 1978. Mineral Nutrition. In Plant Physiology. 2nd Edition. W. A. Jenson (Editor). Wadsworth Publ. Co., Inc.: Belmont, California.
- Shearer, L. A., B. J. Jahn, and L. Lenz. 1969. Deterioration of duck foods when flooded. J. Wildlife Mgt. 33:1012-1015.
- Smith, D. L. 1971. Nuclear changes in the cotyledons of <u>Pisum</u> arvense L. during germination. Ann. Bot. 35:511-521.
- Smith, D. L. 1973. Nucleic acid, protein, and starch synthesis in developing cotyledons of Pisum arvense L. Ann. Bot. 37:795-804.
- Statistical Analysis System User's Guide. 1979. J. T. Helwig and K. A. Council (Editors). Statistical Analysis Systems Institute, Inc.: Raleigh, N. Carolina.

VITA /

Robert Keith Berg, Jr.

Candidate for the Degree of

Master of Science

Thesis: SOIL FERTILITY EFFECTS ON GROWTH, NODULE PARAMETERS, AND NITROGENASE ACTIVITY (C2H2 REDUCTION) OF AUSTRIAN WINTER PEA (PISUM SATIVUM SUBSPECIES ARVENSE (L.) POIR)

Major Field: Agronomy

Biographical:

- Personal Data: Born in Warner Robins, Georgia, on December 15, 1952, the son of Mr. and Mrs. Robert K. Berg, Sr. Married to Pamela Kay (Koehn) Berg on November 11, 1972. The father of Robin Kay Berg (daughter) born April 22, 1976, and Ryan Keith Berg (son) born January 21, 1981.
- Education: Graduated from Enid High School, Enid, Oklahoma, in May, 1971; attended Northwestern Oklahoma State University, Alva, Oklahoma, 1971-72; received the Bachelor of Science in Agriculture degree from Oklahoma State University, Stillwater, Oklahoma, in May, 1981; completed the requirements for the Master of Science degree in Agronomy at Oklahoma State University, Stillwater, Oklahoma, in May, 1982.
- Professional Experience: Block Machine Operator, Farmland Industries, 1972-78; Rented 80 acres of wheat and native grass, 1977-78; Engineering Assistant, USDA Soil Conservation Service, 1978; Soil Microbiology Research Laboratory Assistant, Department of Agronomy, Oklahoma State University, 1979-81; Received a H. F. Murphy Memorial Scholarship, Department of Agronomy, Oklahoma State University, 1979-81; Received a Scholarship in Conservation, Soil Conservation Society of America, 1980; Graduate Research Assistant in Soil Microbiology, Department of Agronomy, Oklahoma State University, 1981-82.
- Professional Organizations: American Society of Agronomy, Society for Range Management, Soil Conservation Society of America, Soil Science Society of America, Crop Science Society of America, Phi Kappa Phi, Sigma Xi.