Vol. 63 JULY 1, 1941 No. 1

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION
OF LAND-GRANT COLLEGES AND
UNIVERSITIES

For sale by the Superintendent of Documents, Washington, D. C. - See page 2 of cover for prices

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal:	Domestic, $3.25 a year (2 volumes)
	Foreign, $4.75 a year (2 volumes)
Single numbers:	Domestic, 15 cents
	Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. When purchased in quantity of 100 or more a reduction of 25 percent in the cost will be made. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 63 WASHINGTON, D. C., JULY 1, 1941 No. 1

CERTAIN ENVIRONAL AND NUTRITIONAL FACTORS AFFECTING APHANOMYCES ROOT ROT OF GARDEN PEA [1]

By PAUL G. SMITH, formerly *assistant in plant pathology and agronomy*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

The *Aphanomyces* root rot (*Aphanomyces euteiches* Drechs.) has been an important disease of pea (*Pisum sativum* L.) in the canning crop in Wisconsin and numerous other States for the past 20 years or more. Earlier studies by Jones and associates (*9, 10*)[2] and by members of the New Jersey Agricultural Experiment Station (*6*; *13, Rpt. 52*) emphasized the importance of soil moisture as well as temperature in their influence upon the development of the disease. More recently Walker and Musbach (*20*) found little or no infection in the greenhouse in soil from a severely diseased field when the moisture level was kept low while at higher moisture contents the disease was very severe.

Efforts to find a practicable control for this disease have been only partially successful. Investigators at the New Jersey Station in a series of reports (*5, 11, 12, 13, 14*) have shown that heavy applications of complete fertilizers are effective in reducing the severity of infection. They also found that the nitrogen compounds used in the fertilizer mixtures were much more influential in reducing root rot than the phosphorus and potassium components. No benefit resulted when the fertilizer was applied after infection had taken place. Walker (*19*) in Wisconsin and Geach (*3*) in Australia have obtained similar decreased infection with the use of complete fertilizers. Walker and Musbach (*20*) showed that applications of fertilizer containing nitrogen reduced root rot damage in the field somewhat more than corresponding applications without nitrogen. They also found that greater protection was effected when the fertilizer was applied in the drill at the time of planting than when it was applied in a furrow at one side of but removed a slight distance from the seed.

The purpose of the present investigation was to examine more closely the relation of certain environmental factors to the development of the disease, and to study the effect of mineral nutrition on its incidence and severity.

MATERIALS AND METHODS

In the study of the effect of nutrition on the disease a constant-flow sand-culture method was used. The nutrient solutions were allowed to drip at the rate of 600 to 800 cc. per day into 8-inch flowerpots filled

[1] Received for publication January 20, 1941. This investigation is part of a broader project on the Nature of Disease Resistance in Plants, supported jointly by the Wisconsin Agricultural Experiment Station and the Wisconsin Alumni Research Foundation. The writers are indebted to Prof. B. M. Duggar for continued interest and advice. Routine assistance was provided for some aspects of the work by the personnel of the Works Progress Administration, project No. 65-1-53-2349.

[2] Italic numbers in parentheses refer to Literature Cited, p. 19.

with washed, sterilized silica sand. The inside surface of the pot was varnished to prevent diffusion of salts. Each pot was equipped with an intermittent siphon which allowed the nutrient solution to rise within 2 inches of the surface before draining. The time required for each pot to fill and drain varied from 24 to 36 hours.

The nutrient solution used throughout was a modification of that described by Hoagland and Snyder (7). In addition to the "normal" solution, solutions containing extra quantities of one of the elements N, P, and K and solutions lacking one of these elements were used. The ratio of each element in the "excess" solutions to that in the normal solution was approximately: N, 1.15 to 1; P, 2.0 to 1; and K, 1.28 to 1. A trace of each of the essential minor elements was added to all solutions. The reaction of all solutions was maintained between pH 5.5 and 6.2. The composition of the various nutrient solutions used is given in table 1. In the experiments involving higher concentrations of the nutrients, NaCl was added so that the proportions of the major elements could be varied without changing the total salt concentration.

TABLE 1.—*Chemical composition of the nutrient solutions at the basic concentration used in the sand-culture experiments*

Chemical	Cubic centimeters of molar solutions used to make up 10 liters of nutrient						
	Normal	+K	−K	+P	−P	+N	−N
$Ca(NO_3)_2$	[1] 50.0	50.0	50.0	50.0	50.0	50.0	
$CaCl_2$							50.0
KNO_3	50.0	50.0		50.0	50.0	50.0	
KCl		16.7			10.0		50.0
$NaNO_3$			50.0			22.2	
NaCl [2]	22.2	5.5	22.2	12.2	22.2		22.2
$MgSO_4$	20.0	20.0	20.0	20.0	20.0	20.0	20.0
KH_2PO_4	10.0	10.0		10.0		10.0	10.0
NaH_2PO_4			10.0	10.0			

[1] 50 cc. of a M/1 salt solution in 10 liters equals 0.005 M concentration.
[2] Not used in the dilute nutrient-solution experiments.

Inoculum was obtained by growing the organism in pea decoction in 250 cc. Erlenmeyer flasks. About 25 wrinkled-type pea seeds were placed in each flask, 100 cc. of water was added, and the flasks were plugged and autoclaved for 2 hours. Each flask was inoculated with a fragment of an agar culture of the fungus, and the organism was allowed to grow for 10 to 12 days. The zoospore suspension was obtained by removing the mycelial mats, washing them first in running tap water for 2 hours, and rinsing them in distilled water. They were then placed in shallow pans with just enough water to cover them. Abundant zoospore formation followed within 6 to 8 hours.

Inoculation was made by adding 100 cc. of the zoospore suspension to each pot. In the first experiments the siphon drains were plugged at the time of inoculation and the sand was flooded with the nutrient solution. After 24 hours the plugs were removed and the nutrient was allowed to flow through in the normal manner. In the experiments in which the higher salt concentrations were included, small 1 mm. bore siphons were placed over the edges of the pots after the sand was saturated and the nutrient was allowed to continue dripping into the pots. With the ends of the small siphons placed under the sand, the siphon column was not broken and the nutrient solution continued to flow into the pots without causing them to flood. The pots were drained after 24 hours.

The Wisconsin Perfection variety of pea was used throughout all experiments, except where indicated. Eighteen seeds were planted in each pot.

DISEASE SYMPTOMS AND METHOD OF DISEASE RATING

In sand cultures the first symptoms of the disease appear on the roots within 4 or 5 days after inoculation. Water soaking spreads above and below the initial infection zone without appreciable discoloration in the area of infection. The water-soaked tissue is firm at first, but it gradually becomes yellowish and soft, darkening with age and eventually collapsing and disintegrating. About 4 days after the underground symptoms may be found, water soaking appears in the stem above the sand level and progresses an inch or more up the aerial portion of the stem.

The extent of disease development was recorded when all or nearly all plants in the dilutest solution in the respective series showed severe above-ground symptoms. Plants were removed from the sand and divided into five arbitrary classes based on the severity of disease and designated as follows: O, No disease; 1, slight water soaking on epicotyl or on primary or secondary roots; 2, moderate water soaking of primary root or epicotyl, with or without slight darkening of the infected tissue, which remains firm; 3, infected areas extensive, darkened, and becoming soft, but without collapse of tissue; 4, extensive water soaking and discoloration with collapse and disintegration of part or all of the infected tissue; plants dying previous to inspection were rated in this class.

A disease index calculated on the basis of the above classes appeared to be a more accurate indication of the extent of infection than the percentage of infected plants, since it afforded a means of indicating the relative severity in combination with the percentage of plants affected. The index was calculated by using the class figures as weighted values. To obtain the index the number of individuals in each class was multiplied by the class number, the sum of the products of each class multiplied by 100, and this figure divided by 4 times the total number of plants. Thus, when all the plants were healthy, the rating was 0, and when all plants showed severe infection, the rating was 100, while intermediate grades were represented by intermediate index figures.

ENVIRONMENTAL STUDIES

TEMPERATURE IN RELATION TO GROWTH OF THE FUNGUS

Plates of potato-dextrose agar adjusted to a neutral reaction were inoculated in the center with 5-mm. disks from the periphery of an actively growing 4-day-old Petri-dish culture of *Aphanomyces euteiches*. Four replicates were placed at each of eight temperatures ranging in 4° intervals from 8° to 36° C., inclusive. Increments of radial growth were measured daily.

The results are shown graphically in figure 1. The greatest daily increase and total colony growth occurred at 28°. No growth occurred at either extreme temperature. From the minimum to the optimum there was a steady increase in rate of growth with increase in temperature. Above 28° the growth rate dropped rapidly. The straight-line trends in the graph indicate that growth was not retarded by the accumulation of staling or other metabolic products of the fungus.

REACTION OF MEDIUM IN RELATION TO GROWTH OF THE FUNGUS

The reaction series was set up by using various quantities of H_3PO_4, and of mono-, di-, and tri-sodium phosphate solutions, each with 0.1

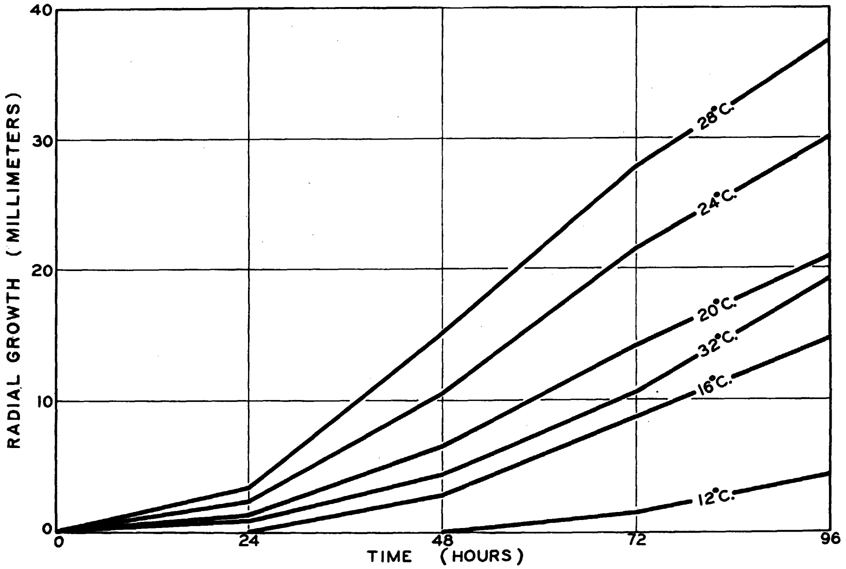

FIGURE 1.—The effect of temperature on growth of *Aphanomyces euteiches* on potato-dextrose agar.

cc. of concentrated H_2SO_4 added, according to the method described by Tilford (*16*). The quantity of phosphates for each pH level was 20 cc.; the amount of each fraction was varied to produce the desired pH level. Each 20-cc. phosphate mixture and a 200-cc. quantity of potato-dextrose agar (1.7 percent agar) was sterilized separately and thoroughly mixed just before pouring the plates. One plate of each pH level was melted, diluted with three parts of distilled water, and the pH determined with a glass electrode. The plates were inoculated in the same manner as in the temperature series. Increments of growth were measured daily and the curve of growth plotted after 4 days.

The results are shown graphically in figure 2. The fungus exhibited a tolerance to a wide range of acidity, with the limits at about pH 3.4 and slightly above pH 8.0. A broad optimum range from about pH 4.5 to 6.5 occurred, with an apparent isoelectric point at pH 5.9. The daily increment of growth at each pH value remained quite constant, indicating no apparent drift in the reaction of the medium or any interference of staling or other metabolic products of the fungus.

EFFECT OF SOIL TEMPERATURE ON INFECTION

The soil-temperature range at which root rot infection can occur has been found by Jones and Drechsler (*9*) to lie between 10° and 30° C., with an optimum somewhere between 15° and 30°. Further study of this important environmental factor was undertaken to determine more exactly the rate of disease development at different soil temperatures. Twenty-four soil-temperature cans were filled to within 1½ inches of the top with clean, sterile silica sand, and each can was equipped with a siphon for drainage purposes. Fifteen seeds were

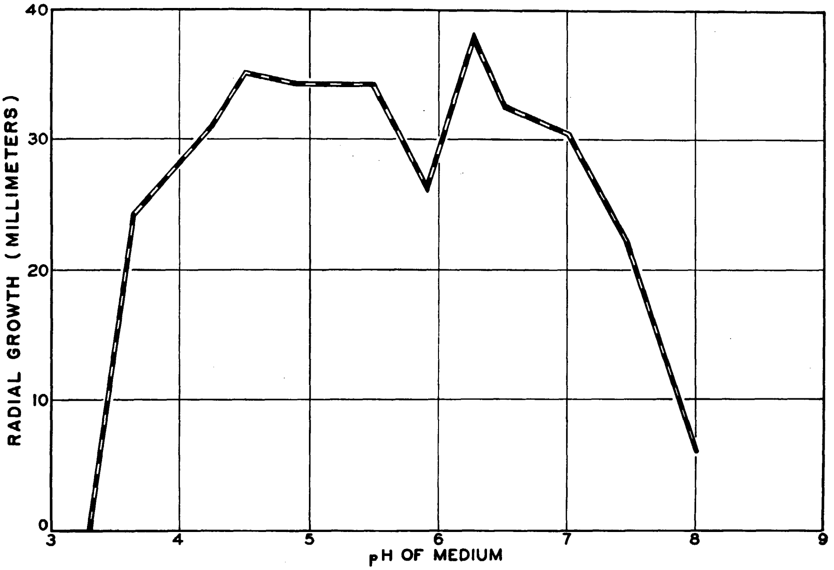

FIGURE 2.—The effect of initial pH of potato-dextrose agar on growth of *Aphanomyces euteiches*.

planted in each can and watered with a balanced nutrient solution of one-tenth the salt concentration described in table 1. All cans were placed at a temperature of approximately 24° in order to secure uniform plant growth. Every 3 days these were drained and fresh nutrient was added. Two days before inoculation four cans were placed in each of six soil-temperature tanks in which the temperatures were maintained at approximately 12°, 16°, 20°, 24°, 28° and 32°, so that the sand in each case did not vary more than 1°. The air temperature varied between 20° to 24°.

Fourteen days after planting, each can was saturated with nutrient and 100 cc. of a zoospore suspension were added. The cans were then partially drained in order to draw the inoculum around the roots, but sufficient moisture was left at the surface for infection to take place. Notes were taken in the manner described for the nutrient studies.

In the 24° and 28° tanks infection was visible 4 days after inoculation. The experiment was terminated 11 days after inoculation when nearly all plants at these two temperatures were infected. The results (table 2) show a definite optimum for disease development at 24° and 28°. No infection was found on plants at 12°, while at 16° some infection did occur. The rate of disease development at the various sand temperatures appeared to parallel quite closely the rate of growth of the organism on agar.

TABLE 2.—*The effect of sand temperature on Aphanomyces root rot development as shown by the extent of disease development 11 days after inoculation*

Disease index at the temperature (°C.) indicated					
12°	16°	20°	24°	28°	32°
0	12.5	51.6	90.4	86.4	55.6

EFFECT OF SOIL MOISTURE ON INFECTION

Others (*5, 9, 10, 20*) have noted that root rot infection is most severe when the soil moisture is high. Observations in Wisconsin during the past three years (1938, 1939, 1940) have indicated that the disease has been more severe in the seasons of abundant rainfall.

For the purpose of obtaining more information on the effect of soil moisture on root rot, an experiment employing two moisture levels was made. The soil was taken from a naturally infected field near Clyman, Wis. It was mixed thoroughly and two portions were adjusted to 45 and 75 percent of the water-holding capacity, respectively. The saturation point of the soil was reached when the moisture content was 43 percent of its dry weight. To maintain a fairly constant moisture content, Wisconsin soil-temperature cans watered every third day were used. A 2-inch layer of ground cork was placed above the soil to reduce evaporation from the surface. Six replicates were included at each moisture level and the arrangement of the cans was randomized. The cans were kept at 20° to 25° C. after 15 seeds had been planted in each. The plants were dug for inspection 30 days later.

TABLE 3.—*The effect of high and low soil moisture on Aphanomyces root rot infection as shown by the extent of disease development 30 days after planting*

[Cans kept at 20° to 25° C. after 15 seeds had been planted in each]

Soil moisture in percentage of water-holding capacity	Percentage of plants infected	Disease index
45	[1] 1.4	0.7
75	[2] 72.0	52.7

[1] 70 plants in 6 replicates.
[2] 82 plants in 6 replicates.

The results are shown in table 3. A statistical analysis of the results was not made because of the extreme difference in the amount of infection between the two series. It is evident from both the disease index and the percentage of infected plants that the higher moisture markedly favored disease development. At 45-percent water-holding capacity, there was a very small amount of infection, indicating that this moisture content of the soil is approximately the minimum at which infection can take place in this soil. This is somewhat higher than the results of Haenseler (*5*), who found that the minimum for infection occurred at about 30 percent of the water-holding capacity of the soil he used, when inoculated by means of a zoospore suspension. Although not determined experimentally, a somewhat higher moisture level than 45 percent of the water-holding capacity could undoubtedly have been maintained in soil used in the present investigation without severe infection.

NUTRITIONAL STUDIES

Although the beneficial action of fertilizers in reducing root rot has been demonstrated in infected soil, the nature of this action has not been explained. Carpenter (*2*) and LeBeau (*8*) showed that high nitrogen increased the root rot of sugarcane caused by *Pythium* spp., and that adequate phosphorus decreased it. Vanterpool (*17, 18*) demonstrated that the browning root rot of wheat occurred in phosphorus-deficient soil and that applications of phosphorus reduced its severity. Broadfoot and Tyner (*1*), studying the *Helminthosporium* root rot of wheat under controlled conditions, observed that any treatment which resulted in the production of undernourished plants favored disease development. The work of these investigators indicates that unbalanced nutrition predisposes sugarcane and wheat to the causal organisms concerned. Workers at the New Jersey Experiment Station (*11, Rpt. 57*) in field studies over a period of several years found that increase in yield of peas on root-rot-infested soil was almost directly proportional to the amount of fertilizer applied even when the rate was as high as 2,400 pounds per acre. This was interpreted as indicating that the control of root rot was a contributing factor to higher yield in addition to the direct effect of nutrients on plant growth, especially at the high fertilizer levels. Because a more adequate explanation of the relation of host nutrition to root rot development in peas was desirable, further investigation of this question was undertaken.

RELATION OF SOIL FERTILITY TO DISEASE DEVELOPMENT

In order to obtain a further check on the effect of nitrogenous fertilizers, a field test was made in 1940. A 1-acre area was chosen in the field just mentioned in which root rot had caused a complete crop failure the previous year. Each plot was 24 by 100 feet in dimension; each treatment was made on three replicates arranged in randomized order. The treatments consisted of an unfertilized check, 2–12–6 fertilizer at the rates of 333, 534, 760, and 1,108 pounds per acre, respectively, and 0–12–6 at the rate of 1,094 pounds per acre. The 2–12–6 fertilizer was a commercial preparation, and the 0–12–6 mixture was compounded from Tennessee Valley Authority superphosphate and commercial 50-percent K_2O. The fertilizers were applied broadcast on recently prepared soil and harrowed in promptly about 2 weeks before planting. Profusion variety was planted on May 8 by means of a seed drill. Throughout the season rains were frequent and the soil was thus kept at a high moisture content, a condition which favored root rot development.

On June 12 random samples, each containing from 40 to 50 plants, were dug from each plot and rated on the same scale as was used in the sand-nutrient series. Twelve days later, on June 24, a second sample, and on July 9 a third sample of plants was dug and rated. The results of the first two inspections were analyzed statistically and are given in table 4.

TABLE 4.—*Effect of fertilizer treatments on naturally infested soil in the field on the development of Aphanomyces root rot on Profusion peas sown on May 8*

Fertilizer treatment	Rate of application in pounds per acre	Mean disease index— 35 days after planting	Mean disease index— 47 days after planting
None		63.70	97.50
2-12-6	333	43.50	97.50
2-12-6	534	45.53	94.53
2-12-6	760	28.50	89.93
2-12-6	1,108	21.20	81.30
0-12-6	1,094	46.57	92.80
Minimum significant difference (5-percent point)		20.77	5.82

The results at 35 days show clearly that disease development in the unfertilized check was significantly greater than that which occurred when 2–12–6 fertilizer was applied at the rates of 760 and 1,108 pounds per acre. At the lower rates of 2–12–6 and at the 1,094-pound application of 0–12–6 there was a little less disease than in the unfertilized plots, but the difference was not significant. A comparison of the 0–12–6 fertilizer application with the corresponding 2–12–6 treatment shows a significant difference in favor of the nitrogen-bearing fertilizer. At 47 days the root rot had increased markedly and the decrease in disease at the 760- and 1,108-pound treatments with 2–12–6 was barely perceptible. At the time of the third field inspection all plots were completely and severely infected, although even at that time the plants in the plots receiving 1,108 pounds of 2–12–6 fertilizer were visibly taller and greener than those in the other plots. The crop was so poor in all plots, however, that yields were not taken. Although from the standpoint of commercial control the results were disappointing, the trend was nevertheless in general agreement with those previously observed (*3*, *5*, *11*, *12*, *13*, *14*, *19*, *20*).

DILUTE NUTRIENT-SOLUTION EXPERIMENTS

In the first series of sand-culture experiments the previously described solutions were applied at one-tenth the basic concentration. In addition to the solutions lacking N, P, and K, a solution lacking S was also employed. Although the nutrient solutions used were quite dilute, it was hoped that the degree of variation in the balance between the major elements would show whether the incidence of root rot was influenced by the nutrition of the host plant.

Four pots were used for each nutrient. Two weeks after planting all were inoculated with a zoospore suspension of *Aphanomyces euteiches* of an undetermined spore concentration. At the end of the first experiment the series was repeated. Inoculation this time was made 3 weeks after planting. In both experiments, above-ground symptoms appeared within 7 days and all plants were dead within 14 days after inoculation. Regardless of the nutrient employed, infection was equally rapid and severe.

Peas were again planted in the pots used in the second experiment without sterilizing and washing the sand. In this case the inoculum was largely in the form of oospores in the numerous fine roots left in the sand when the previous crop of infected peas was removed, rather than the introduced zoospores which brought about the initial infection. The seedlings emerged normally, but infection was visible 5 days after emergence. All plants were dead within 12 days. Again no effect of nutrient variation was discernible.

On the assumption that the form of nitrogen might have an influence on the disease, one experiment was set up to determine the effect of NH_4 ions as compared with NO_3 ions. The NO_3 solutions was the same as the normal solution used in the experiments described above. In the NH_4 solution the $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl, respectively, and an equal number of nitrogen atoms was added in the form of NH_4Cl. The pots were inoculated 2 weeks after planting. Disease development and severity were equal in both nitrate and ammonium solutions, indicating that at the nutrient concentrations used, neither form of nitrogen had any effect on disease development.

From the foregoing experiments, it was evident that with the nutrient concentration employed, the development and severity of the disease were independent of the nutrient-ion balance, or of the two types of nitrogen ions.

RELATION OF INCREASED NUTRIENT CONCENTRATION AND ION BALANCE TO ROOT ROT INFECTION

With the failure of the low-concentration experiments to show any influence on root rot, it became obvious that the fertilizer action was dependent upon factors other than nutrient-ion balance alone. Since the previous experiments had been made at a very low concentration of nutrients, it seemed advisable to determine whether differences resulting from varied nutrient-ion balance might not occur at higher concentrations. In order first to observe the effect of increased nutrient concentrations, only the complete nutrient solution was used.

In the previous experiments the basic solutions (table 1) were diluted to one-tenth concentration. The concentrations hereafter are referred to as multiples of the basic solution. Thus, the dilute solution mentioned above is designated as 0.1H, the basic as 1H, double concentration as 2H, etc. The arrangement of the sand cultures was the same as in the previous experiments. The inoculation technique used has already been described, and in the following experiments the concentration of the inoculum was standardized at 7,500 to 8,000 zoospores per centimeter of inoculum. One pot in each group of four was left uninoculated.

In the first experiment normal nutrient solution at concentrations of 0.1H, 1H, 2H, and 3H were used. Thus, the highest concentration was 30 times as strong as the nutrient used in the previous sand-culture experiments. As soon as infection appeared it became evident that at the highest nutrient concentration there was a distinct decrease in amount of disease as compared with that in the 0.1H nutrient concentration (fig. 3). The plants were dug for inspection when all the

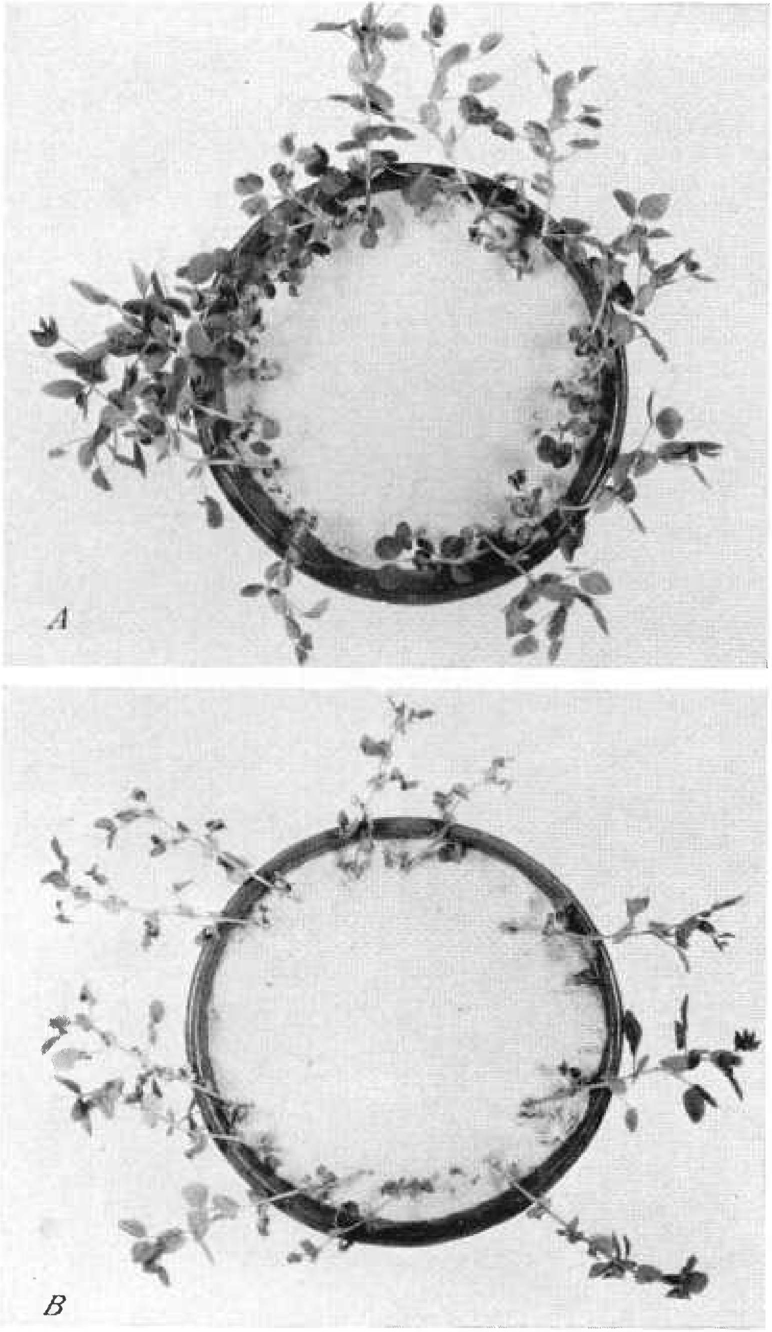

FIGURE 3.—Pea plants 18 days after inoculation with *Aphanomyces euteiches*: *A*, Plants grown in a high concentration of nutrient solution (3H); *B*, plants grown in a low concentration of nutrient solution (0.1H). The plants in *B* are all severely diseased and most have already died; of those in *A* only one showed any above-ground signs of disease at the time of the photograph.

plants in the 0.1H nutrient showed above-ground disease symptoms. The results (fig. 4, experiment 1) show a striking decrease in the amount and severity of root rot infection at the higher nutrient concentrations. The decrease in the severity appeared in direct proportion to the concentration of the nutrient.

In order to verify the findings in the above experiment, another series was run. Again the same relation between the severity of disease and the concentration of nutrients resulted (fig. 4, experiment 2). Since the 3H solution did not completely control the disease, however, still another experiment was made in which the concentrations 0.1H, 1H, 2H, 3H, 4H, and 5H were used. The same

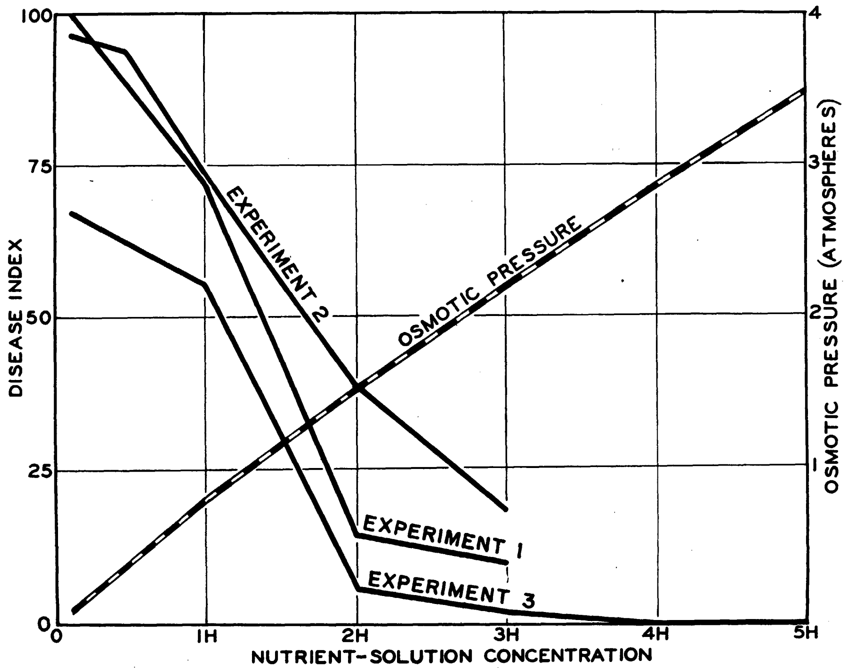

FIGURE 4.—The relation of salt concentration to osmotic pressure of the nutrient and to the development of *Aphanomyces* root rot of pea. Representative plants from experiment 3 are shown in figure 5. Explanation of nutrient-solution concentration symbols will be found in the text.

relative decrease in disease with increased concentration occurred. At the two higher concentrations, 4H and 5H, no infection whatsoever could be found (fig. 4, experiment 3; fig. 5). In this experiment the infection was not so severe in any of the nutrients, although the experiment was allowed to run about 10 days longer than the first two. For this reason the possibility remains that a small amount of infection might take place at the 4H and 5H concentrations. However, from the results of this experiment and the general relation found between concentration and infection in all three experiments, it is highly probable that the zero point for infection, under the conditions of these experiments, would be found at a concentration between 3H and 5H.

The marked reduction in the amount and severity of root rot with increase in concentration of the solution again raised the question of the relation of the nutrient elements to the disease. Although the relative proportions of all elements in the normal solution were the

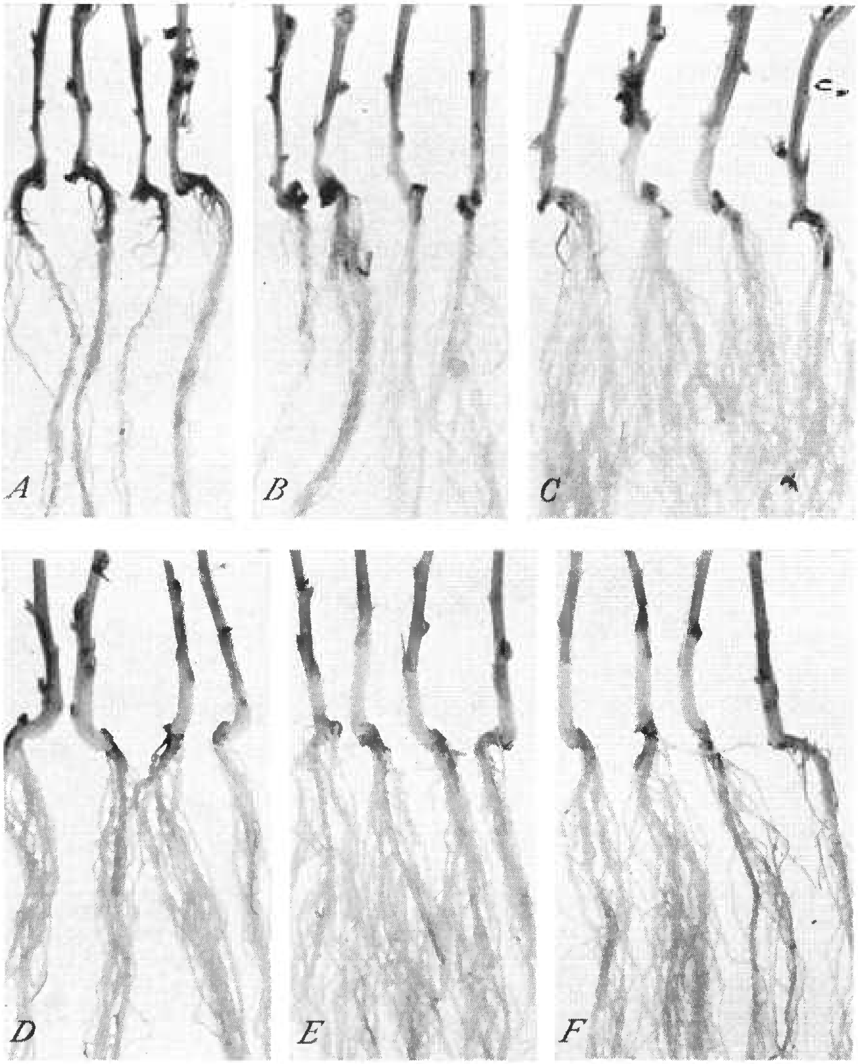

FIGURE 5.—The relation of salt concentration of the nutrient solution to the development of *Aphanomyces* root rot of peas grown in silica-sand culture. See experiment 3 in figure 4. Photographed 27 days after inoculation. The concentrations used were as follows: *A*, 0.1H; *B*, 1H; *C*, 2H; *D*, 3H; *E*, 4H; *F*, 5H (see further explanation in the text). Plants in *A* are severely diseased; many fibrous roots have been killed and cortical decay of the upper taproot and the lower stem is evident. In *B* root decay is moderate and there is considerable cortical decay in the stems. In *C* decay in the root and stem is slight. In *D* there is very slight root and stem discoloration. In *E* and *F* no signs of disease were found.

same, the possibility that one of the major elements was the effective agent still existed. In order to determine whether this was the case, each of the three elements N, P, and K was varied in the same proportions as in the experiments in which the low concentration was used. This was done at each of the three concentration levels 1H, 2H, and 3H. With this range any effect of a single element should be detected readily. With the exception of the experiment with varying nitrogen at the 1H level, a 0.1H check was run with each experiment for purposes of comparison. The results are shown in table 5.

TABLE 5.—*The effect of varied proportions of nitrogen, potassium, and phosphorus at different nutrient concentration levels on the development of Aphanomyces root rot of pea, as indicated by the disease index*

Relative level of ion being varied	N varied at concentration [1]—			K varied at concentration [1]—			P varied at concentration [1]—		
	1H	2H	3H	1H	2H	3H	1H	2H	3H
High	86.0	3.4	3.1	39.1	3.4	0.7	33.7	4.9	0.0
Normal	61.2	3.2	.6	41.0	3.2	.6	36.4	3.2	.6
Minus	77.2	3.3	.7	43.8	8.0	.7	35.6	0	0
0.1H control	--------	82.1	82.1	75.0	82.1	81.4	84.4	82.1	81.4

[1] See text for explanation of symbols indicating nutrient-solution concentration.

On the basis of the previously discussed field experiments made by other investigators, and the one made by the authors, it was expected that the quantity of nitrogen in the nutrient would have considerable influence on the severity of the root rot. The results of these experiments indicate clearly that the variations in the amount of nitrogen used, from none to 15 percent more than in the normal solution, had little or no effect on the degree of infection at any of the three nutrient concentrations. At each concentration level, nitrogen-deficiency symptoms were clearly visible in the series lacking nitrogen before the experiments were terminated, eliminating the possible influence of nitrogen carried in the seed. Since the disease development was so rapid in the lower nutrient levels, there seems to be no reason to believe that the food stored in the seed had any effect on the results of any experiment.

Although the field experiments have shown very little reduction in root-rot damage by the potassium and phosphorus portions of the fertilizers, these two elements were tested in sand culture at the same nutrient concentrations as the nitrogen portion. The results failed to show any influence of high or low amounts of either element as compared with the normal solution on the amount of disease at any of the three nutrient concentrations (table 5).

At the three nutrient levels used in the experiments on elements in varied proportions the same general decrease in disease occurred with increased concentrations, as shown in the experiments involving increased concentrations of the normal nutrient solution. However, it must be kept in mind that at any given nutrient concentration, the total salt concentration of the nutrient solutions remained essentially constant, regardless of the lack or excess of any one element. It is evident, from the results of this set of experiments, that development of the disease is not dependent upon an adequate nutrient balance, but that the reduction in disease is directly correlated with the increased concentration of the nutrient salts in the root zone of the host plants.

White and Ross (*21*) in a study of the effect of fertilizer salts have found the nitrogen components of present-day fertilizers to be much more active in raising the salt concentration of the soil solution than equivalent amounts of salts of potassium and phosphorus. It seems probable, then, that the favorable action of the nitrogen-bearing fertilizers in reducing root rot in the field is due to the greater activity of the nitrogen fractions of the fertilizer in increasing the salt concentration of the soil solution.

In order to determine the osmotic pressure of the various nutrient concentrations used, the freezing point depression method was employed. A Drucker-Burian microthermometer was used, and with the equation $P=12.06\triangle-0.021\triangle^2$, the osmotic pressure, P, in atmospheres was calculated.[3] The values obtained are shown in figure 4. It is possible that these values could be used as a guide in soil experiments designed to determine the fertilizer requirements for field control of root rot.

The results of the above experiments substantiate the suggestion made by workers at the New Jersey Agricultural Experiment Station (*11, Rpt. 59*) that the increased fertilizer concentration of the soil was responsible for reduced disease when fertilizers were applied. The observations made by Walker and Musbach (*20*) that less root rot developed when a complete fertilizer was placed with the seed than when it was placed away from the seed at the time of planting would seem to be explained by the fact that a greater fertilizer concentration is present at the zone of major root development when the fertilizer is placed with the seed.

EFFECT OF INCREASED NUTRIENT CONCENTRATION AFTER INOCULATION

The investigators at the New Jersey Station (*13, Rpt. 55; 11, Rpts. 56, 57, 59; 14, Rpt. 58*) have found that fertilizers must be applied before infection has occurred in order to be effective in reducing root rot damage. An experiment was carried out to determine the effect on incidence of root rot of increasing the salt concentration from a low to a high level at definite intervals of time after inoculation. The purpose was to find the length of time over which infection takes place under ideal conditions in the sand culture, and to observe whether the increased concentration of nutrients would decrease disease development after infection had taken place. Eight rows of four pots each were started with a normal solution at the 0.1H concentration and one row at the 3H concentration. Two weeks after planting, three pots in each row were inoculated while the fourth was left uninoculated. One row of the 0.1H concentration and the one at the 3H concentration were continued throughout the experiment. Plants in a second row of 0.1H concentration were removed periodically to determine progress of the disease. Of the other six rows, the first was changed to 3H 1 day after inoculation, the second after 2 days, etc. When the 3H solution was applied, all pots in the row were flushed with this solution in order to bring the concentration of

[3] This equation is from Gortner (*4, pp. 317–336*): $\triangle=\triangle'-0.0125\triangle' U$, when $\triangle'$ is the difference in ° C between the freezing point of the solution and that of pure water, while U represents the difference in ° C between the freezing point and the point of undercooling at which crystallization occurs.

the nutrient in the pots to the high level immediately. The results are shown graphically in figure 6.

Daily inspection of the roots of plants in the 0.1H solution disclosed no visible signs of infection until 4 days after inoculation. However, delay of the rise in nutrient concentration for 2 days after inoculation resulted in a marked increase in infection, while delay until the fourth day resulted in little reduction in disease. Thus the shift in concentration of nutrient to a level that would otherwise prevent severe disease development was of no avail if made after infection had occurred. The results of this experiment are in agreement with the field observations at the New Jersey Station, in which

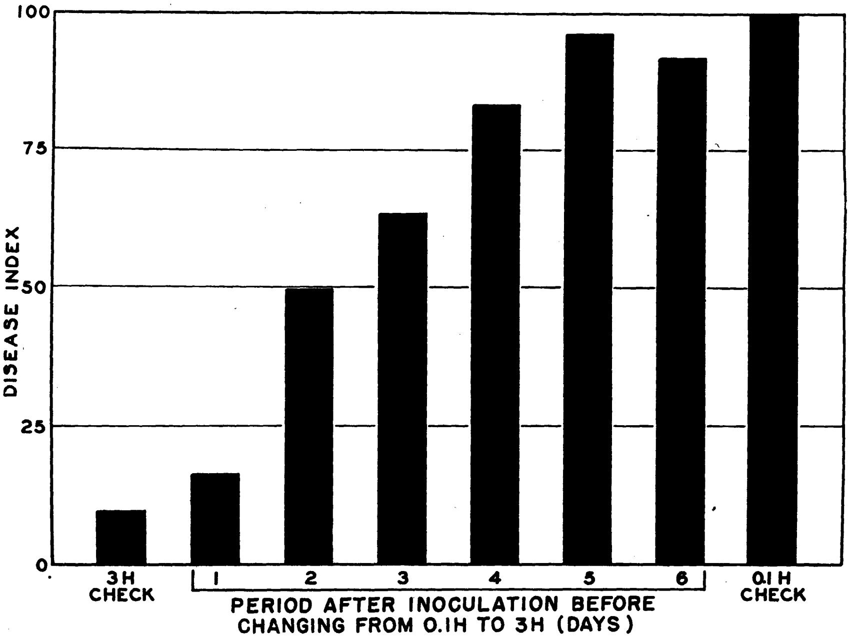

FIGURE 6.—The development of root rot (in terms of disease index) in plants grown at high (3H) and low (0.1H) concentrations of nutrient solution, compared with those started at 0.1H and shifted to 3H at various intervals after inoculation. Note the steady increase in the disease index as the interval between the inoculation and the shift to the high-concentration nutrient was increased.

the beneficial effects of fertilizer did not prevail if the fertilizer was applied after infection had taken place (*13*, *Rpt. 55*).

EFFECT OF NUTRIENT CONCENTRATION ON GROWTH OF THE FUNGUS

The direct inhibiting effect of high salt concentrations of the nutrient solution on the growth of the organism would seem to be a possible explanation for decreased severity of the root rot disease. In order to determine the effect of the nutrient concentration on the growth of the fungus, agar plates of the normal solutions at the 0.1H, 1H, 2H, 3H, 4H, and 5H levels were poured and inoculated with the fungus. The agar was prepared by making up the nutrient at double strength and combining with an equal volume of 4 percent agar. The ingredients were sterilized separately and mixed just prior to the pouring of plates. At the higher concentrations 3 to 4 drops of M/1 NaOH per 200 cc. of agar solution were added when necessary to bring the reaction to pH 6.0 or 6.2. The separate sterilization of agar and nutrient solutions was found necessary when previous attempts to sterilize them after mixing had resulted in a strongly acid reaction in the more concentrated nutrients.

The plates were inoculated in the center by 2-mm. disks of agar taken from the periphery of a vigorously growing 4-day-old culture of *Aphanomyces euteiches*. Six plates were used at each nutrient concentration. Daily measurements of radial growth were made. Because of the absence of all organic and other nutrient materials, except for that occurring in the agar, growth was slow and sparse. Nevertheless, growth did occur at the higher nutrient concentrations at approximately the same rate as in the lower, except at the 5H level at which slightly reduced growth was observed. From these results it is apparent that the growth of the organism is not inhibited at the nutrient concentrations at which no infection occurred in the sand cultures.

Geach (*3*) made a brief study of the growth of *Aphanomyces euteiches* in cultures containing various nitrogen compounds. He found growth of the organism to be retarded on pea-decoction agar containing any one of the following compounds: 0.5 percent urea, 1.1 percent NH_4NO_3, and 1.4 percent $NaNO_3$. On Shear's corn-meal agar containing 2.8 percent $NaNO_3$ no growth occurred. The apparent contradiction between these results and those of the authors is readily explained when the total salt concentrations of the sand-culture nutrient solutions and the agar cultures employed by Geach are noted. The osmotic values for the different concentrations of nutrient solutions are shown in figure 4. The calculated osmotic values for the 1.4 percent $NaNO_3$ in the pea-decoction agar used by Geach is approximately 6.8 atmospheres. This is roughly twice the osmotic value of the 5H solution employed in the sand-culture studies, and far in excess of that at which disease control was obtained. Evidently the salt concentration of the 2.8 percent $NaNO_3$ was sufficiently high to prevent growth of the organism. From the previously discussed nutrient studies, it has been shown that the presence or absence of nitrogen has no effect on the severity of root rot. Thus the inhibitory action of nitrogen in some form, as implied by the work of Geach, cannot be considered as the true cause for the control of pea root rot.

DISCUSSION

The *Aphanomyces* root rot of pea is a widespread and often destructive disease of this crop. Up to the present time, the environmental relations and disease control studies have been confined, for the most part, to field observations and experiments. Disease development is favored by high soil moisture, and occurs within a temperature range of about 10° to 30° C. Studies on the control of this disease, made largely by Haenseler at the New Jersey Agricultural Experiment Station and confirmed by other investigators, have shown that applications of complete fertilizers to infested soil tend to reduce root rot. It has been suggested that the nitrogen fraction in the fertilizer is the most important one in the reduction of disease.

The root rot organism in potato-dextrose-agar culture grows at temperatures from 12° to 32°, inclusive, with an optimum at 28°, while the disease develops in plants grown in inoculated sand maintained at constant temperatures from 16° to 32° with an optimum at 24° and 28° C. No infection was found at 12°, although Jones and Drechsler (*9*) with experiments in soil, involving a longer period of time, found a small amount of infection at 10°. The rate of disease development in sand and the rate of growth of the organism on agar approximate each other quite closely at the various temperatures used.

In naturally infected soil practically no root rot occurred when the moisture was maintained at 45 percent of the water-holding capacity of the soil, while at 75 percent of the water-holding capacity 72 percent of the plants were infected. The soil-moisture relationship found here is in agreement with the observations of other investigators. Since the experiment was not terminated until a month after planting, it would appear probable, under certain field conditions, that a single or an occasional heavy rain would not result in complete root rot infection, but rather that a prolonged condition of high soil moisture is required to produce this condition. This, of course, could happen in poorly drained fields, or under conditions of frequent heavy rainfall, as was the case in Wisconsin in 1940 when root-rot damage was widespread. This is further supported by the results of the 1940 fertilizer trial, for, on June 12, 35 days after planting, 37.8 percent of the plants in the unfertilized check plots were still free from root rot, although rainfall had been abundant. On June 24, after a 12-day period of almost continuous rainfall, all plants were infected, but 6.8 percent were still classed as slightly diseased.

During 1940 the fertilizer trial, mentioned above, was made on a field known to be heavily infested with the root rot organism. Applications of 2–12–6 fertilizer were made at various rates up to 1,108 pounds per acre. One treatment of 0–12–6 at 1,094 pounds per acre was also included. The two highest applications of 2–12–6 (760 and 1,108 pounds per acre) resulted in significantly less disease than in the unfertilized plots. Lesser rates of 2–12–6 and the 1,094-pound rate of 0–12–6 were ineffective in decreasing root rot damage. Since the phosphorus and potash content of the two treatments (2–12–6 at 1,108 pounds, and 0–12–6 at 1,094 pounds) were the same, it is evident that these two components are less active in reducing root rot infection than is the nitrogen fraction. The rainfall during this season was extremely heavy; had there been less rain, it is probable that the degree of control would have been greater in the plots receiving nitrogen in the fertilizer.

When the nutrient solution was used in continuous-flow sand-culture experiments at the concentration described by Hoagland and Snyder (*7*), and in concentrations up to five times that described, infection and disease severity decreased in direct proportion to the increase in the concentration of the nutrient solution. When severe infection occurred in the one-tenth dilution, only a small amount occurred at three times the basic concentration, and none whatsoever

408742—41——2

occurred when the nutrient concentration was raised to four and five times the basic concentration.

At the basic concentration and at two and three times that concentration, solutions each having N, P, and K in excess, and lacking each of these elements were tested. The total salt concentration was maintained constant at each concentration level. The same relation as previously described was observed. The disease decreased as the total salt concentration of the nutrient solution increased; the favorable or unfavorable balance of any of the three essential elements in the nutrient solution in no way modified the disease response.

This apparent disagreement with the results of the fertilizer investigations appears to be explained when the effects of the various fertilizer fractions on the concentration of the soil solution are investigated. White and Ross (*21*), as a result of their study of the effects of fertilizers on the salt content of the soil solution, have found that the nitrogen compounds $NaNO_3$, $(NH_4)_2SO_4$, and NH_4NO_3 increase the salt concentration of the soil solution much more than corresponding quantities of the potassium and phosphorus salts now used in commercial fertilizers. If this is the case, the greater degree of control obtained with nitrogenous as compared with nonnitrogenous fertilizers could probably be attributed directly to the greater increase in the salt concentration of the soil solution by the nitrogen fractions of the fertilizers than by the potassium and phosphate fractions. The possible detrimental effect of nitrogen alone on the organism is ruled out for the reason that the disease development was equally inhibited in nutrient solutions of high salt concentration whether nitrogen was present in excess or lacking entirely.

Under favorable conditions for infection in the sand culture, all plants may become infected within 5 days after inoculation. After infection has occurred, and before any but the slightest macroscopic symptoms are visible, changing the solution concentration from a low level to one sufficiently high to largely inhibit infection, if done at the outset, does not delay the development of the disease within the host plant. For this reason it would appear that the action of the high concentration of the nutrient salts is that of preventing infection, either by direct action of some kind on the organism, or by rendering the host more resistant. In the latter case the promotion of host resistance is much less pronounced after infection has taken place.

On agar made from nutrient solutions ranging from one-tenth to five times the basic concentration, the organism appeared to grow equally well. Only a slight depression in growth rate was noted at the highest nutrient concentration. Therefore it appears likely that the mechanism by which the root rot is prevented when in the presence of high salt concentrations is due to some other cause than concentrations of nutrient salts inhibitory to the organism.

A possible explanation for the lack of root rot at the high nutrient concentrations is to be found in the work of Nightingale and Farnham (*15*). In a study of the effect of the nutrient concentration on the anatomy of the sweet pea (*Lathyrus odoratus* L.) they have found the roots of plants growing in dilute solutions to be highly succulent and lacking in mechanical strength. The roots of plants grown in high nutrient concentrations, on the other hand, were typically woody, mechanically strong, and distinctly lacking in succulence. A similar condition occurred in the stem tissue. Since the range in nutrient concentration employed by them (0.5 to 3.0 atmospheres osmotic pressure) closely paralleled that used in the present investigations (0.12 to 3.5 atmospheres), it may possibly be, in the case of pea root rot, that the host plants acquire a morphological resistance as a direct result of the high concentration of the nutrient when grown in such solutions.

SUMMARY

The investigations comprise a study of temperature, moisture, and nutrition in relation to root rot (*Aphanomyces euteiches* Drechs.) of peas.

On potato dextrose agar the most rapid radial expansion of the organism occurred at 28° C. No growth occurred at either 8° or 36°. The optimum temperature for disease development on plants grown in sand was found at 24° and 28°. No infection was noted at 12° during an 11-day period, while nearly all plants at the optimum temperature were severely affected in that period.

On phosphate-buffered potato-dextrose agar, the pH limits for growth were about pH 3.4 and slightly above pH 8.0. The optimum, as measured by radial expansion of the organism, occurred between pH 4.5 and 6.5. An apparent isoelectric point appeared at pH 5.9.

In infested soil, practically no infection occurred when the moisture was maintained at 45 percent of the water-holding capacity. At 75 percent of moisture-holding capacity, infection was quite severe.

Under conditions of controlled nutrition in a continuous-flow sand culture, inoculated by means of a zoospore suspension, the severity of disease decreased in direct proportion to the increase in total salt concentration of the nutrient solution. No infection occurred at the highest concentrations employed, while all plants were severely diseased at the lowest concentration. Varying the ratio of each of the elements, N, P, and K from complete absence to an excess of that in the balanced solution had no effect on disease development, whether in dilute or concentrated nutrient solutions.

When conditions are favorable in the sand culture, all plants may become infected within 5 days. Once infection takes place, high nutrient concentrations do not appear to inhibit the development of the disease.

On agar cultures made from the nutrient solutions employed in the sand culture, the organism grew readily on the concentrations at which infection was prevented in the sand culture.

LITERATURE CITED

(1) BROADFOOT, W. C., and TYNER, L. E.
1938. STUDIES ON FOOT AND ROOT ROT OF WHEAT. V. THE RELATION OF PHOSPHORUS. POTASSIUM, NITROGEN, AND CALCIUM NUTRITION TO THE FOOT- AND ROOT-ROT DISEASE OF WHEAT CAUSED BY HELMINTHOSPORIUM SATIVUM P. K. AND B. Canad. Jour. Res., Sect. C, 16: 125–134, illus.

(2) CARPENTER, C. W.
1934. PREDISPOSING FACTORS IN PYTHIUM ROOT ROT. Hawaii. Planters Rec. 38: 279–388, illus.

(3) GEACH, W. L.
1936. ROOT ROT OF GREY PEAS IN TASMANIA. Austral. Council Sci. & Indus. Res. Jour. 9: 77–87.

(4) GORTNER, ROSS AIKEN.
1938. OSMOTIC PRESSURE AND ELECTRICAL CONDUCTIVITY. *In his* Outlines of Biochemistry. Ed. 2, 1017 pp., illus. New York and London.

(5) HAENSELER, C. M.
1926. STUDIES ON THE ROOT ROT OF PEAS (PISUM SATIVUM) CAUSED BY APHANOMYCES EUTEICHES, DRECHSLER. N. J. Agr. Expt. Sta. Ann. Rpt. (1924–25) 46: 467–484, illus.

(6) ———
1931. THE USE OF FERTILIZERS IN REDUCING LOSSES FROM PEA-ROOT ROT CAUSED BY APHANOMYCES EUTEICHES. (Abstract) Phytopathology 21: 116–117.

(7) HOAGLAND, D. R., and SNYDER, W. C.
1934. NUTRITION OF STRAWBERRY PLANT UNDER CONTROLLED CONDITIONS: (A) EFFECTS OF DEFICIENCIES OF BORON AND CERTAIN OTHER ELEMENTS; (B) SUSCEPTIBILITY TO INJURY FROM SODIUM SALTS. Amer. Soc. Hort. Sci. Proc. 30: 288–294, illus.

(8) LEBEAU, FRANCIS J.
1939. THE RELATION OF ENVIRONMENTAL FACTORS AND ANTAGONISTIC ORGANISMS TO ROOT ROT OF SUGARCANE AND CORN. Internatl. Soc. Sugar Cane Technol. Cong. Proc. (1938) 6: 342–347, illus.

(9) JONES, FRED REUEL, and DRECHSLER, CHARLES.
1925. ROOT ROT OF PEAS IN THE UNITED STATES CAUSED BY APHANOMYCES EUTEICHES (N. SP.) Jour. Agr. Res. 30: 293–325, illus.

(10) ——— and LINFORD, M. B.
1925. PEA DISEASE SURVEY IN WISCONSIN. Wis. Agr. Expt. Sta. Res. Bul. 64, [31] pp., illus.

(11) NEW JERSEY AGRICULTURAL EXPERIMENT STATION.
1928–38. PEA ROOT ROT. N. J. Agr. Expt. Sta. Ann. Rpt. (1927–28) 49: 55–56, 1928; (1929–30) 51: 48, 1930; (1934–35) 56: 68, 1935; (1935–36) 57: 73–74, 1936: (1937–38) 59: 83, 1938.

(12) ———
1929. FERTILIZERS VS. PEA ROOT ROT. N. J. Agr. Expt. Sta. Ann. Rpt. (1928–29) 50: 41.

(13) ———
1931–34. PEAS. N. J. Agr. Expt. Sta. Ann. Rpt. (1930–31) 52: 50, 1931; (1931–33) 53–54: 61, 1933; (1933–34) 55: 67–68, 1934.

(14) ———
1937–39. PEA ROOT ROT INVESTIGATIONS. N. J. Agr. Expt. Sta. Ann. Rpt. (1936–37) 58: 80, 1937; (1938–39) 60: 93, 1939.

(15) NIGHTINGALE, G. T. and FARNHAM, R. B.
1936. EFFECTS OF NUTRIENT CONCENTRATION ON ANATOMY, METABOLISM, AND BUD ABSCISSION OF SWEET PEA. Bot. Gaz. 97: 477–517, illus.

(16) TILFORD, PAUL E.
1936. THE RELATION OF TEMPERATURE TO THE EFFECT OF HYDROGEN- AND HYDROXYL-ION CONCENTRATION ON SCLEROTINIA FRUCTICOLA AND FOMES ANNOSUS. SPORE GERMINATION AND GROWTH. Ohio Agr. Expt. Sta. Bul. 567, 27 pp., illus.

(17) VANTERPOOL, T. C.
1935. STUDIES ON BROWNING ROOT ROT OF CEREALS. III. PHOSPHORUS-NITROGEN RELATIONS OF INFESTED FIELDS. IV. EFFECTS OF FERTILIZER AMENDMENTS. V. PRELIMINARY PLANT ANALYSES. Canad. Jour. Res., Sect. C, 13: 220–250, illus.

(18) ———
1940. STUDIES ON BROWNING ROOT ROT OF CEREALS. VI. FURTHER CONTRIBUTIONS ON THE EFFECTS OF VARIOUS SOIL AMENDMENTS ON THE INCIDENCE OF THE DISEASE IN WHEAT. Canad. Jour. Res., Sect. C, 18: 240–257, illus.

(19) WALKER, J. C.
1933. RELATION OF SOIL FERTILITY TO INCIDENCE OF APHANOMYCES ROOT ROT OF PEA. (Abstract) Phytopathology 23: 36.

(20) ——— and MUSBACH, F. L.
1939. EFFECT OF MOISTURE, FERTILITY, AND FERTILIZER PLACEMENT ON ROOT ROT OF CANNING PEAS IN WISCONSIN. Jour. Agr. Res. 59: 579–590, illus.

(21) WHITE, LAWRENCE M., and ROSS, WILLIAM H.
1939. EFFECT OF VARIOUS GRADES OF FERTILIZERS ON THE SALT CONTENT OF THE SOIL SOLUTION. Jour. Agr. Res. 59: 81–99, illus.