STUDIES ON THE NUTRITION OF BARTLETT PEAR TREES (PYRUS COMMUNIS L.) AND FIRE BLIGHT (ERWINIA AMYLOVORA, BURR.)

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Lowell N. Lewis

1960

This is to certify that the

thesis entitled

STUDIES ON THE NUTRITION OF BARTLETT PEAR TREES (PYRUS COMMUNIS L.) AND FIRE BLIGHT (ERWINIA AMYLOVORA, BURR.)

presented by

LOWELL N. LEWIS

has been accepted towards fulfillment of the requirements for

_____ **degree in** _____

DOCTOR OF PHILOSOPHY

Department of Horticulture

C. F. Kennedy

Major professor

Date November 4, 1960

O-169

STUDIES ON THE NUTRITION OF BARTLETT PEAR TREES (PYRUS COMMUNIS L.) AND FIRE BLIGHT (ERWINIA AMYLOVORA, BURR.)

By

LOWELL N. LEWIS

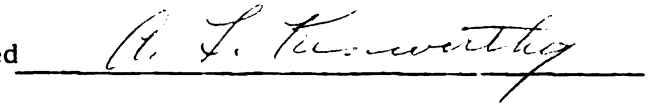
AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1960

Approved 

LOWELL N. LEWIS

ABSTRACT

Bartlett pear trees were grown in a nutrient solution sand culture at various levels of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, boron, manganese, copper, and molybdenum during the 1958, 1959 and 1960 seasons. These trees were used to study the effect of nutrition on the mineral composition of the leaves, on the free amino acid levels in the growing tips and older leaves, and on the susceptibility of pear trees to fire blight.

As an auxiliary study, the causal organism of fire blight, Erwinia amylovora, Burr. was grown in synthetic media to ascertain its ability to utilize natural and synthetic amino acids and amides as a source of nitrogen. Various other synthetic amides were tested for their bactericidal effect.

The studies on the effect of nutrition on the mineral composition of the leaves showed that decreasing the amount of an element in the nutrient solution resulted in a corresponding decrease in the leaf concentration of that element in all cases except iron, zinc, copper, and molybdenum. Increasing the amount of an element in the nutrient solution increased leaf concentration except for calcium, zinc, and molybdenum. In a few instances the concentration of one element seemed to be dependent on the concentration of another. Magnesium, calcium, and potassium concentrations seemed to be closely interrelated. Potassium and phosphorus showed an indirectly proportional relationship. There were other instances of low magnitude elemental interactions.

The free amino acid composition of the growing tip of a pear tree also seemed to be related to the nutrient element composition of the leaves. Increasing or decreasing total leaf nitrogen resulted in a similar change in the free amino acid concentration. High levels of phosphorus or potassium in the leaf were associated with an increase in total free amino acids similar to that found for trees high in nitrogen. Either increasing leaf boron or decreasing leaf magnesium resulted in amino acid levels equally as low as those in the minus nitrogen trees.

The free amino acid levels were also studied in the mature leaves from non-growing shoots of trees grown in solutions deficient in nitrogen, phosphorus, potassium, iron, and boron. Minus boron leaves contained more total amino acids than the check leaves, but low potassium leaves accumulated the highest total free amino acid level. Arginine accumulated only in the leaves deficient in phosphorus.

The most promising effect of nutritional environment on the susceptibility of pear trees to E. amylovora was found on trees grown in a high level of calcium. Neither the plus calcium nor the minus boron trees became infected with the bacteria. Those trees deficient in phosphorus or iron as well as the check trees appeared to be quite resistant. Trees grown in high levels of molybdenum, copper, potassium and nitrogen appeared to be the most susceptible to an E. amylovora infection and spread.

Studies on the bacteria, itself, showed that E. amylovora readily utilized aspartic acid, glutamic acid, asparagine, glutamine, beta-alanine, and gamma-amino butyric acid as a sole source of nitrogen in a synthetic culture medium. However, there appeared to be no correlation between the foliar concentration of the individual amino acids or the total amino acid level and the susceptibility of the tree to a fire blight infection.

Tests on the bactericidal effect of certain synthetic amides indicated that bromoacetyl valinamide may be a good bactericide for E. amylovora. This compound gave better control than agrimycin under the conditions of this experiment and showed no phytotoxic effect on the tree.

STUDIES ON THE NUTRITION OF BARTLETT PEAR TREES (*PYRUS COMMUNIS* L.) AND FIRE BLIGHT (*ERWINIA AMYLOVORA*, BURR.)

By

LOWELL N. LEWIS

A THESIS

Submitted to the School for Advanced Graduate Studies of Michigan
State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1960

7/6/61

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. A. L. Kenworthy for his encouragement and guidance throughout the course of this program.

The author also desires to express his thanks and appreciation to Dr. N. E. Tolbert, Dr. E. J. Klos, Dr. G. P. Steinbauer, and Dr. A. E. Mitchell for serving on the guidance committee and for their help and advice throughout the investigation. Special thanks also goes to Dr. D. R. Dilley and Dr. C. L. Bedford for their generous assistance and the use of their laboratory facilities.

Special acknowledgement is also made to the author's wife, Alice, for her help in typing and editing the manuscript, her assistance in the laboratory work, and her constant inspiration and encouragement throughout the entire period of college training.

TABLE OF CONTENTS

TABLE OF CONTENTS CONT'D

TABLE OF CONTENTS CONT'D

INTRODUCTION

The production of Bartlett pears (Pyrus communis, L.) in Michigan could be greatly increased if it were not for the constant threat of the fire blight organism (Erwinia amylovora, Burr.). This disease limits production because of the damage it does to the trees, and because of management practices used by many growers to prevent its occurrence. In many instances the pear orchards are grown in sod with little or no fertilizer. Fire blight, therefore, has cut heavily into the potential of the pear industry in Michigan and many other states.

Certain notations in the literature (34, 40, 58) suggest that the restrictions in growth of pear trees to reduce fire blight infection may result in a reduced level of certain naturally occurring nitrogen compounds and thus reduce growth of the fire blight organism after inoculation.

Therefore this investigation was planned to study (1) the nutrition of pears as related to fire blight susceptibility, and (2) the requirements of the organism in culture as a possible lead to a means of controlling Erwinia amylovora.

REVIEW OF LITERATURE

Pear Nutrition

Nutrient element balance is a most essential factor in plant growth; and any violation of this balance may result in a deficiency or toxicity of the elements involved. Many studies have been conducted on the problem of elemental balance in pears especially with nitrogen, phosphorus, potassium, magnesium, iron, manganese, boron, zinc, and copper.

Early work on the nitrogen, phosphorus, and potassium requirements of pears was done by L. Proebsting (49). He concluded that nitrogen was the only fertilizer required for pear trees grown under the conditions of his experiment. The trees which received high nitrogen were lower in phosphorus but not low enough to indicate phosphorus deficiency. This nitrogen-phosphorus relationship was also observed by E. Proebsting (50). Neither potassium nor phosphorus fertilizers increased the level of their respective elements in the leaves. Baxter (2) was not able to increase the nitrogen in pear leaves even though large amounts of nitrogen were applied to the tree. According to Boynton (10), pears can usually tolerate a low phosphorus level. Bryant and Gardner (15) reported one instance of pears responding to phosphorus fertilizer. These trees were growing in a soil which was believed to contain little available phosphorus.

Wallace (67) described potassium deficiency in pears as a marginal

leaf scorch appearing first on older leaves and noted the similarity of the symptoms on pear with those on apple. Reuther (51) found that the leaves of Bartlett pears showed deficiency symptoms when the potassium level was less than 0.5 percent[1]. Baxter (2) showed that the potassium content of pear leaves was lowered by soil applications of nitrogen, magnesium or calcium. Conversely he noted that calcium and potassium applications lowered the percent magnesium in the leaves. However, the sum of magnesium, calcium and potassium remained about the same over a wide variation in the nutrient status of the tree. Vidal and Herrera (65) noticed the potassium/magnesium (K/Mg) interaction in their work too. They found that a K/Mg ratio of 3 to 4 indicated a potassium deficiency, while a potassium excess might cause a K/Mg ratio as high as 23.

Harley (23) also commented on the K/Mg relationship. He found that leaves showing symptoms of magnesium deficiency contained about 0.05 percent magnesium. Leaf potassium was about 50 percent higher in the magnesium deficient trees than in the normal appearing trees. Harley described magnesium deficiency symptoms on pear leaves as oblong islands of almost black tissue arranged in a rather orderly fashion between the parallel veins on both sides of the midrib.

From a study of Japanese pears grown in sand culture, Sato (55) observed the following concentrations of major elements in leaves showing

---

[1] All percent and ppm values in this thesis are based on dry weight unless otherwise indicated.

deficiency sumptoms: nitrogen - 0.83%, phosphorus - 0.07%, potassium - 0.27 to 0.41%, calcium - 0.66%, and magnesium - 0.09 to 0.11%. Kenworthy's data (33) indicated that the optimum level for major elements in healthy orchard grown Bartlett pear leaves was: nitrogen - 2.50%, phosphorus - 0.135%, potassium - 1.45%, calcium - 1.90%, and magnesium - 0.397%. Sato (56) found about the same levels of leaf nitrogen, phosphorus and potassium in healthy pear trees grown in sand culture.

The problem of lime induced chlorosis has made iron the focal point of many studies. Boynton (10) has described iron deficiency in pears as contrasting green leaf veinlets and main veins followed by yellow leaves and finally white leaves with brown necrotic lesions; the tree may also exhibit thin shoot growth and considerable dieback. Several workers (37, 63, 66) have noticed the high level of potassium and low level of calcium in iron deficient plants. Linder and Harley (37) pointed out, however, that the potassium-calcium interaction was not associated with iron deficiency due to low iron but rather with iron deficiency induced by lime, iron-manganese balance upset or phosphorus-iron balance upset. Vidal and Herrera (65) also studied the importance of the iron-manganese ratio. They found that iron deficiency resulted in a diminuation of the ratio compared to a healthy tree. The data of Kenworthy (33) showed this ratio to be about one in healthy pear leaves. Vidal and Herrera also noticed that iron or manganese deficiencies resulted in high levels of nitrogen, phosphorus and potassium. Bennett (3) also reported that iron chlorosis was accompanied by a high level of nitrogen in the leaf.

Manganese toxicity symptoms have been described by Grasmanis (20) from observations on the Josephine pear. There was an internal bark necrosis, cracking of the outer bark, and rolling of bark cuticle. Tissue showing these symptoms contained 23 ppm of manganese on a fresh weight basis compared with 5 ppm in the healthy leaves.

Boron deficiency symptoms have been described by many researchers. Keinholz (32) described them as shortened shoot growth, fewer basal leaves, dwarfed terminal leaves, defoliation except for the terminal leaves, twig die-back, and bark cankers on the younger branches. Kobernuss (35) similarly described the symptoms and added possible swelling of the root tips. The actual level at which boron becomes deficient may be debatable. According to Compton (16), the foliar level of boron in deficient trees was 14 ppm. Woodbridge, Carney and McLarty (70) found that leaves from healthy trees contained 10.3 ppm of boron while leaves from deficient trees had 0.5 to 1 ppm. They pointed out that low soil moisture may accentuate boron deficiency.

Bruno (14) found that inadequate moisture may also cause signs of a zinc deficiency to appear when the zinc supply seems adequate. An abundance of water may retard development of the zinc deficiency symptoms. Mulder (42) showed that high calcium or phosphorus levels in the soil tend to bring on zinc deficiency. According to Bollard (6), Bould et al. (8), and Trocme (64), the threshold value for zinc deficiency is about 10 to 14 ppm. Healthy trees had 16 to 30 ppm of zinc in their leaves. Woodbridge (69) described zinc deficiency

as a cause of delayed bud opening, modified rosette, small, elongated and uniformly yellow leaves with the symptoms often distributed irregularly on the tree.

Leaves from pear trees deficient in copper may contain between 3 and 7 ppm according to Oserkowsky and Thomas (45). Bould et al. (9) found that the value for copper deficiency was about 5 ppm. They found yellow to necrotic leaf tips, and dieback of shoots with retention of dead terminal leaves typical of copper deficiency. Dieback of the terminal shoots with copper deficiency was also described by Jones (31) and Harris (24).

Kenworthy (33) also established levels of essential trace elements in Bartlett leaves: manganese - 133 ppm, iron - 140 ppm, copper - 54 ppm, and boron 23 ppm.

Amino Acid Levels as Related to Nutrition

Most of the previous work on the relationship between mineral nutrition and amino acid levels has been with crops other than pears.

Bollard's work (6) on amino acids found in the xylem sap of angiosperms and gymnosperms showed that the following amino acids were almost always present: aspartic acid, asparagine, glutamic acid, glutamine, serine, threonine, methionine, valine, leucine, and gamma-aminobutyric acid. Arginine, alanine, tyrosine and phenylalanine were sometimes present in trace amounts. In a study of the amino acids found in the xylem sap of apples, he found that aspartic acid, asparagine, and glutamine predominated.

Boynton et al. (11) studied the effects of potassium and magnesium deficiencies on amino acid levels in Sparkle strawberry leaflets. Control plants contained a total of 502 ug of amino acids per gram of dry tissue, the magnesium deficient plants contained 504 ug per gram and the potassium deficient plants, 1392 ug per gram. In the control treatment about 90 percent of the amino acids were gamma-aminobutyric acid (27.7%), alanine (15.7%), arginine (11.8%), glutamic acid (11.6%), serine (7.4%), and threonine (5.0%). Glycine, asparagine, glutamine, histidine, lysine, proline, valine, tyrosine and pipicolic acid made up the other 10 percent.

Plants deficient in magnesium contained about one-half as much gamma-aminobutyric acid, about 50 percent more serine and glutamic acid, and slightly more asparagine and pipecolic acid. All others were present in small amounts as they were in the control. The leucines were present in magnesium deficient plants but not in the controls. In potassium deficient plants only 6.2 percent of the total amino acids was gamma-aminobutyric acid, 36.1 percent was arginine, 7.0 percent glutamic acid, and 16.1 percent aspartic acid. The concentrations of the other amino acids were similar to the checks except for the presence of the leucines in the deficient plants.

Deficiencies of the elements, calcium, magnesium, potash and phosphorus, caused a sharp increase in the total free amino acids in tobacco according to Steinberg et al. (59). Several other workers (18, 21, 52, 54) have observed an increase in total free amino acids in potassium deficient barley and alfalfa plants. Richards and

Berner (52) and Richards and Coleman (53) found an accompanying decrease in aspartic and glutamic acids. Richards and Coleman also noted the accumulation of putrescine in potassium deficient barley leaves.

Richards and Templeman (54) reported that the most pronounced characteristic of phosphorus deficiency in barley leaves is an accumulation of amides accompanied by an increase in the total free amino nitrogen. They also found a general drop in all amino acids with nitrogen deficiency. Similar results were obtained by Gregory and Sen (21).

Possingham (47) reported that tomato plants deficient in zinc, copper, manganese and iron contained more total free amino acids than control plants, but the molybdenum deficient plants were about the same as the controls in this regard. The following table summarizes Possingham's results on the increase in specific amino acids which accompany various nutrient deficiencies:

Deficient Element	Amino Acids Increased
Iron	Asparagine, glutamine and pipecolinic acid
Zinc	Asparagine, glutamine and beta-alanine
Manganese	Pipecolinic and other amino acids except the amides
Molybdenum	Beta-alanine

Certain variations from these results have been reported by other workers. Steward and Pollard (60) found an accumulation of arginine in iron deficient blueberry and apple. Brown (13) suggested that arginine accumulation was typical of iron deficient plants.

Margolis (39) found that tomato plants deficient in manganese accumulate the amide, asparagine, and Hewitt et al. (28) found an accumulation of both amides in manganese deficient cauliflower plants. Hewitt (27) later reported that glutamic and aspartic acids were generally the principal amino acids accumulated by manganese deficient plants.

Most workers (28, 39, 47) agreed that a molybdenum deficiency may be characterized by a reduced concentration of amino acids.

The relation between amino acids and nutrition has been summarized by Possingham (47): Except for amall anomalies, the array of amino acids remains the same regardless of the nutrient status, but quantitative relations do change considerably.

Fire Blight Susceptibility as Related to Nutrition

According to Heald (25) the fire blight disease was first reported in the New York Hudson River Highlands about 1780 and has spread from that area throughout the United States and southern Canada. In 1878, Burrill, in Illinois, established the bacterial nature of the disease. The bacteria is presently known as Erwinia amylovora (Burr.) and will be referred to as E. amylovora in this report.

The close relationship between the succulent growth of the tree and its susceptibility to fire blight quickly became apparent. Hildebrand and Heinicke (30) reported an increase in fire blight on shoots of Delicious, Cortland and Rhode Island Greening apple trees following application of nitrogen fertilizer.

They also noted that orchard cultivation increased growth and fire blight. Fisher (17) reported that mulching increased leaf nitrogen and the development of fire blight in the trees. He observed no correlation between application of phosphorus and/or potassium and fire blight development; however, the leaf potassium level in his study did not exceed 1.45 percent. Other workers (12, 44, 57, 61) have also commented on the close relation between susceptibility and the vigor of the trees.

Nightingale (44) felt the balance between carbohydrates and nitrogen was more important than nitrogen alone in determining the susceptibility of the trees to fire blight. E. amylovora grew poorly on agar slants of the juices from trees high in carbohydrate and low in nitrogen, but slants from trees low in carbohydrates and high in nitrogen favored bacterial growth. The addition of asparagine to the medium from the low nitrogen high carbohydrate tree also favored bacterial growth. The amino acids tyrosine, cystine, and glutamic acid did not improve bacterial development. Link and Wilcox (38) also noted the importance of the carbohydrate-nitrogen balance in fire blight resistance.

The use of asparagine to increase tree susceptibility to fire blight was also studied by Ark (1). When a dormant Bartlett pear tree was inoculated with asparagine before a bacterial inoculation, the shoots became infected with E. amylovora, while those inoculated without an injection of asparagine showed no infection. All E. amylovora isolates studied by Ark utilized asparagine as a sole source of nitrogen in media studies. A few were able to use alanine, leucine, and proline,

but none of the isolates utilized glycine, valine, iso-leucine, cystine, cysteine, tyrosine, tryptophane, or glutamic acid.

Spraying to Control Fire Blight

The use of spray materials has grown from bordeaux sprays in pre-war days to the present day use of various antibiotics and organic compounds, as well as the copper sprays. A few of these compounds and their effectiveness are listed below:

Compound	Control	Reference
Bioquin 1	Fair	(48)
Dithane D-14	Fair	(62)
2-Pyridinethione-1-oxide	Best	(22)
Naban	Fair	(58)
Tersan	Good	(58)
Dithane Z-78	Fair	(26, 62, 68)
Streptomycin	Good	(41, 68)
Terramycin	Good	(68)
Agrimycin	Fair	(17, 19)

In spite of all this work, Goodman (19) summarizes the results as negative, "It is suggested that antibiotic sprays, although capable of providing blossom type control are ineffective against the late or mid-season form of twig infection."

GENERAL PROCEDURE - EXPERIMENTS I, II and III

Bartlett pear trees were grown in various nutrient solutions to supply material for studies on the effect of nutritional environment on the concentration of certain elements in the leaves, the concentration of amino acids in shoots and leaves, and the susceptibility of the tree to E. amylovora. Twenty-three nutrient solutions of high, low, and intermediate levels of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, boron, manganese, copper and molybdenum were used, and each treatment was replicated three times.

Nutrient solutions for the various treatments were obtained by using the Hoagland (29) solution as a check and varying its composition to supply the other nutrient levels (Table I). Minus solutions were void of the element being studied except for low nitrogen. This treatment was composed of only 20 percent of the nitrogen found in the check solutions. In 1958, high level solutions of nitrogen, phosphorus, potassium, calcium, and magnesium contained five times as much of their respective element as the check solution. The trace elements, iron, zinc, boron, manganese, copper, and molybdenum, were 25 times as concentrated in their respective high level solutions as the check solution. In 1959 the level of some of the plus solutions was dropped to keep the trees alive. High nitrogen was dropped to a three-fold concentration, and high iron, zinc, and boron were dropped to a fifteen-fold concentration.

Stock solutions were prepared by dissolving C.P. grade chemicals in deionized water, and the nutrient solutions were made from these.

One-year-old Bartlett pear trees on a French seedling rootstock were

TABLE I

Composition of Nutrient Solution used in 1959 and 1960

Treatment	Ppm										
	N	P	K	Ca	Mg	Fe	Mn	B	Cu	Zn	Mo
Check	210	31	235	200	49	5	.5	.5	.02	.05	.01
-N	42	31	235	200	49	5	.5	.5	.02	.05	.01
3N*	630	31	235	200	49	5	.5	.5	.02	.05	.01
-P	210	-	235	200	49	5	.5	.5	.02	.05	.01
5P	210	155	235	200	49	5	.5	.5	.02	.05	.01
-K	210	31	-	200	49	5	.5	.5	.02	.05	.01
5K	210	31	1175	200	49	5	.5	.5	.02	.05	.01
-Ca	210	31	235	-	49	5	.5	.5	.02	.05	.01
5Ca	210	31	235	1000	49	5	.5	.5	.02	.05	.01
-Mg	210	31	235	200	-	5	.5	.5	.02	.05	.01
5Mg	210	31	235	200	245	5	.5	.5	.02	.05	.01
-Fe	210	31	235	200	49	-	.5	.5	.02	.05	.01
15Fe*	210	31	235	200	49	75	.5	.5	.02	.05	.01
-Mn	210	31	235	200	49	5	-	.5	.02	.05	.01
25Mn	210	31	235	200	49	5	12.5	.5	.02	.05	.01
-B	210	31	235	200	49	5	.5	-	.02	.05	.01
15B*	210	31	235	200	49	5	.5	7.5	.02	.05	.01
-Cu	210	31	235	200	49	5	.5	.5	-	.05	.01
25Cu	210	31	235	200	49	5	.5	.5	.50	.05	.01
-Zn	210	31	235	200	49	5	.5	.5	.02	-	.01
15Zn*	210	31	235	200	49	5	.5	.5	.02	.75	.01
-Mo	210	31	235	200	49	5	.5	.5	.02	.05	-
25Mo	210	31	235	200	49	5	.5	.5	.02	.05	.25

*These treatments were 5N, 25Fe, 25B, and 25Zn in 1958.

selected for this study. The trees were pruned to reduce weight variation to 105±15 grams and to a standard tree height of 24 inches.

In May 1958, the trees were planted in 12-inch clay pots using coarse quartz sand according to the procedure described by Bergman (5).

One quart of the proper solution was applied to each tree twice a week. The trees were watered with deionizing water as often as needed to prevent wilting, or about three times a week. The deionized water prevented the toxic accumulation of minerals in the quartz media. During the winter the trees were stored in a 38 to 42° F storage. They received about one pint of distilled water per month to prevent dessication. The trees were grown under these conditions for three years -- 1958, 1959 and 1960.

Experiment I

Procedure

The influence of nutritional environment on the concentration of elements in the leaf was studied using the Bartlett pear trees described on page 14 Most of the leaves were removed from each tree in early September in 1958 and 1959 for nutrient element analysis.

The samples from 1958 were analyzed for potassium with flame photometry in the Department of Horticulture. All further analyses on the 1958 samples were made in laboratories of the Department of Agricultural Chemistry. Nitrogen was analyzed by the Kjeldahl method. Phosphorus, calcium, magnesium, iron, boron, manganese, and copper were determined spectrographically.

Samples from 1959 were analyzed by the Department of Horticulture. Nitrogen was determined by the Kjeldahl method and potassium by flame photometry. Phosphorus, calcium, magnesium, iron, zinc, boron, manganese, copper, molybdenum, and aluminum were analyzed with a "quantograph"[1] (34).

The leaf composition data was treated statistically as a completely randomized block. Difference between treatment means was based on a minimum required difference (MRD). The MRD is obtained by multiplying the standard deviation of the treatment mean by the value in the studentized range table corresponding to the degree of freedom for the error term (f) and the number

---

[1] Photoelectric spectrometer as manufactured by Applied Research Laboratories, Glendale, California.

of averages (a) being compared. For this study 'f' was 44 and 'a' was 23. All values were compared with those of the check treatment for significance.

Results

1. Nutrient Element Composition of Leaves

Data pertaining to the mineral composition of the leaves harvested in 1958 and 1959 are given in Table II for nitrogen, phosphorus and potassium; Table III for calcium, magnesium and iron; Table IV for boron, manganese and copper; Table V for zinc, molybdenum and aluminum.

In the following elaboration of the data, all comparisons were based on a composition difference between the particular treatment and the check.

Nitrogen: The concentration of leaf nitrogen was higher in 1958 than in 1959 regardless of the treatment. The greatest decrease in leaf nitrogen occurred for the minus nitrogen treatment which did not significantly affect the concentration of nitrogen in the leaf in 1958, but did have a significant influence in 1959. The concentration of nitrogen in the leaf was not significantly altered by any of the other treatments in 1958 or 1959.

In 1959, trees grown with a minimum of nitrogen had the lowest concentration of leaf nitrogen (1.34%); while the check trees had 2.06 percent. The high nitrogen trees contained 3.28 percent, which was the highest concentration of nitrogen observed.

Trees grown in minus calcium solutions were also higher in nitrogen

TABLE II

Influence of Nutritional Environment on Leaf Composition*
(Percent Dry Weight)

Treatment	Nitrogen		Phosphorus		Potassium	
	1958	1959	1958	1959	1958	1959
Check	2.81	2.06	.18	.13	2.24	1.65
-N	2.52	1.34	.26	.16	2.47	1.89
3N**	3.66	3.28	.29	.16	1.52	0.96
-P	2.60	2.09	.12	.07	2.25	2.08
5P	2.79	2.33	.32	.55	1.41	0.75
-K	2.39	2.07	.32	.22	0.37	0.49
5K	2.47	1.80	.21	.14	3.64	4.68
-Ca	3.27	2.50	.29	.15	2.33	1.99
5Ca	2.67	2.06	.17	.11	1.96	1.76
-Mg	2.71	2.22	.23	.16	2.46	1.98
5Mg	2.80	2.07	.21	.13	1.43	1.39
-Fe	2.47	2.08	.22	.14	1.79	1.65
15Fe**	2.49	1.85	.27	.14	1.28	1.24
-Mn	2.72	2.06	.24	.12	2.10	1.85
25Mn	2.43	2.09	.27	.15	1.83	1.53
-B	2.58	1.90	.22	.13	2.01	1.52
15B**	2.80	2.21	.23	.14	1.53	1.53
-Cu	2.60	1.94	.25	.12	1.71	1.21
25Cu	2.75	2.10	.20	.12	2.08	1.83
-Zn	2.84	2.18	.21	.12	2.38	1.59
15Zn**	2.60	2.12	.23	.13	1.87	1.57
-Mo	2.50	2.17	.23	.14	2.30	1.79
25Mo	2.82	2.10	.23	.14	2.30	1.79
MRD 5%	.68	.36	N.S.	.03	.69	.35
MRD 1%	N.S.	.48	N.S.	.04	.91	.46

*All treatments are an average of three figures except -Ca and 15 Fe for 1959 which are an average of two figures.

**These treatments were 5N, 25Fe, 25B, and 25 Zn in 1958.

TABLE III

Influence of Nutritional Environment on Leaf Composition*
(Percent Dry Weight)

Treatment	Calcium		Magnesium		Iron	
	1958	1959	1958	1959	1958	1959
Check	1.40	0.83	.33	.24	.0364	.0164
-N	0.86	0.75	.34	.17	.0268	.0153
3N**	0.68	1.14	.35	.36	.0348	.0183
-P	1.27	0.67	.33	.21	.0259	.0170
5P	0.78	1.00	.45	.32	.0291	.0149
-K	1.01	1.05	.54	.34	.0303	.0139
5K	0.53	0.41	.22	.08	.0227	.0152
-Ca	0.63	0.44	.31	.32	.0886	.0202
5Ca	1.31	1.03	.35	.24	.0234	.0173
-Mg	1.00	0.81	.14	.04	.0201	.0154
5Mg	1.06	0.84	.79	.37	.0218	.0167
-Fe	1.31	1.31	.36	.36	.0211	.0137
15Fe**	1.07	0.98	.64	.30	.0886	.0544
-Mn	0.99	0.81	.32	.24	.0242	.0157
25Mn	1.10	0.81	.32	.25	.0241	.0156
-B	1.22	0.82	.34	.25	.0222	.0153
15B**	1.30	1.12	.48	.26	.0307	.0145
-Cu	1.02	0.94	.50	.25	.0213	.0146
25Cu	1.55	0.86	.44	.32	.0238	.0148
-Zn	1.31	0.96	.36	.22	.0268	.0149
15Zn**	1.06	0.73	.35	.22	.0230	.0148
-Mo	1.04	0.89	.44	.25	.0271	.0170
25Mo	1.28	0.73	.38	.25	.0250	.0165
MRD 5%	.47	.36	.18	.16	.0163	.0061
MRD 1%	.63	.47	.25	.21	.0247	.0081

*All treatments are an average of three figures except -Ca and 15Fe for 1959 which are an average of two figures.

**These treatments were 5N, 25Fe, 25B, and 25Zn in 1958.

TABLE IV

Influence of Nutritional Environment on Leaf Composition*
(Ppm Dry Weight)

Treatment	Manganese		Boron		Copper	
	1958	1959	1958	1959	1958	1959
Check	32	29	32	29	13	7
-N	39	50	40	34	15	9
3N**	40	62	28	21	15	9
-P	66	39	35	32	14	13
5P	49	58	32	38	15	7
-K	95	54	34	47	14	8
5K	25	26	29	33	16	7
-Ca	52	78	34	33	15	10
5Ca	45	23	29	23	16	10
-Mg	60	49	35	38	12	8
5Mg	41	30	25	29	17	7
-Fe	53	58	35	31	13	9
15Fe**	91	64	31	31	15	10
-Mn	21	14	26	31	15	9
25Mn	225	311	36	29	16	9
-B	38	30	17	13	13	8
15B**	52	39	112	155	17	7
-Cu	37	34	25	30	12	7
25Cu	49	31	31	29	22	11
-Zn	49	29	31	27	13	11
15Zn**	40	28	32	25	24	8
-Mo	43	37	27	31	16	8
25Mo	49	36	32	33	17	7
MRD 5%	49	24	16	11	4	4
MRD 1%	65	31	21	15	7	N. S.

*All values are an average of three figures except -Ca and 15Fe for 1959 which are an average of two figures.

**These treatments were 5N, 25Fe, 25B, and 25Zn in 1958.

TABLE V

Influence of Nutritional Environment on Leaf Composition*
(Ppm Dry Weight - 1959)

Treatment	Zinc	Molybdenum	Aluminum
Check	26	3.5	136
-N	27	3.9	146
3N	29	4.9	146
-P	31	4.0	193
5P	26	5.1	99
-K	32	5.6	105
5K	26	2.4	143
-Ca	30	3.7	170
5Ca	26	4.6	174
-Mg	25	4.0	128
5Mg	27	4.5	181
-Fe	27	6.3	162
15Fe	27	4.6	177
-Mn	23	3.9	204
25Mn	28	3.2	153
-B	23	4.2	183
15B	24	4.6	126
-Cu	25	4.7	139
25Cu	27	3.4	107
-Zn	24	4.8	213
15Zn	35	3.0	177
-Mo	27	4.5	151
25Mo	22	2.6	209
MRD 5%	N.S.	1.4	73
MRD 1%	N.S.	1.9	96

*All values are an average of three figures except -Ca and 15Fe which are an average of two figures.

than the check trees. This increase was not significant in 1958, but it was by 1959.

Phosphorus: The concentration of phosphorus in the leaf was slow to respond to the various treatments. In 1958 there was no significant difference between the concentrations of leaf phosphorus over the entire experiment. Some trends were evident and these became significant in 1959. The concentration of phosphorus was generally higher in 1958 than it was in 1959.

Treatments void of phosphorus resulted in the lowest concentration of leaf phosphorus (0.07%) while the check trees contained 0.13 percent. When an abundance of phosphorus was supplied to the tree, the phosphorus in the leaves reached the highest concentration (0.55%).

The leaf level of phosphorus was higher than the check in the trees grown in either an excess or deficiency of nitrogen. Both of these treatments resulted in a leaf concentration of 0.16 percent in 1959.

The data also indicated that trees grown in a potassium deficient environment accumulated phosphorus in their leaves. These trees contained 70 percent more phosphorus than the check trees.

Potassium: The concentration of leaf potassium was higher in almost all trees in 1958 than 1959. In some instances response to treatments were similar for both years, but in others the data from the two years did not indicate the same effects.

The greatest effect on the concentration of potassium in the leaf was

observed on trees grown in the absence of potassium or in an excess of potassium. In 1959, trees grown in the absence of potassium contained only 0.49 percent leaf potassium. Trees grown in check solutions contained 1.65 percent leaf potassium, and the high potassium trees contained 4.68 percent leafpotassium. In 1958 when the check value was 2.24 percent, the high potassium leaves contained 3.64 percent and the minus potassium leaves contained 0.37 percent.

In 1959, the percent potassium in the leaves increased when trees were grown in solutions lacking nitrogen, phosphorus or calcium. These effects were not evident in 1958.

The percent potassium in the leaves decreased under the influence of high levels of nitrogen, phosphorus, and iron in 1958 and 1959. In 1958, high boron trees also contained less leaf potassium, but the 1959 data did not indicate this effect. In 1959 trees grown in a minus copper solution contained less potassium than the check trees while the 1958 data did not show this effect.

Calcium: The check trees contained more calcium in 1958 than they did the following year, but this trend was vague for other treatments.

Data for both seasons showed that the lowest concentrations of leaf calcium resulted when trees were grown in solutions deficient in calcium or with an excess of potassium.

There appeared to be quite a difference between the effect of the nitrogen treatments on calcium in 1958 and 1959. In 1958, both the trees grown in high

nitrogen (5N) and those grown in low nitrogen contained less leaf calcium than the check trees. The 1959 high nitrogen treatment (3N) tended to increase the concentration of calcium in the leaves. The minus nitrogen treatments for 1958 and 1959 and the checks for 1959 were almost equal.

Minus iron trees contained about the same percent of leaf calcium as the check trees in 1958 (1.34% and 1.40%, respectively). In 1959, however, the leaves from trees grown in an iron deficient nutrient solution contained 1.31 percent calcium compared with 0.83 percent for the checks.

Magnesium: In 1959, the only treatments which appeared to significantly alter the concentration of leaf magnesium were minus magnesium and high magnesium. Leaves from these two treatments contained 0.04 percent and 0.87 percent magnesium respectively, and the check leaves contained 0.24 percent. The 1958 data indicated a similar response to variations in the magnesium level, although the general concentration was higher for most treatments.

In 1958, the minus potassium treatment increased the magnesium concentration in the leaf to 0.54 percent, compared with the 0.33 percent in the check leaves. This trend was evident in 1959, but it was not significant.

High iron treatments (125 ppm) in 1958 resulted in almost twice the concentration of leaf magnesium as that found in the check leaves. However, high iron (75 ppm) in 1959 did not seem to effect the leaf magnesium concentration.

Iron: During both years the high iron treatment resulted in an accumulation of iron in the leaf; however, the greatest accumulation occurred in 1958. In 1959, the leaf concentration of iron was less for all treatments. Low levels of iron decreased the percent of iron in the leaves, but the difference was not significant either year.

In 1958 the minus calcium trees accumulated almost as high a foliar concentration of iron as the high iron trees. Minus calcium leaves tended to contain more iron than the check leaves in 1959, but the difference was not significant.

Manganese: The data for both years showed that the highest concentration of manganese resulted from the high manganese treatment. Minus manganese treatments tended to decrease the leaf manganese concentration, but the differences were not significant.

The concentration of leaf manganese was also increased when the environment was deficient in potassium, calcium, or iron, or when an excess of nitrogen, phosphorus, or iron was available. Only the effects of minus potassium and high iron were significant in both 1958 and 1959. The effects of minus calcium, minus iron, and high nitrogen and phosphorus were significant only in 1959.

Boron: In 1958, the only treatment which significantly affected the concentration of boron in the leaves was high boron. The 1959 data showed an increase in the concentration of leaf boron due to the application of solutions

high in boron or low in potassium. Trees grown in the absence of boron contained only 13 ppm of boron in their leaves in 1959, as compared to 29 ppm from the check treatment.

Copper: The concentration of copper in the leaves in 1958 was almost twice that found for most treatments in 1959. In 1958 the highest concentration of copper was in the leaves from the high zinc treatment (24 ppm), second was the high copper treatment (22 ppm) and the check trees contained 13 ppm. In 1959, the check trees contained only 7 ppm, high copper trees - 11 ppm, and minus phosphorus trees - 13 ppm.

Zinc: The concentration of zinc in the leaves of Bartlett pear trees was not altered significantly by any of the treatments.

Molybdenum: The highest concentration of this element was found in trees grown in an absence of iron. Other treatments which increased the concentration of molybdenum were minus potassium, high phosphorus, and high nitrogen. None of the treatments significantly decreased leaf molybdenum below the check value.

Aluminum: The check trees contained 136 ppm of aluminum. The highest concentration (213 ppm) was found in trees grown in the absence of zinc. High molybdenum trees were next with 209 ppm. None of the treatments decreased leaf aluminum below the concentration found in the check treatment.

2. Deficiency and Toxicity Symptoms of Stems and Leaves

During 1958 and 1959 observations were made on the influence of nutritional environment on the general appearance of the pear trees grown in various nutritional environments. Some of the trees exhibited symptoms which could be associated with the excess or deficiency of a certain element.

Nitrogen: Trees grown in a low nitrogen solution were generally retarded in stem and leaf growth. The leaves were smaller than the checks, and light green to yellow in color. High nitrogen trees had very dark leaves of good size, growth was about the same as that of the check trees.

Phosphorus: The low phosphorus trees developed foliar symptoms in 1959. The leaves were small and the adaxial faces tended to fold together along the midrib. There was some irregular yellowing of the leaves with necrotic patches. Defoliation began in late August on lower limbs and moved apically. There was little twig growth in 1959 or in 1960. Axial buds in 1959 became visible much later than in the check trees, and in 1960 there was no bloom although the check trees did flower.

High phosphorus trees had a foliar color that was extremely dark. Growth was slightly less than on the check trees.

Potassium: Potassium deficient trees showed an inward rolling of the leaves and a dull gray to brown marginal scorch. The symptoms were most prevalent at the base of a twig and diminished toward the tip.

High levels of potassium resulted in foliar symptoms resembling those of magnesium deficient trees.

Magnesium: Leaves on the minus magnesium trees showed patches between the lateral ribs that were either chlorotic or necrotic. These areas formed a pattern similar to a "Christmas tree" shape. Symptoms seemed to be most prevalent at the base of the stems.

High magnesium trees had a very desirable appearance.

Calcium: Calcium deficient leaves resembled potassium deficient leaves except for the color of their necrotic areas. On the low calcium leaves this area was more of a bright brown.

High calcium trees appeared healthy and made good growth. The leaves tended to roll slightly and defoliation preceded that of the check trees by 2 to 3 weeks. The petioles and midribs became red in August.

Iron: Low iron trees exhibited less growth and branching than check trees. By mid-July there was also a prevalence of bright yellow leaves. Some leaves were yellow only along the margins, while other leaves were entirely yellow. Usually the area next to the midrib remained green.

High iron trees were severely stunted in twig and leaf growth. The leaves showed patches of necrosis most of which were between the lateral leaf veins at the leaf's margin. The midrib and the fruit showed an abundance of red coloring.

Copper: One tree which had a leaf copper value of 5 ppm, showed symptoms of copper deficiency in 1960. There was from two to three inches of dieback on each of the shoot terminals. The dead twig and attached leaves

were black and curled, but the leaves did not fall off.

Other Treatments: Low levels of boron, zinc, manganese and molybdenum resulted in growth inhibition, reduced branching and small leaves. There were no symptoms which were specific for any one element.

High levels of boron, zinc, manganese, copper and molybdenum inhibited growth slightly but did not result in any specific symptoms.

3. Deficiency and Toxicity Symptoms on Roots

Most of the roots from the trees grown in the various nutritional treatments appeared uniform in their growth and were dark brown in color when examined after the 1960 growth period.

High calcium trees seemed to have the best root system. The roots were extensive, well branched, fine, and colored similar to the check trees.

Trees from the high nitrogen treatment had a very poor root system. The roots had grown very little since planting, they were coarse and the branching was sparse.

The poorest roots were on the trees grown in a high iron solution. They had made almost no growth, and branching was limited to short wiry laterals. The root system was very coarse and black in color.

Minus copper treatments also resulted in poor root growth although the texture and color were about the same as the check roots.

High phosphorus treatments did not alter root growth appreciably, but the color was more of a reddish brown.

Experiment II

Procedure

The amino acid composition of the growing shoots and the mature leaves from the pear trees described on page 12 was studied to ascertain the effect of nutritional environment on the amino acids in the tree.

On June 6, 1960, a two-gram sample of the growing shoots were collected from most of the pear trees[1]. The tissue was immediately placed in 50 ml of 70 percent alcohol at 80° C for 10 minutes. The alcohol solution was decanted and 50 ml of water added. After ten minutes at 80° C, this solution was decanted and added to the alcohol extract, the combined extract filtered, and brought to volume with 80 percent alcohol in a 200 ml volumetric flask. Four gram samples of mature leaves were taken from selected treatments on August 3, 1960, after all terminal growth had ceased. Alcohol and water extracts were also prepared from these samples.

To permit the development of better chromatograms a portion of the sample extracts were then purified by use of the following modification of the procedure described by Plaisted (46):

1. 25 ml of the sample was added to 9 ml of Dowex 50 x 12 resin in a 250 ml wide mouthed flask.
2. Resin and sample were agitated for 10 minutes; the resin was allowed to settle and the solution decanted.
3. The resin was washed twice with 25 ml of 80% alcohol. Samples were agitated for two minutes per washing and solutions were decanted after the resin settled.

---

[1] Because of insufficient terminal growth not all trees were sampled.

4. The resin which then contained the amino acids was washed twice with 25 ml of 4N ammonium hydroxide in 80% alcohol. For each washing, samples were agitated two minutes. After the resin settled, the solutions were decanted and saved for analysis.*
5. The resin was again washed with 25 ml of 80% alcohol with a two minute agitation. The resin was allowed to settle, and this solution was decanted and saved for analysis. A similar wash was then done with water, and this was also saved.*
6. The resin was then regenerated with a hydrochloric acid washing.

The purified sample was dried under vacuum in a freeze-dry apparatus consisting of a "Cenco High Vac 14" vacuum pump hooked through a glass lyophylization trap to an approximately 4 liter "Machlett and Sons freezing trap". The dry sample was dissolved in a 10 percent isopropyl alcohol and brought to a 1 ml volume.

100 ul of the concentrated sample (equivalent to 50 milligrams of fresh tissue) was chromatographed on sheets of 18 by 22 inch Whatman No. 1 paper with water saturated phenol in the first direction and n-butanol-propionic acid-water in the second direction (4).

Identification of the developed amino acid spots was based on three methods (4): (1) Identification of the amino acid spot with the specific ninhydrin color; (2) comparison of the relative Rf values as stated in the literature; and (3) by cochromatography with known amino acids and known C^{14} labeled amino acids. To develop the ninhydrin color, each chromatogram was dipped in 40 ml

*The additional washes were used to insure complete removal of the amino acids.

of an acetone solution containing 2 ml of glacial acetic acid and 80 mg of ninhydrin, and dried in an ethanol atmosphere at 60° C for 15 minutes (40). After identification, a quantitative analysis was performed on each spot (40). The spot was cut into approximately 1 cm squares, and allowed to stand for 30 minutes in 0.6 ml of a 5% ninhydrin in butanol solution in a tightly stoppered test tube. To each tube, 5 ml of 70% alcohol containing 70 mg of $CaSO_4 \cdot 5H_2O$ per liter were added and the tubes were shaken for 15 minutes at a rate sufficient to agitate the paper squares in the tube. The optical density of each solution was determined at 515 mu[1] with a Coleman Spectrophotometer Junior. Colorimeter tubes were standardized previously to an optical density reading of .269±.005.

A standard curve was established for each amino acid by spotting known amounts of the amino acid on the chromatographic paper and following the same method of development and elution. These data were then used to translate optical density readings into micrograms of amino acids.

The data for treatments for which all replicates were sampled were statistically analyzed for minimum required differences (MRD). These treatments were check, high nitrogen, high phosphorus, minus potassium, minus and high calcium, high magnesium, high iron, minus and high zinc, minus and high boron, minus and high manganese, and high molybdenum.

---

[1]The 515 mu maximum absorption peak for the eluted amino acid solutions was determined with a Beckman Model DK-2 Spectrophotometer.

Results

Data from the samples of growing shoots taken for amino acid analysis in June, 1960 indicated a wide variation within treatments. This variation made it difficult to observe amino acid variations that might have resulted from nutrition. The results of the August, 1960 sampling seemed to be more uniform within treatments.

In most cases the following amino acids[1] were present in pear shoots and leaves: aspartic acid, asparagine, glutamic acid, glutamine, serine, glycine, threonine, alanine, and amino acids at the chromatographic location of gamma-amino butyric acid, leucine, cysteine, and arginine. Because of the large amounts of glutamic acid and glutamine, the trace amount of ninhydrin color at the Rf of gamma-amino butyric acid has been designated as this derivative of glutamic acid (43).

1. June Samples of Growing Shoots

Variations in tree nutrition significantly affected the concentration of glutamic acid, serine, alanine and the total concentration of the free amino acids (Table VI). The concentrations of asparagine, glutamine, cysteine, and arginine (Table VII), and aspartic acid, glycine, threonine, gamma-amino butyric acid, and leucine (Table VIII) did not vary significantly because of insufficient sample and wide biological variation. However, trends among these

---

[1] In all instances, reference to the presence of amino acid means only that a chromatographic spot, believed to be synonymous with the indicated compound, was present.

TABLE VI

Effect of Nutrition on Amino Acid Levels* in Pear Shoots - 1960
(Micrograms per 100 Milligrams Fresh Tissue)

Treatment	Total Amino Acids	Glutamic Acid	Serine	Alanine
Check	100	12	8	6
-N**	44	14	8	6
3N	188	26	22	8
-P**	98	10	6	4
5P	192	22	18	20
-K	102	10	8	6
5K**	224	28	20	20
-Ca	80	14	8	8
5Ca	98	26	16	18
-Mg**	46	10	8	4
5Mg	102	22	10	12
-Fe**	66	10	4	6
15Fe	96	20	8	16
-Zn	104	18	10	10
15Zn	104	12	10	12
-B	76	12	10	8
15B	42	8	8	10
-Mn	94	14	8	8
25Mn	72	14	10	12
-Cu**	90	20	10	10
25Cu**	102	20	12	14
-Mo**	116	26	12	12
25Mo	80	20	8	14
MRD 5%	72	12	8	8

*Data given are an average of three numbers.
**Data were not included in statistical analysis as all replicates could not be sampled.

TABLE VII

Effect of Nutrition on Amino Acid Levels* in Pear Shoots - 1960
(Micrograms per 100 Milligrams Fresh Tissue)

Treatment	Asparagine	Glutamine	Cysteine	Arginine
Check	58	2	tr	tr***
-N**	4	2	tr	tr
3N	72	22	tr	--
-P**	60	2	tr	tr
5P	94	18	tr	--
-K	64	2	tr	tr
5K**	42	32	tr	--
-Ca	32	2	tr	tr
5Ca	38	6	tr	--
-Mg**	6	4	tr	tr
5Mg	40	4	tr	--
-Fe**	30	2	tr	--
15Fe	26	14	tr	--
-Zn	44	4	tr	tr
15Zn	54	4	--	--
-B	26	4	tr	tr
15B	6	4	tr	--
-Mn	40	10	tr	--
25Mn	16	6	tr	--
-Cu**	28	6	tr	--
25Cu**	32	10	tr	--
-Mo**	38	8	--	--
25Mo	18	12	tr	--

*Data given are an average of three numbers. Statistical analysis indicated no significant differences.

**Data were not included in statistical analysis as all replicates could not be sampled.

***The symbol tr indicates a visible but unmeasurable spot on the chromatogram.

TABLE VIII

Effect of Nutrition on Amino Acid Levels* in Pear Shoots - 1960
(Micrograms per 100 Milligrams Fresh Tissue)

Treatment	Aspartic Acid	Glycine	Threonine	Gamma-amino butyric	Leucine
Check	10	2	2	tr	tr***
-N**	10	tr	tr	tr	tr
3N	26	8	4	tr	tr
-P**	12	2	2	tr	tr
5P	14	2	2	tr	tr
-K	10	2	tr	tr	tr
5K**	60	8	10	tr	tr
-Ca	10	6	tr	tr	tr
5Ca	10	2	2	tr	tr
-Mg**	12	2	tr	tr	tr
5Mg	10	2	2	tr	tr
-Fe**	12	tr	2	tr	tr
15Fe	8	2	2	tr	tr
-Zn	14	2	2	tr	tr
15Zn	8	2	2	tr	tr
-B	10	2	2	tr	tr
15B	4	2	-	tr	tr
-Mn	14	tr	tr	tr	tr
25Mn	10	2	2	tr	tr
-Cu**	12	2	2	tr	tr
25Cu**	10	2	2	tr	tr
-Mo**	16	2	2	tr	tr
25Mo	6	2	tr	tr	tr

*Data given are an average of three numbers. Statistical analysis indicated no significant differences.

**Data were not included in statistical analysis as all replicates could not be sampled.

***The symbol tr indicates a visible but unmeasurable spot on the chromatogram.

latter amino acids are interesting and suggestive for future analysis.

The high potassium trees had more amino acids than all others with a total of 224 ug per 100 mg of fresh tissue; this figure represents only one sample so its value is questionable. High phosphorus with 192 ug of amino acids and high nitrogen with 188 ug had the next highest concentrations. The check contained 100 ug of amino acids per 100 mg of tissue. Low concentrations of total amino acids were found in trees grown with high levels of boron and with low levels of manganese.

Nutrition of the trees seemed to alter the concentration of glutamic acid. This was significantly so in high nitrogen and high calcium trees where the concentration of glutamic acid was almost twice that of the check. High potassium and minus molybdenum trees showed the same trend.

Samples of the shoots from the trees grown in high levels of nitrogen, phosphorus, and calcium contained 22, 18 and 16 ug of serine per 100 grams of fresh tissue. These values were significantly larger than the concentration of serine in the check trees (8 ug). The high potassium treatment also seemed to increase the concentration of serine in the growing tip.

High levels of phosphorus, calcium, and iron resulted in a significantly greater accumulation of alanine than was present in the check trees. Check samples contained 6 ug of alanine while high phosphorus trees contained 20 ug, high calcium - 18 ug, and high iron - 16 ug. High potassium and copper trees also seemed to accumulate alanine.

Although the asparagine concentration was not significantly altered by nutrition, some trends were evident. The minus nitrogen, minus magnesium, and plus boron trees were lower in asparagine than other treatments. They contained 4, 6, and 6 ug respectively, while the checks contained 29 ug. The plus phosphorus trees had the highest level of asparagine (47 ug).

Glutamine levels also showed trends although they were not significant. High nitrogen, phosphorus and potassium trees were all higher in glutamine concentration than the check trees.

The cysteine spot was not evident in the samples from trees grown in a solution deficient in molybdenum or containing an excess of zinc, but was present for all other treatments.

The check and several of the treatments seemed to contain a trace of arginine. This was true with the minus nitrogen, phosphorus, potassium, calcium, magnesium, zinc and boron treatments.

2. August Samples of Mature Leaves (Table IX)

Aspartic acid levels were similar in the minus nitrogen, phosphorus, potassium and iron treatments. In the check trees, however, there was only a trace of aspartic acid and in the minus boron trees 3 ug were measured.

Glutamic acid was similarly affected. Check trees contained about 1 ug and minus boron trees about 5. 5 ug. Minus nitrogen, phosphorus, and potassium treatments were intermediate.

Low levels of potassium seemed to account for an accumulation of

TABLE IX

Effect of Nutrition on Amino Acid Levels* in Mature Pear Leaves - 1960
(Micrograms per 100 Milligrams Fresh Tissue)

Amino Acid	Treatment					
	Check	-N	-P	-K	-Fe	-B
Aspartic acid	tr**	0.5	1.0	0.7	1.5	3.0
Glutamic acid	1.0	2.3	2.7	3.4	3.3	5.5
Serine	0.4	0.5	0.4	3.9	0.5	1.7
Glycine	0.2	tr	0.2	0.2	tr	0.4
Threonine	tr	tr	tr	tr	tr	tr
Alanine	4.7	3.0	1.5	3.9	1.8	3.2
Arginine	--	--	1.5	--	tr	tr
Asparagine	--	--	--	13.2	tr	--
Glutamine	tr	tr	tr	7.9	tr	tr
Gamma-amino butyric acid	tr	tr	tr	tr	tr	tr
Leucine	tr	tr	tr	tr	tr	tr
Cysteine	--	--	--	tr	tr	tr
Total Amino Acids	6.2	6.3	7.0	32.9	7.0	13.2

*Each figure is an average of three values except minus nitrogen and minus iron which are averages of two figures.

**The symbol tr indicates a visible but unmeasurable spot on the chromatogram.

serine. A deficiency of other elements did not seem to affect the serine concentration, but the minus potassium trees contained 3.9 ug compared with 0.4 ug in the check trees.

Glycine and threonine did not seem to be affected by variations in the nutritional program.

The arginine spot was very evident in the samples from minus phosphorus trees. Samples from the check trees did not show this spot and minus iron and minus boron had only traces of it. The minus phosphorus trees averaged 1.5 ug per 100 mg of fresh tissue.

Alanine seemed to be less abundant in the minus phosphorus trees (1.5 ug) and the minus iron trees (1.8 ug) than in the checks (4.7 ug). The other amino acids observed were not greatly affected by nutrition.

Both of the amides, asparagine and glutamine, also accumulated under the condition of low potassium. Check trees showed only a trace of glutamine and no asparagine, but the minus potassium samples contained 13.2 ug of asparagine and 7.9 ug of glutamine.

Gamma-amino butyric acid and leucine were present in trace amounts in all treatments. Cysteine was not present in a noticeable quantity in the check, minus nitrogen, and minus phosphorus treatments. A trace of cysteine was present in minus potassium, minus iron, and minus boron trees.

The total amino acid levels were highest in the potassium deficient trees (32.9 ug) next highest in the minus boron trees (13.2 ug) and about the same in the minus nitrogen, phosphorus, iron, and check samples.

Experiment III

Procedure

This experiment was conducted to study the influence of nutrition on the susceptibility of pear trees to E. amylovora. On June 7, 1960, three actively growing shoots on each of the pear trees described on page 12 were inoculated with E. amylovora. The culture of bacteria used for the inoculation was 24 hours old and had been established by seeding plates of Emerson's medium (Table X) with infected plant tissue. The culture was transferred to the trees by use of the stab type inoculation described by Lamb (36).

Scoring for susceptibility to E. amylovora was based on the percent of inoculations which became infectious in combination with the percent of the total length of twig, limb, and trunk infected by the disease. The sum of these two percentages was designated as a susceptibility value. The percent of infectious inoculations was counted 30 days after inoculation, and the percent of the tree infected was measured 60 days after inoculation.

Results

The susceptibility values used to indicate the ease with which E. amylovora infected the trees in each nutritional treatment are shown in Table XI as the sum of the percent of infection based on total inoculations and the percent of infected wood. A graphic representation of the susceptibility values as they compare with average susceptibility for this experiment is shown in Figure 1.

TABLE X

Composition of Media for E. Amylovora

Medium	Components	Amounts
Synthetic*	K_2HPO_4	500 mg
	$MgSO_4$	250 mg
	KCl	250 mg
	Fe-Versenol	7.5 mg
	Thiamine hydroxide	.08 mg
	Sucrose	15 g
	Water	500 ml
Emerson's (liquid)**	NaCl	125 mg
	Peptone and beef extract	500 mg
	Yeast extract	50 mg
	Dextrose	50 mg
	Water	100 ml
Emerson's (solid)**	Same components as liquid plus 1.5% agar	

*This medium was derived from Czapek's medium, Difco Manual.

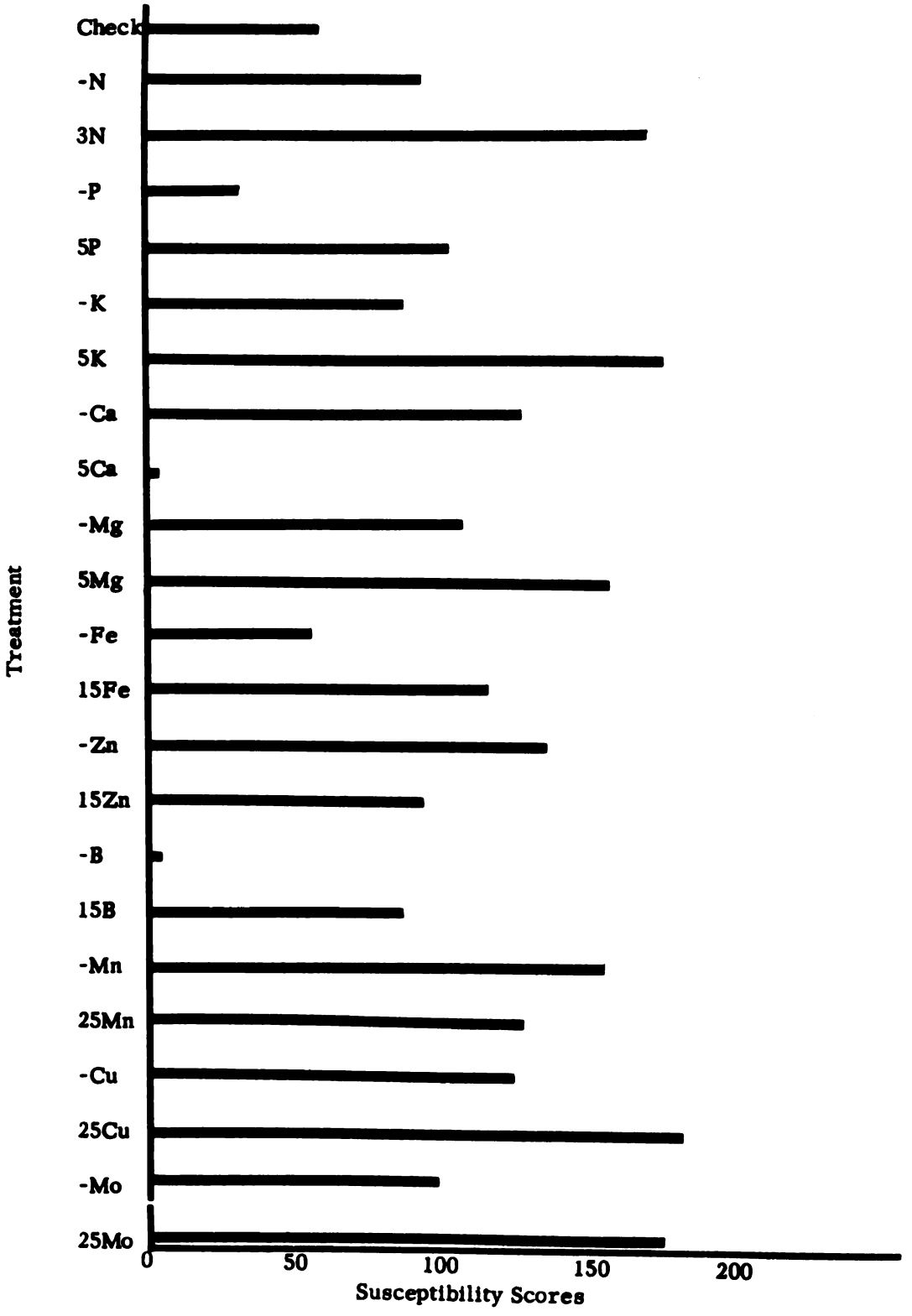
**Emerson's medium was recommended by Dr. Dwight Powell, University of Illinois.

TABLE XI

Influence of Nutritional Environment on the Susceptibility of Pear Trees to *E. Amylovora*

Treatment	Percent Take of Inoculations	Percent of Wood Infected	Susceptibility Source
Check	33	25	58
-N	67	26	93
3N	100	69	169
-P	22	9	31
5P	56	46	102
-K	44	42	86
5K	84	91	175
-Ca	44	83	127
5Ca	0	0	0
-Mg	33	73	106
5Mg	78	78	156
-Fe	33	22	55
15Fe	44	71	115
-Zn	67	68	135
15Zn	67	26	93
-B	0	0	0
15B	50	36	86
-Mn	89	65	154
25Mn	67	60	127
-Cu	50	74	124
25Cu	89	92	181
-Mo	50	48	98
25Mo	100	75	175
Mean	52	55	107

Figure 1. Effect of Nutritional Environment of Fire Blight Susceptibility



Low susceptibility was found for 12 of the 23 treatments. None of the inoculations on the high calcium or low boron trees developed into an infection. The minus phosphorus trees also indicated a high degree of resistance. The check trees, which received a balanced nutrient solution, and the minus iron trees were about equally resistant, and both were well below the rest of the treatments in the experiment.

The four most susceptible groups of trees resulted from high levels of nitrogen, potassium, molybdenum and copper. However, trees from treatments deficient in manganese or zinc, and the high magnesium trees were also highly susceptible.

Experiment IV

Procedure

Various amino acids were used as a sole source of nitrogen in synthetic liquid medium (Table X) inoculated with E. amylovora to determine which amino acids could be utilized by the bacteria as a source of nitrogen. The medium was devised to insure that no other source of nitrogen was available to the bacteria except one of the amino acids being studied.

After the amino acid had been added to the medium, the mixture was adjusted to a pH of 6.9 ± 0.1 with sodium hydroxide or hydrochloric acid and sterilized. Heat sterilization of the medium appeared to result in deamination of the amino acids so the medium was sterilized by filtering it through a Sietz germicidal suction filter equipped with pads of 0.5 micron porosity. The solution was transferred to a 125 ml flask and allowed to stand 24 hours. If turbidity did not develop during this period, the medium was assumed to be sterile and ready for inoculation.

All glassware was autoclaved for 30 minutes at 20 pounds pressure and 120° C. Other techniques necessary to ensure asceptic conditions were followed.

A stock of the bacteria[1] was maintained on a slant of Emerson's solid medium (Table X). Twenty-four hours prior to inoculating the media being studied, a tube of Emerson's liquid medium was seeded from the stock. A four millimeter diameter loop of this medium was used to inoculate each

---

[1]The original culture of E. amylovora used in these studies was obtained from Dr. Dwight Powell, University of Illinois.

flask of synthetic media containing separate amino acids.

The infected flasks were incubated for 48 hours at room temperature. After incubation, the growth of the bacteria was measured by recording the percent transmittance of the medium in a colorimeter at 525 mu using sterile medium as a standard.

Seventeen naturally occurring L-amino acids, some D- forms of these acids and five synthetic amides were studied (Table XII). The concentration of each compound was adjusted to provide 210 ppm nitrogen. Tests were replicated at least three times.

Results

Data showing the ability of E. amylovora to utilize various amino acids as a source of nitrogen are given in Tables XIII and XIV.

The first results obtained from this experiment indicated that only the amides, asparagine and glutamine, would support the growth of E. amylovora, and that aspartic and glutamic acid would not. It is believed that these results were erroneous. The first tests were conducted on a medium that contained sodium carbonate as a buffer to obtain the desired pH. Autoclaving the medium resulted in a return to a pH of about 4 (typical of a water solution of aspartic or glutamic acids) which appeared to be too low for E. amylovora to grow.

When this experiment was performed as previously described (page 45) the bacteria grew well (Table XIII) when the source of nitrogen was asparagine,

TABLE XII

Compounds Tested for Bactericidal Effect and/or Support for Bacterial Growth

Amides of Amino Acids	Moles per Liter x 10^{-2}*
Glycyl glycine amide	0.61
Alanyl asparagine	1.52
Glycine amide	1.52
Nicotinamide	1.52
Chloracetamide	3.00
Acetamide	1.32
D-asparagine	0.76
L-asparagine	0.76
DL-asparagine	0.76
L-glutamine	0.76
DL-glutamine	0.76

Amino Acids	Moles per Liter x 10^{-2}*
Leucine	1.52
Arginine	0.38
Lysine	0.76
Glycine	1.52
Alpha-Alanine	1.52
Serine	1.52
Phenylalanine	1.52**
Tyrosine	1.52
Cysteine	1.52
Histidine	1.52
Valine	1.52**
Tryptophane	0.76
Beta-Alanine	1.52
D-aspartic acid	1.45
L-aspartic acid	1.45
DL-aspartic acid	1.45
Gamma-amino butyric acid	1.45
Alpha-amino butyric acid	1.45
L-glutamic acid	1.43
DL-glutamic acid	1.43
Ammonium chloride	1.52

*Concentration adjusted to add about 210 ppm of nitrogen to the medium.

**Partially insoluble.

TABLE XIII

Compounds which Supported Growth of E. Amylovora

Compound	Percent Transmittance*
D-asparagine	58
L-asparagine	38
DL-asparagine	47
L-glutamine	66
DL-glutamine	47
L-glutamic acid	58
DL-glutamic acid	35
D-aspartic acid	82
L-aspartic acid	38
DL-aspartic acid	33
Gamma-amino butyric acid	35
Beta-alanine	5
Alanyl asparagine	64
Glycine amide	81
Ammonium chloride	58

*Values are an average of three readings.

TABLE XIV

Compounds which did not Support Growth of E. Amylovora

Compound	Percent Transmittance*
Cysteine	100
Serine	90
Tryptophane	91
Glycine	92
Alpha-alanine	90
Phenylalanine	90
Histidine	90
Leucine	95
Valine	88
Alpha-amino butyric acid	100
Nicotinamide	88
Acetamide	95
Chloracetamide	98
Glycyl glycine amide	95
Arginine	88

*Values are an average of three readings.

glutamine, aspartic acid or glutamic acid. A comparison of the D- and L-forms of these amino acids showed slight variations in their ability to support growth. The organism appeared to favor L- asparagine and L- aspartic acid over their D- forms, and DL- glutamine or glutamic acid over their L- forms.

The two other amino acids, gamma-amino butyric and beta-alanine, supported the growth of E. amylovora in culture. To a lesser degree, alanyl asparagine and glycine amide were satisfactory nitrogen sources.

The rest of the amino acids and amides tested apparently were not satisfactory sources of nitrogen for the organism (Table XIV).

Experiment V

This experiment was performed to test the bactericidal activity of a host of natural and synthetic nitrogenous compounds through the use of culture studies and field studies.

1. Culture Studies

Procedure

The synthetic liquid medium (Table X) used for these studies was identical to the one used in Experiment IV except for the addition of one gram of asparagine per liter of medium. The medium thus prepared, satisfactorily supported the growth of E. amylovora.

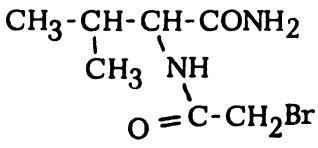
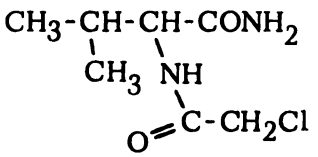
The bactericidal action of each compound was tested by adding it to the complete medium and inoculating the mixture with E. amylovora. Sterilization, inoculation, and pH adjustment were all carried out as described in Experiment IV by the use of germicidal filters. If the medium remained clear after being incubated at room temperature for 48 hours, the compound was considered to have bactericidal activity. The development of any turbidity in the medium was evidence enough to drop the compound from further consideration.

All amino acids which did not support growth of E. amylovora in Experiment IV were tested for bactericidal qualities in the same concentration used in the studies in Experiment IV. In addition, a group of 48 compounds

obtained from the Union Carbide Chemicals Company, were tested for bactericidal qualities. The concentration of each compound (Table XV) used in the test was established so that each compound would add 3.44 mg of amide nitrogen to 40 ml of the medium.

Results

Of all the compounds tested, seven appeared to have bactericidal activity. The rest of the compounds did not inhibit growth of the bacteria at the concentrations used. The active compounds were checked further to determine their minimum effective concentration. These compounds and their minimum effective concentrations are listed in the table below. Those marked with an asterisk (*) were active at the lowest concentrations, and so were used in the following field studies.

Compound	Concentration (Moles per liter)
	
*Bromoacetyl valinamide	5.25×10^{-5}
	
Chloroacetyl valinamide	5.60×10^{-3}

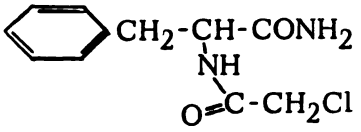
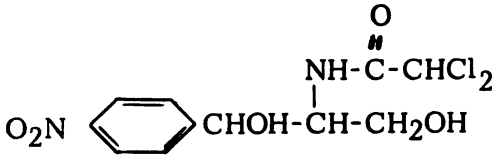
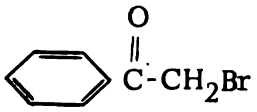
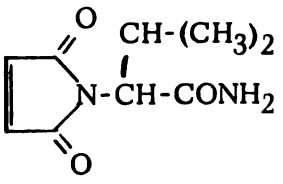
Compound	Concentration (Moles per liter)
	
Chloroacetyl phenylalaninamide	5.20×10^{-3}
$ClCH_2\text{-}CONH_2$	
Chloroacetamide	5.15×10^{-3}
	
*Chloramphenicol	1.25×10^{-5}
	
*Bromacetophenone	1.25×10^{-6}
	
*Alpha-maleimido isovaleramide	5.35×10^{-4}

TABLE XV

Compounds Tested for Bactericidal Effect on E. Amylovora

Compound	Moles per Liter (x 10^{-3})
DL-methioninamide crotate	5.35
DL-bromoacetyl valinamide	5.40
N(n-Butyl)-N'-(alpha-(-p-chloro-phenylglycinamido)) urea	5.45
DL-alpha-Aminobutyramide hydrochloride	5.45
DL-Stearoyl valinamide	5.35
DL-Alaninamide hydrochloride	5.45
DL-N-Carbamyl valinamide	2.52
DL-alpha-Chloropropionyl valinamide	5.35
N-(2-(3-Methyl) butyramido) carboxamidomethyl pyridinium chloride	5.35
Sarcosinamide hydrochloride	5.45
2-(3-Phenyl-5-isopropyl) thiohydantoin	5.45
Chloroacetylglycyl valinamide	5.45
N-(Ethylexalyl) valinamide	5.35
DL-alpha-Chloropropionyl leucinamide	5.35
2-(3-Ethyl-5-isopropyl) thiohydantoin	5.40
N-Phenyl-N'-(alpha-isovaleramide) urea	5.45
Alpha-(Maleimido) isovaleramide	5.35
DL-Maleylvalinamide	5.40
DL-alpha-hydroxybutyramide	5.35
Orotic acid salt of DL-valinamide	5.35
N-Benzoyl-DL-valinamide	5.35
N-Acetyl-DL-valinamide	5.05
DL-Chloroacetylvalinamide	5.60
DL-Norvalinamide hydrochloride	5.40
N-carbethoxy valinamide	5.30
Chloroacetyl-DL-phenylalaninamide	5.20
DL-p-chlorophenyl glycinamide hydrochloride	5.35
2-(3'-Indoleacetamido) acetamide	5.50
DL-Serinamide hydrochloride	5.35
DL-Methioninamide hydrochloride	5.45
1-Methyl-2, 6-dicarboxamido piperidine hydrochloride	2.74
DL-Valinamide hydrobromide	5.45
DL-Stearoylglycinamide	5.35
DL-Phenylglycinamide hydrochloride	5.40
DL-Sorbyl phenylalaninamide	5.25
DL-Glycylvalinamide hydrochloride	5.30

TABLE XV Cont'd

Compounds Tested for Bactericidal Effect on E. Amylovora

Compound	Moles per Liter (x 10^{-3})
DL-Valinamide hydrochloride	5.40
N-Carboxamidomethylmorpholine hydrochloride	5.55
DL-O-ethylserinamide hydrochloride	5.35
DL-Isoleucinamide hydrochloride	5.40
DL-Leucinamide hydrochloride	5.40
2-Methylalaninamide hydrochloride	5.45
2-(3'-Indoleacetamido)-4-methyl valinamide	5.30
DL-sorbylglycinamide	5.35
Lauroylsarcosinamide	5.35
DL-Phenylalaninamide hydrochloride	5.40
DL-alpha-hydroxyphenylacetamide	5.40
DL-alpha-hydroxypropionamide	5.35
*N-Chloroacetyl asparagine	5.60
*Benzoyl glycine amide	5.20
*Chloramphenicol	6.25
*Bromacetophenone	0.63

*All compounds were supplied by the Union Carbide Chemicals Company except those marked with an asterisk.

2. Field Studies

Procedure

The compounds which appeared to have the best bactericidal qualities were checked further in a field study in the following manner: A randomized block experiment was established in a block of newly set one-year-old Bartlett pear trees. One-half of the treatments were sprayed on the trees prior to inoculation with E. amylovora and one-half after inoculation. Treatments made before and after inoculation consisted of two concentrations of each compound being tested (Table XVI). Agrimycin was applied at the recommended rate before and after inoculation to afford a comparison with a material being used commercially. All treatments were replicated twice. Four trees were inoculated but left unsprayed to serve as checks.

The before inoculation sprays were applied on May 25, 1960. Thirty-six hours later each tree was inoculated in one growing shoot as described in Experiment III. Sixty hours after inoculation the second half of the treatments were sprayed on the trees. Each tree was sprayed with about 75 ml of solution. The results of this study were recorded ten days after inoculation. Trees were graded as either infected or non-infected.

Cold weather and rain which prevailed made it desirable to repeat the experiment. Care was taken to asceptically remove all fire blight from infected trees as well as the inoculated shoot on trees which did not become infected. Each tree received the same compound in both experiments to

TABLE XVI

Treatments for Bactericidal Sprays in Field Studies

Compound	1st Spray[1] Conc. - ppm	2nd Spray[2] Conc. - ppm	3rd and 4th Sprays[3] Conc. - ppm
Chloramphenicol	200	800	800
Chloramphenicol	29	80	-
Bromoacetyl valinamide	200	1250	1250
Bromoacetyl valinamide	20	125	-
Alpha-maleimido isovaleramide	400	1000	1000
Alpha-maleimido isovaleramide	200	200	-
Bromacetophenone	25	250	250
Bromacetophenone	2.5	25	-
Agrimycin	300	300	300

[1]The concentrations in this column were used only in the pre-inoculation spray in the first field study.

[2]The concentrations in this column were used in the post-inoculation in the first field study.

[3]The concentrations in this column were used in the pre- and post-inoculation sprays in the second field study.

prevent an interaction or carry-over effect. Only one concentration (Table XVI) of each compound was used and each treatment including check was replicated four times. The agrimycin treatments were replicated three times.

On June 6, 1960, the before inoculation sprays were applied. Thirty-six hours later three growing shoots on each tree were inoculated with E. amylovora. Thirty-six hours after inoculation the post inoculation treatments were applied. Eleven days after inoculation the results were recorded on the basis of percent of inoculated shoots infected per tree.

Results

May Field Test: The check trees in this test were infected at every inoculation point. None of the trees sprayed with agrimycin were infected. The trees sprayed with bromoacetyl valinamide after inoculation showed no signs of infection. When this compound was sprayed on before inoculation, there was one point of infection with the high concentration and one point with the low concentration.

Bromacetophenone sprays after inoculation also resulted in an absence of infection. When sprayed on before inoculation, only one of the trees receiving the low concentration was infected.

All of the trees sprayed with chloramphenicol after inoculation were infected. None of those treated with this compound before inoculation were infected regardless of the concentration.

Trees sprayed with alpha-maleimido isovaleramide after inoculation were infected 100 percent, and 75 percent of those sprayed before inoculation became infected.

June Field Test: Three of the four check trees were infected at each of the three points on inoculation. The fourth tree was not infected. Spraying with agrimycin after inoculation prevented development of fire blight in two of the three trees. The third tree was infected at all points of inoculation. When agrimycin was sprayed on before inoculation, one twig of a possible six became infected.

None of the inoculated twigs sprayed with bromoacetyl valinamide showed signs of becoming infected. This was true for the trees sprayed before as well as after inoculation.

Chloramphenicol sprayed twigs showed no signs of infection when the trees were sprayed prior to inoculation. Of those trees sprayed after inoculation, one tree appeared to be infected at all three points of inoculation.

Three twigs on one tree sprayed with alpha-maleimido isovaleramide became infected. Two trees sprayed after inoculation became infected at all points of inoculation.

Three of the four trees sprayed with bromacetophenone before inoculation became infected at all points of inoculation. Two of the four trees sprayed after inoculation also became infected at all points of inoculation.

DISCUSSION

Nutrient Absorption and Response

The complete Hoagland solution appeared to be generally satisfactory as a nutrient source for pear trees. The following comparison of the leaf composition of nutritionally healthy pear trees grown in Michigan orchards (33) and the pear trees from this experiment suggests that the trees received an adequate amount of most elements except calcium, magnesium, manganese and copper from the nutrient solution. The level of calcium, magnesium, manganese and copper in the leaves suggests that the concentration of these nutrients should be increased in the basic Hoagland solution.

Element	Kenworthy's Data	Check Treatment
Nitrogen - %	2.50	2.06
Phosphorus - %	0.13	0.13
Potassium - %	1.45	1.65
Calcium - %	1.90	.83
Magnesium - %	0.40	.24
Manganese - ppm	133	29
Iron - ppm	140	164
Copper - ppm	54	7
Boron - ppm	23	29

In general an increase or decrease in the concentration of an element in the nutrient solution resulted in a similar change in the leaf concentration of that element. These changes were not always significant, especially in the trace element category, but the trends usually followed the treatment. There were also many instances, especially within the major elements, when an interaction between two or more elements seemed to be present.

Varying nitrogen in the nutrient solution affected the concentrations of leaf nitrogen and potassium. As the concentration of foliar nitrogen increased from 2.06 to 3.28 percent the concentration of potassium decreased by the same factor from 1.65 to 0.96 percent. A similar trend was noticed by Baxter (2). Low leaf concentrations of nitrogen did not seem to alter the potassium concentration in the leaves, and the potassium concentration did not appreciably alter the percent nitrogen in the leaves.

Proebsting (49) noticed that applications of nitrogen fertilizers on Bartlett pear trees decreased the phosphorus content of the leaves, but a nitrogen-phosphorus interaction was not observed in this study.

Nitrogen deficiency symptoms were recognized in this experiment on trees with leaves containing 1.34 percent nitrogen. Sato (55) observed nitrogen deficiency at 0.83 percent leaf nitrogen in the Josephine pear.

The concentration of phosphorus in the nutrient solution seemed to influence the concentration of leaf phosphorus and potassium. Minus phosphorus trees contained an average of 0.07 percent phosphorus. This level was

accompanied by an appearance of deficiency symptoms which were probably attributable to the low concentration of phosphorus. This value agrees with that established by Sato (55) for the phosphorus deficiency level.

The concentration of potassium seemed to be indirectly related to the concentration of phosphorus. However, only a potassium deficiency increased the phosphorus concentration; an excess of potassium did not seem to affect the leaf phosphorus level.

Potassium levels in the leaf were generally dependent on the quantity of potassium in the nutrient solution. Leaves from the minus potassium treatments exhibited deficiency symptoms as described by Wallace (67) when the percent potassium was 0.49. Reuther (51) observed potassium deficiency symptoms at the 0.5 percent level, and Sato (55) stated that symptoms may occur in leaves having between 0.27 and 0.41 percent potassium.

Toxicity symptoms observed on the high potassium trees could have been due either to a potassium excess or to a magnesium deficiency. The magnesium level (0.08%) was below the level (.09 to .11) set by Sato (55) and approaches the level observed in the minus magnesium trees (0.04) of this experiment.

Calcium was the only major element which was not increased significantly in the leaf by an increase in the quantity of calcium in the solution. The high calcium solution contained 1002 ppm of calcium of which about 40 percent was in the calcium sulfate form. According to Bergman (5), high levels of

calcium sulfate did not increase the concentration of calcium in grape petioles. Perhaps this form is not suitable for nutrient solutions. Minus calcium treatments resulted in a calcium level of 0.44 percent and in the occurrence of deficiency symptoms. Sato (55) observed deficiency symptoms in pears at the 0.66 percent level. Trees grown in the high calcium solution showed symptoms which may have been due to the accumulation of the relatively insoluble calcium sulfate in the root zone.

Low magnesium treatments reduced the concentration of magnesium in the leaves to 0.04 percent. These trees exhibited deficiency symptoms similar to those described by Harley (23) for trees which contained 0.05 percent magnesium. Sato (55) felt that leaves containing 0.09 to 0.11 percent magnesium or less would show deficiency symptoms.

Baxter (2) reported that soil applications of magnesium and calcium decreased leaf potassium and that applications of potassium and calcium had a similar effect on magnesium. He also felt that the sum of the potassium, calcium, and magnesium concentrations in the leaves remained about the same. Since high calcium concentrations were not attained in the leaves, it was difficult to evaluate the effect of calcium. However, the influence of potassium and magnesium seemed to follow the pattern described by Baxter in that the concentration of potassium in the leaf increased as the level of magnesium in the solution decreased. The reverse was true when the magnesium level of the nutrient solution was increased.

The sum of the potassium, calcium and magnesium concentrations in the check trees was 2.72 percent. Most treatments did not vary from this figure by more than 10 percent of this sum. The most unequal sums of these three elements were found in the trees deficient in potassium (sum - 1.88%) or with an excess of potassium (sum - 5.17%). The potassium excess was so great that both magnesium and calcium would have had to be less than zero to maintain the equality. Trees grown in an iron deficient nutrient solution seemed to have a high sum (3.32%) and trees with an excess of phosphorus had a low sum (2.07%).

Low iron treatments did not seem to affect the level of iron in the leaf, but high iron treatments instigated a toxic accumulation of iron (544 ppm). Root and top development were very poor in the high iron trees, and necrotic spots were evident on the leaves.

Interestingly, the trees grown in a nutrient solution lacking iron exhibited an accumulation of calcium. These trees exhibited some chlorotic symptoms which have been associated with lime induced iron deficiency in other reports. Here it would seem that the calcium accumulated as a result of iron deficiency rather than a cause of it.

Either a high or a low level of iron in the solution resulted in an increase in leaf manganese. The manganese level was still less than the value indicated as essential by Kenworthy (33). However, the manganese level in the nutrient solution did not seem to affect the concentration of iron in the leaf.

Minus iron treatments also accounted for the largest accumulation of molybdenum observed in this study (6.3 ppm).

Nutrient solutions containing 25 times the normal amount of manganese resulted in the accumulation of 311 ppm of the element in the leaves. According to Grasmanis (20) this should have induced toxicity symptoms, but none were observed. Withholding manganese from the trees lowered leaf manganese to 14 ppm, but it did not induce any particular symptom other than reduced top and leaf growth. This symptom was typical of most of the trees grown on low or high levels of trace elements.

Trees grown in high and low levels of boron exhibited no particular symptoms of deficiency or toxicity even though they ranged in concentration from 13 to 155 ppm.

Oserkowsky and Thomas (45) felt that 7 ppm was the threshold value for copper deficiency. The check trees and minus copper trees in this experiment averaged 7 ppm in 1959, and may have been dangerously low in copper. In 1960, one minus copper tree developed symptoms similar to those described by Jones (31) and Harris (24). This tree contained only 5 ppm of foliar copper in 1959. Thus, it appears that the critical value for the copper level in pears grown under these conditions was about 5 ppm. Increasing the copper level in the nutrient solution 25 times over that of the check solution raised the foliage concentration to 11 ppm. Trees deficient in phosphorus had the highest concentration of copper on a percentage basis. Since these trees made almost no

growth, there was probably less dilution of the copper absorbed and therefore only appeared that there was an accumulation of copper.

Zinc concentrations in the trees were not affected significantly by the level of zinc in the nutrient solution, but trees grown in an absence of zinc accumulated the highest level of aluminum in their leaves. Aluminum was also more concentrated in the trees grown in a high molybdenum solution. The amount of molybdenum in the nutrient solution did not significantly alter the concentration of molybdenum in the leaf.

There is often no explanation for the interactions between elements that are evident under various nutritional environments. It is important, however, to recognize their existence in diagnosing nutritional disorders and making fertilizer recommendations.

Amino Acid Accumulation as Related to Nutrient Environment

Growing Shoots: Variations in the nutrient solution content also affected the accumulation of free amino acids present in the growing tips of pear trees. There seemed to be little qualitative variation of the amino acids under these conditions, but there were quantitative responses. The amino acids present in the most generous concentrations were aspartic acid, glutamic acid, serine, glycine, threonine, alpha-alanine, asparagine and glutamine. Cysteine, gamma-amino butyric acid, and leucine were present in trace amounts in most samples. Traces of arginine were present in the check trees and a few minus treatments.

Trees with high concentrations of nitrogen, phosphorus, and potassium accumulated the largest total amounts of free amino acids. The high nitrogen trees achieved this accumulation through an increase in concentration of each of the more plentiful amino acids. High phosphorus trees accumulated all of the plentiful amino acids except glycine and threonine. The high potassium value represented only one sample and so should not be emphasized.

The reason for the large amounts of total free amino acids in the shoots of pear trees growing on high levels of phosphorus and potassium is not known. It is possible that a high phosphorus and potassium nutritional condition may have stimulated the photosynthetic production of the amino acids, but that the resultant nutritional inbalance prevented complete utilization of the amino acids for protein synthesis.

Steinberg (59) found an accumulation of total amino acids in tobacco plants deficient in phosphorus, calcium, magnesium or potassium. Other reports (11, 18, 21, 52, 54) also have shown this to be true with potassium deficient plants. The accumulation of free amino acids in the high phosphorus trees could be partially due to the low concentration of potassium in its leaves. The minus magnesium and plus potassium trees did not follow this pattern.

The lower concentrations of free amino acids occurred in trees high in boron, low in magnesium or low in nitrogen. The low level of free amino acids in these trees was due mainly to the low level of asparagine. There was about 90 percent less asparagine in these trees than in the check trees.

Since asparagine usually serves as an ammonia detoxification mechanism in plants, the trees low in nitrogen and magnesium or high in boron may not have had any excess of ammonia. The low nitrogen trees probably did not because nitrogen was not available to be absorbed in large quantities. The minus magnesium and high boron trees contained just as much total nitrogen as the check trees or high phosphorus trees. Thus, the condition of these trees must have favored a rapid assimilation of the nitrogen into amino acids and thence into protein.

Mature Leaves: The nutritional environment also induced variations in the amino acid levels in mature leaves from pear shoots which had ceased growth. These variations were not always consistent with those in the actively growing shoot.

Those trees grown without boron or without potassium had the highest total free amino acid levels in their leaves. An increase in the acidic amino acids, aspartic acid and glutamic acid, accounted for most of the increase due to low boron. Potassium deficient trees accumulated asparagine, glutamine and serine. These amino acids, and especially the two amides probably accumulated as an ammonia detoxification mechanism. Thus, it would appear that the potassium deficient pears were either not utilizing nitrogen for protein synthesis or that the low potassium level was equivalent to a starvation effect and protein breakdown resulted in an excess of ammoniacal nitrogen.

Phosphorus deficient trees were also very interesting in that they showed

a significant accumulation of arginine. The alanine concentration was one-third of that in the check trees, and there were 1.5 ug of arginine compared to none in the checks. None of the other treatments showed an accumulation of arginine.

Many of the changes in amino acid composition of pear leaves or shoots as a consequence of an abnormally high or low level of an essential element cannot be explained. Detailed investigations on certain of these changes might contribute to an understanding of the biochemical function in vivo of the respective element. It is also possible that variations in amino acid levels could be an indication of an approaching element imbalance.

Susceptibility of Trees to E. amylovora

Trees grown in a nutrient solution containing an excess of calcium and those grown without boron did not become infected with E. amylovora even though each tree was inoculated three times. Minus phosphorus, minus iron and the check trees were also quite resistant to the disease.

The high calcium and the check treatments resulted in similar trees with a healthy appearance. The other treatments which resulted in resistant trees would be impractical as the trees grew poorly. The high calcium trees did not accumulate an excess of calcium in their leaves; in fact, the high calcium trees were not significantly different than the check trees for any nutrient element. Neither was there any difference in the concentration of the total free amino acids between these two groups. It should not be assumed

that there was no physiological difference between the check and high calcium trees. It is possible that the high level of calcium altered the metabolism of the tree sufficiently to increase resistance to E. amylovora.

The striking effect of the minus boron and high calcium treatments point out the importance of further study with these treatments to learn more about their effect on resistance of pear trees to E. amylovora.

The trees most susceptible to E. amylovora were those grown with high levels of molybdenum, copper, potassium and nitrogen. The high nitrogen trees contained the largest concentration of leaf nitrogen and so follow the observations of several workers (17, 30, 59, 61) that high nitrogen levels accentuate a fire blight infection. However, the level of nitrogen in the other most susceptible trees was no different from the level found in the least susceptible trees. In fact the minus nitrogen trees which contained the lowest concentration of leaf nitrogen were about average in susceptibility. Thus, the axiom that excess nitrogen will increase susceptibility to fire blight does not include the prevalent feeling that a minimum of nitrogen will lessen susceptibility. The same can be said for the other elements as a deficiency of any element except phosphorus, iron, boron, and potassium resulted in trees of average or above average susceptibility.

No obvious difference in elemental composition of the leaves was observed which would explain the extremes of susceptibility which seemed to result when the nutrition of the tree was altered. The only two treatments

which exhibited similar levels of resistance with no significant differences in the elemental composition of the leaves were high calcium and check. Perhaps the balance of elements found in the check and high calcium trees is the best pattern for a resistant tree.

Dependence of E. amylovora Bacteria on Amino Acids

The experiments to determine which nitrogen sources would support the growth of E. amylovora were initiated because of reports in the literature (1,44) that this organism had a nearly obligated requirement for asparagine. For the present research, it was postulated that the growth of an organism whose nitrogen requirement was so specificshould be controlled through the inhibitory action of an analogous compound. Furthermore, it was postulated that the spread of E. amylovora inthe plant might be correlated with the availability of asparagine in the growing shoots and leaves. Asparagine was found to be present in large amounts in the pear shoots (Experiment III) and it was felt that the organism utilized this reservoir of asparagine directly for its growth.

However, E. amylovora was able to grow not only on asparagine but also on glutamine, aspartic acid, glutamic acid, gamma-amino butyric acid, beta-alanine, alanyl asparagine, glycine amide, and ammonia (Table XIII). This disagreement with Ark's data (1) is believed to be due to a more careful control of pH.

There is, however, a remarkable correlation in structure of the amino acids which the organism can utilize. Aspartic acid and glutamic acid are two very similar dicarboxylic amino acids, and asparagine and glutamine are their corresponding amide derivatives. These four compounds were found in Experiment III to be major amino acids in the shoots which would be available to the organism. Further, the utilization of gamma-amino butyric acid and beta-alanine by the organism may be directly related. Gamma-amino butyric acid is formed in plants and micro-organisms from glutamic acid by enzymatic decarboxylation (4) and beta-alanine would be the decarboxylation product of aspartic acid; however, this pathway for the formation of beta-alanine in plants has not been found (43).

The growth of the organism on alanyl asparagine and glycine amide was poor and these compounds are not naturally occurring substrates. Growth on ammonia was good, but little or no nitrogen exists in plants in the ammonia form. Therefore, the growth of E. amylovora on six amino acids seems related strikingly in that aspartic acid, asparagine and beta-alanine are chemical analogs and glutamic acid, glutamine, and gamma-amino butyric acid are similarly related to each other, and all are among the constituents of the dicarboxylic amino acids. The lack of growth on all the other amino acids accentuates the uniqueness of the growth on the dicaroxylic amino acids.

Future research should be directed toward a study of the metabolism of E. amylovora to determine the reason for its obligated requirement for the

dicarboxylic amino acids. These amino acids are common constituents produced from the citric acid cycle in most organisms, and it is unusual to find an organism that requires an independent source of them for growth.

Since four of the amino acids utilized by the bacteria made up the majority of the total free amino acids in the pear shoots, it was suspected that only the total concentration of the free amino acids in the shoots and leaves might be an important factor in regulating the susceptibility of the trees to E. amylovora. However, the data indicated that neither the quantitative nor qualitative aspects of the free amino acid level were factors in fire blight susceptibility.

Three of the treatments containing the highest total level of amino acids were high nitrogen, phosphorus and potassium. High nitrogen and potassium trees were of about average susceptibility. The lowest levels of total free amino acids were found in minus nitrogen, minus magnesium, and high boron, and these trees were of average susceptibility or a little lower, but were not lowest in susceptibility.

Thus it seems that there was usually enough of one of the utilizable amino acids present to support growth of the bacteria regardless of the nutritional program.

Possible Bactericides for E. amylovora

The working hypothesis that a chemical analog of asparagine might inhibit the growth of E. amylovora was not possible after the discovery that other related amino acids would also support growth. However, the structural relationships between these amino acids (page 72) were so similar that the inhibitory effect of unnatural amide structures was explored. These compounds were selected because they contained an amide, and, in general, a substituted constituent on or near the amino group. It was hoped that a structure somewhat similar to asparagine would be accepted by a specific and limiting enzyme in the organism and block its action and thus block the growth of the organism. This hypothesis was tested first in synthetic culture studies and then in field studies.

Of all the potential bactericides checked, the only one which seemed to be as good as the material presently being used by orchardists (agrimycin) was bromoacetyl valinamide.

When agrimycin sprays were applied after bacterial inoculation, three twigs of the eleven inoculated became infected. Sprays prior to inoculation resulted in one infected twig of a possible eight. Altogether, 21 percent of the inoculated twigs became infected. Similar bromoacetyl valinamide treatments reulted in one infected twig of a possible sixteen when the sprays were applied before inoculation; the same ratio resulted when the treatment was applied after inoculation. In this case about 12 percent of the inoculated twigs were infected.

Check trees, which were not sprayed with a bactericide, had 13 infected twigs out of the 16 inoculated or about 81 percent infection.

Chloramphenicol acted as a good preventive bactericide; none of the twigs inoculated became infected. However, when it was applied after inoculation, about 31 percent of the inoculated twigs became infected.

None of the compounds tested exhibited any phyto-toxicity in the quantities used.

All of the compounds which showed bactericidal effects had several interesting structural similarities. (1) In general, the compound contained a chloro or bromo substituent on a carbon adjacent to a carbonyl group. This halogen could carry a partial positive charge and would be reactive with sulfhydryl amino acids or -SH sites on a protein molecule. (2) This active halogen is adjacent to the amide group with which it conceivably might react to form a ring structure which in turn might be responsible for the biological activity. (3) Chloramphenicol is an inhibitor of growth because it blocks the incorporation of the amino acid-RNA complex into protein. Thus bromacetyl valinamide may likewise be blocking growth at some step in amino utilization.

Future work on these compounds should include investigations on the site of action of bromacetyl valinamide not only for E. amylovora inhibitors but also as an inhibitor in studies on the biochemistry of protein synthesis. Similar compounds are available, and they should be checked for possible bactericidal activity.

When a compound is perfected which will penetrate pear leaf and stem tissue and kill E. amylovora without damaging the tree or the fruit, growers will be able to grow pears without the fear of fire blight. Until that time, the present spray program, coupled with a well balanced and substantial nutritional program should result in a minimum of fire blight problems.

SUMMARY

Bartlett pear trees were grown in a nutrient solution sand culture at various levels of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, boron, manganese, copper, and molybdenum during the 1958, 1959 and 1960 seasons. These trees were used to study the effect of nutrition on the mineral composition of the leaves, on the free amino acid levels in the growing tips and older leaves, and on the susceptibility of pear trees to fire blight.

As an auxiliary study, the causal organism of fire blight, E. amylovora, was grown in synthetic media to ascertain its ability to utilize natural and synthetic amino acids and amides as a source of nitrogen. Various other synthetic amides were tested for their bactericidal effect.

The following paragraphs contain a summary of these studies.

1. Decreasing the amount of an element in the nutrient solution resulting in a corresponding decrease in the leaf concentration of that element in all cases except iron, zinc, copper, and molybdenum. Increasing the amount of an element in the nutrient solution increased leaf concentration except for calcium, zinc, and molybdenum. In a few instances the concentration of one element seemed to be dependent on the concentration of another. Magnesium, calcium, and potassium concentrations seemed to be closely interrelated. Potassium and phosphorus showed an indirectly proportional relation-

ship. There were other instances of low magnitude elemental interactions.

2. The free amino acid composition of the growing tip of a pear tree also seemed to be related to the nutrient element composition of the leaves. Increasing or decreasing total leaf nitrogen resulted in a similar change in the free amino acid concentration. High levels of phosphorus or potassium in the leaf were associated with an increase in total free amino acids similar to that found for trees high in nitrogen. Either increasing leaf boron or decreasing leaf magnesium resulted in amino acid levels equally as low as those in the minus nitrogen trees.

3. The free amino acid levels were also studied in the mature leaves from non-growing shoots of trees grown in solutions deficient in nitrogen, phosphorus, potassium, iron, and boron. Minus boron leaves contained more total amino acids due mainly to an increase in the concentration of glutamic acid and aspartic acid. Low potassium leaves accumulated large quantities of asparagine and glutamine and thus had the highest total free amino acid level. Arginine accumulated in the leaves deficient in phosphorus but not in any of the other treatments.

4. There was some variation in susceptibility to E. amylovora based on the nutrient element environment of the trees. Trees grown in a high level of calcium did not become infected with E. amylovora at all, but these trees did not indicate any accumulation of calcium in their leaves. Trees deficient in boron did not become infected either. Those trees deficient in

phosphorus or iron as well as the check trees appeared to be quite resistant. Trees grown in high levels of molybdenum, copper, potassium and nitrogen appeared to be the most susceptible to an E. amylovora infection and spread.

5. E. amylovora readily utilized aspartic acid, glutamic acid, asparagine, glutamine, beta-alanine, and gamma-amino butyric acid as a sole source of nitrogen in a synthetic culture medium. Consistent with these findings, there appeared to be no correlation between the foliar concentration of individual amino acids or the total amino acid level and the susceptibility of the tree to a fire blight infection.

6. Culture studies and field tests indicated that bromoacetyl valinamide may be a good bactericide for E. amylovora. This compound gave better control than agrimycin under the conditions of this experiment and showed no phytotoxic effect on the tree.

LITERATURE CITED

1. Ark, F. A. 1937. Variability in the fire blight organism, Erwinia amylovora. Phytopath. 27: (1) 10-12.

2. Baxter, P. 1957. Fertilizer trials on apple and pear orchards in Southern Victoria. Jour. Agr. Victoria 55: 351-359. (Hort. Abstr. 28, No. 131).

3. Bennett, J. P. 1945. Iron in leaves. Soil Sci. 60: 91-105.

4. Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka. 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. Jour. Amer. Chem. Soc. 72: 1710-1718.

5. Bergman, E. L. 1958. Response of Concord grape vine (Vitis labrusca L.) to various levels of essential nutrient elements. Ph. D. Thesis, Michigan State University.

6. Bollard, E. G. 1958. The Physiology of Forest Trees (Thimann, E. V., Ed.) The Ronald Press Co., New York, N. Y. pp. 83-93.

7. _____. 1952. Zinc deficiency in pears. New Zeal. Jour. Sci. and Tech. Ser. A. 34: 548-550.

8. Bould, C., D. J. D. Nicholas, J. A. H. Tolhurst, and J. M. S. Potter. 1953. Zinc deficiency of fruit trees in Great Britain. Jour. Hort. Sci. 28: 260-277.

9. _____ 1953. Copper deficiency of fruit trees in Great Britain. Jour. Hort. Sci. 268-276.

10. Boynton, D. 1954. Fruit Nutrition (Childers, N. F., Ed.) Somerset Press, Somerville, N. J. Chap. XI: 642-661.

11. _____, Y. Lawrence, and S. S. Kwong. 1959. Some factors influencing the intermediary nitrogenous compounds in leaves of the strawberry plant (Fragaria chiloensis var. ananassa). Presented at Colloquium on Plant Analysis and Fertilizer Problems, International Botanical Congress, Montreal, Canada.

12. Brooks, A. N. 1926. Studies of the epidemology and control of fire blight of apple. Phytopath. 16: 665-696.

13. Brown, J. C. 1956. Iron chlorosis. Ann. Rev. Plant Physiol. 7: 171-190.

14. Bruno, A. 1953. La richesse en eau du milieu vital et les signes de carence zincique. C. R. Acad. Agr. Fr. 39: 409. (Hort. Abstr. 23, No. 2967).

15. Bryant, L. R., and R. Gardner. 1943. Phosphorus deficiency in pears. Proc. Amer. Soc. Hort. Sci. 42: 101-103.

16. Compton, O. C. 1957. Boron in Oregon pears. Western Fruit Grower 11: 15. (Hort. Abstr. 28, No. 222).

17. Fisher, E. G., K. G. Parker, N. S. Luepschen, and S. S. Kwong. 1959. The influence of phosphorus, potassium, mulch, and soil drainage on fruit size, yield, and firmness of the Bartlett pear and on development of the fire blight disease. Proc. Amer. Soc. Hort. Sci. 73: 78-90.

18. Gleiter, M. E. 1958. A study of the effects of potassium and phosphorus deficiency on some nitrogen components of alfalfa. Ph. D. Thesis, Purdue University.

19. Goodman, R. N. 1955. Late season twig-infection, a serious limitation to the effectiveness of antibiotic sprays for fire blight control. Plant Disease Reporter 39: 922-925.

20. Grasmanis, V. O. 1958. Manganese excess and bark necrosis in pear. Jour. Austl. Inst. Agr. Sci. 24: 347-349.

21. Gregory, F. G., and P. K. Sen. 1937. Physiology studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. Ann. Bot. (N.S.) 1: 521-540.

22. Hamilton, J. M., and M. Szkolnik. 1957. Ornadine, a promising new organic fungicide for the control of blossom blight of pears. Plant Disease Reporter 41: 301-302.

23. Harley, C. P. 1947. Magnesium deficiency in Kieffer pear trees. Proc. Amer. Soc. Hort. Sci. 50: 21-22.

24. Harris, W. B. 1951. Copper deficiency of fruit trees. Jour. Dept. Agr. S. Austl. 54: 277-279.

25. Heald, F. D. 1943. Introduction to Plant Pathology. McGraw-Hill Book Co. Inc., New York. pp. 365-375.

26. Heuberger, J. W., W. R. Cornegys, and R. R. Romanko. 1956. Captan and zineb, used alone, in alteration, and in combination -- and the control of apple diseases. Plant Disease Reporter 40: 471.

27. Hewitt, E. J. 1951. The role of the mineral elements in plant physiology. Ann. Rev. Plant Physiol. 2: 25-53.

28. _____, E. W. Jones, and A. H. Williams. 1949. Relations of molybdenum and manganese to the free amino acid content of the cauliflower. Nature 165: 681-682.

29. Hoagland, D. C., and D. I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Exp. Sta. Bul. 347.

30. Hildebrand, E. M., and A. J. Heinicke. 1937. Incidence of fire blight in young apple trees in relation to orchard practices. N. Y. Cornell Agr. Exp. Sta. Mem. 203: 1-36.

31. Jones, J. O. 1950. Copper deficiency disease of pear trees. Nature 165: 192.

32. Keinholz, J. R. 1942. Boron deficiency in pear trees. Phytopath. 32: 1082-1086.

33. Kenworthy, A. L. 1950. Nutrient-element composition of leaves from fruit trees. Proc. Amer. Soc. Hort. Sci. 55: 41-46.

34. _____. 1960. Unpublished data. Michigan State University.

35. Kobernuss, E. C. 1951. Untersuchungen zur Ursache und Behebung der Bodenmudigkeit bei Obstgeholzen. Kuhn-Arch. 64: 365-408. (Hort. Abstr. 22, No. 3547).

36. Lamb, R. C. 1959. Testing pears for fire blight resistance. Farm Research, September: 12.

37. Lindner, R. C., and C. P. Harley. 1944. Nutrition interrelations in lime-induced chlorosis. Plant Physiol. 19: 420-439.

38. Link, G. K. K., and H. W. Wilcox. 1936. Relation of nitrogen-carbohydrate nutrition of Stayman apple trees to susceptibility to fire blight. Phytopath. 26: 643-655.

39. Margolis, D. 1958. A study of the effect of the trace elements, manganese and molybdenum, upon the soluble nitrogenous constituents of plants. Ph.D. Thesis, Cornell University.

40. McCombe, C. I. 1960. Personal communication. North Carolina State College.

41. Mitchell, A. E., A. C. Dowdy. and E. J. Klos. 1960. Fruit spraying calendar. Mich. Ext. Bul. 154: 1-56.

42. Mulder, D. 1950. Der Zinkmangel im Europaischen Obstbau. Phytopath. Z. 16: 510-511.

43. Naylor, A. W., R. Rabson, and N. E. Tolbert. 1958. Aspartic-C^{14} acid metabolism in leaves, roots, and stems. Physiologia Plantarum 11: 537-547.

44. Nightingale, A. A. 1936. Some chemical constituents of apple associated with susceptibility to fire blight. N. J. Agr. Exp. Sta. Bul. 613: 1-22.

45. Oserkowsky, J., and H. E. Thomas. 1938. Exanthema in pear and copper deficiency. Plant Physiol. 13: 451-467.

46. Plaisted, P. H. 1958. Clearing plant tissue extract for amino acid analysis. Contrib. Boyce Thompson Inst. 19 (3): 231-244.

47. Possingham, J. V. 1956. The effect of mineral nutrition on the content of free amino acids and amides in tomato plants. Austl. Jour. Biol. Sci. 9: 539-551.

48. Powell, D. 1954. An evaluation of copper 8-quinolate as a fungicide against some pome fruit diseases. Plant Disease Reporter 38: 76-79.

49. Proebsting, E. L. 1934. A fertilizer trial with Bartlett pears. Proc. Amer. Soc. Hort. Sci. 42: 101-103.

50. _____. 1953. Certain factors affecting the concentration of N, P, K, Ca and Mg in pear leaves. Proc. Amer. Soc. Hort. Sci. 61: 27-30.

51. Reuther, W. 1941. Studies concerning the supply of available potassium in certain New York orchard soils. N. Y. Cornell Agr. Exp. Sta. Mem. 241.

52. Richards, F. J., and E. Berner. 1954. Physiology studies in plant nutrition. XVII. A general survey of the free amino acids of barley leaves as affected by mineral nutrition, with special reference to potassium supply. Ann. Bot. (N.S.) 18: 15-33.

53. _____, and R. G. Coleman. 1952. Occurrence of putrescine in potassium-deficient barley. Nature 170: 460.

54. _____, and W. G. Templeman. 1936. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. 50: 367-402.

55. Sato, K., M. Ishihara, and R. Harada. 1952. Studies on leaf analysis of fruit trees. Bul. Nat. Inst. Agr. Sci. Hiratsuka (Ser. E.) 1: 60-72. (Hort. Abstr. 24, No. 1208).

56. _____. 1954. Studies on leaf analysis of fruit trees. Bul. Nat. Inst. Agr. Sci. Hiratsuka (Ser. E.) 1: 60-72. (Hort. Abstr. 25, No. 2465).

57. Shaw, L. 1934. Studies on resistance of apple and other rosaceous plants to fire blight. Jour. Agr. Res. 49: 283-313.

58. Sprague, R. 1952. Pear diseases. Plant Disease Reporter, Supplement 213: 131.

59. Steinberg, R. A., J. D. Bowling, and J. E. McMurty. 1950. Accumulation of free amino acids as a chemical basis for morphological symptoms in tobacco manifesting frenching and mineral deficiency symptoms. Plant Physiol. 25: 279-288.

60. Steward, F. C., and J. K. Pollard. 1957. Nitrogen metabolism in plants: Ten years in retrospect. Ann. Rev. Plant Physiol. 8: 65-114.

61. Thomas, H. E., and P. A. Ark. 1939. Some factors affecting the susceptibility of plants to fire blight. Hilgardia 12: 301-322.

62. Thomas, W. D., and W. S. Henderson. 1952. Spray experiments for the control of fire blight on apples and pears, 1947-1950. Plant Disease Reporter 36: 273-275.

63. Thorne, D. W., and A. Wallace. 1944. Some factors affecting chlorosis on high lime soils. I. Ferrous and ferric iron. Soil Sci. 57: 299-312.

64. Trocmé, S., and J. Chabannes. 1952. Observations sur la carence en zinc des pommiers et des poirres dans la region d'Orleans. Ann. Agron. Ser. A. 3: 639-640.

65. Vidal, R. D., and J. M. A. Herrera. 1954. Analysis foliar l. Aplicacion del analysis quimico de la hoja g del metodo de diagnosis visual a la investigacion de deficiencias minerales en relacion con loosuelos en de cultivo. Anales de E Dafologia y Fisiologia Vegetal 13: 339-418.

66. Wallace, T. 1928. Investigations on chlorosis of fruit trees. II. The composition of leaves, bark, and wood of current seasons shoots in cases of lime-induced chlorosis. Jour. Pom. and Hort. Sci. 7: 172-183.

67. _____. 1928. Leaf scorch on fruit trees. Part IV. The control of leaf scorch in the field. Jour. Pom. and Hort. Sci. 7: 1-31.

68. Winter, H. F., and H. C. Young. 1953. Control of fire blight of apples in Ohio in 1953. Plant Disease Reporter 37: 463-464.

69. Woodbridge, C. G. 1954. Zinc deficiency in fruit trees in the Okanagan Valley in British Columbia. Can. Jour. Agr. Sci. 34: 545-551.

70. _____, A. Carney and H. R. McLarty. 1952. A boron deficiency in pears growing in soil having an adequate boron content. Sci. Agr. 32: 440-442.

ROOM USE ONLY