STUDIES ON THE RESISTANCE OF TOBACCO TO A SECOND ATTACK OF *PERONOSPORA TABACINA* ADAM

by

Carroll E. Cox

A Major Thesis Submitted to the Graduate Committee of the
Virginia Polytechnic Institute, Department of Biology

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

PLANT PATHOLOGY

Approved:

Adviser	Head of Department
Dean of Agriculture	Dean of Applied Science

Virginia Polytechnic Institute

1940

TABLE OF CONTENTS

ACKNOWLEDGMENTS

Special acknowledgment is made to R. G. Henderson whose guidance and advice was invaluable in carrying out this investigation. Acknowledgment is also made to Dr. J. G. Harrar, Dr. S. A. Wingard, Dr. G. M. Shear and Dr. F. S. Orcutt for helpful suggestions and use of equipment.

STUDIES ON THE RESISTANCE OF TOBACCO TO A SECOND ATTACK OF *PERONOSPORA TABACINA* ADAM.

INTRODUCTION

Downy mildew caused by *Peronospora tabacina* is a serious plant bed disease in almost all of the tobacco growing areas in North America. Perhaps the most striking phenomenon associated with this disease is the remarkable capacity of badly diseased plants to recover. Certain workers in the field have observed that plants which have recovered and have developed new leaves are not readily subject to a second attack, and this opinion is generally held by tobacco growers. This phenomenon has been interpreted to indicate that recovered plants have acquired a degree of resistance and are, at least for a time, immune to reinfection by the fungus.

In recent years the interest of plant pathologists has been directed toward a study of acquired immunity in plants. A number of instances of acquired immunity following recovery from virus diseases are on record, but there remains some question whether acquired immunity from fungous diseases has been demonstrated in plants. It has been suggested that this tobacco disease might profitably be employed in researches on the subject.

Applications of nitrate of soda to the soil in which diseased tobacco plants are growing has been reported to be beneficial in aiding recovery from downy mildew. There is also some experimental evidence to indicate that plants growing in crocks of sand and peat in the greenhouse are more resistant to the disease and recover from an attack more readily when fertilized with commercial fertilizer

or nutrient solutions high in nitrogen and low in potassium content. The purpose of this preliminary investigation was primarily to find out if any relationship exists between the nitrate-nitrogen and soluble sugar content of tobacco seedlings and their susceptibility to attack by Peronospora tabacina. In the course of the investigation certain other experiments were performed and observations made which shed some light on the nature of the resistance acquired by tobacco seedlings which have recovered from downy mildew.

REVIEW OF LITERATURE

Downy mildew of tobacco has probably been present in Australia since the middle of the nineteenth century (1, 4); definite records of its occurrence there date from 1890. Since it occurs on certain native tobaccos in Australia, it is thought to be indigenous to that country (1, 4, 11). In 1921 the first outbreak of the disease in the United States occurred in Florida and Georgia (29), but it was not again observed until 1931 (29). Since 1931 it has spread to practically all of the tobacco growing areas of North America (28).

In 1932 Angell and Hill (4), describing the disease in the seed beds in Australia, stated that under suitable weather conditions attacked seedlings are killed, but that under certain circumstances some may survive and appear healthy enough to risk transplanting them into the field. They did not claim, however, that these plants had developed a degree of resistance to the disease. In Australia infection of leaves, stems, flowers, seed capsules and even seeds on plants growing in the field is a usual occurrence, which suggests that if a degree of resistance is developed by plants in the seed bed it is not carried over to plants in the field, or at least is of short duration.

In 1933 Clayton and Gaines (7) reported that small plants are liable to be killed outright by the first attack of the disease, but that older plants may lose most of their leaves and still have strength to make new foliage if aided by a fovorable change of weather. Wolf et al. in 1934 (29) made a similar observation and stated that plants which survive may have recovered sufficiently within two weeks

to be transplanted.

In 1935 Wolf (27) stated that seedlings which had survived an attack of downy mildew and developed new leaves did not seem to be readily subject to a second attack. Armstrong and Sumner (5) in the same year reported similar observations. They also described an experiment performed in 1933 in which reinoculation of recovered plants in beds at Florence, South Carolina, failed to produce symptoms of the disease even though it was present in nearby beds and weather conditions were apparently favorable for its development. In another case several recovered plants were transplanted from beds at Florence, S. C. to beds at Oxford, N. C. The disease was just appearing at Oxford, and subsequently attacked almost every plant except those from Florence. These workers were unsuccessful in obtaining infection on several large recovered plants held in temperature-humidity cases but obtained infection on similar plants when the large inoculated leaves were enclosed in cellophane bags. In another experiment, carried out on plants growing in incubators, they were successful in obtaining infection on one group of plants three times in succession and on another group twice in succession. The same workers observed that succulent plants are more susceptible to the disease than nonsucculent ones and that young hardened plants are less susceptible than somewhat older succulent ones. They concluded that conditions within the plant, in addition to conditions of the environment, are important factors in determining infection and the development of the disease.

Anderson in 1937 (2) stated that in Connecticut plants appear to

acquire a certain degree of immunity after the initial attack. In 1938 Clayton and Gaines (8) stated that no matter how mild or severe the initial attack, once recovery has occurred, the plants are so resistant that serious damage from a second attack need not be feared. In 1939 Kincaid and Tisdale (14) reported that plants which had had the disease and had recovered were seldom seriously affected again in the plant bed and when set in the field grew normally.

Wolf, 1939 (28) wrote that in some seasons recovery is followed by a degree of resistance, while in other seasons there may be one or more recurrences of the disease on seedling in the plant bed or soon after they are transplanted. He further wrote that recurrence of the disease appears to be causally correlated with weather factors, but other, as yet unknown, conditions may be primary causes. He suggested that this disease might profitably be studied in researches on immunity in plants.

Most American publications on downy mildew recommend the judicious application of nitrate of soda to the soil of seed beds in which the plants are affected with downy mildew to hasten the recovery of the diseased seedlings (5, 7, 8, 14, 26, 27, 30). Clayton and Gaines in 1933 (7) pointed out that the nitrate merely assists the plants to more rapidly repair the damage caused by the diseases.

It has been pointed out that the use of commercial fertilizers and fertilizer materials to control downy mildew has been uniformly unsuccessful (19, 26) and that claims to the contrary are not supported by experimental results (26). However, Wolf et al. (30) reported that applications of nitrate of soda seem to hasten recovery

by stimulating the formation of new secondary roots near the base of the stem. They further state that several light applications made at intervals are more effective than a single application at time of seeding or after the disease has appeared. They recommend a light application of nitrate of soda before the disease appears followed by another application immediately after the disease is discovered in the plant bed.

Henderson in 1937 (13) reported on rather extensive studies of the effect of various amounts of nitrogen and potassium on susceptibility of tobacco seedlings to downy mildew under greenhouse conditions. He studied the effect of both nutrient solutions prepared from c. p. chemicals and of applications of commercial fertilizers and found that plants supplied with high amounts of nitrogen or with low amounts of potassium were more resistant than plants supplied with low amounts of nitrogen or with high amounts of potassium.

There are some references in the literature on the effect of nutrients on downy mildews of other plants. Quanjer in 1928 (22) concluded that downy mildew of cabbage was more severe on plants lacking potash and he believed that all downy mildews might react in a similar manner. Chupp's (9) observations, however, indicate that in New York both potassium and nitrogen increase the severity of downy mildew on cabbage and cauliflower, but that phosphorus seems to decrease it, and that the decrease is proportional to the increase in application of phosphorus. According to Doran (12), Blattny (6) reported that potash and lime decrease the susceptibility of hop plants

to downy mildew, but Doran found that the susceptibility of cucumber to downy mildew was not affected significantly by potash, lime or complete fertilizer applied to the soil.

MATERIALS AND METHODS

Yellow Mammoth tobacco was used throughout this work except where otherwise indicated. In certain cases hybrids were used, the pedigrees of which are omitted, for the reason that none of them reacted to the disease in a manner different from Yellow Mammoth.

In order to maintain conditions in the greenhouse at all times favorable for the development of the downy mildew, a large shallow metal tank was constructed on one of the greenhouse beds. The tank was large enough to hold twelve three-gallon earthenware crocks in which tobacco seedlings could be raised. A water outlet in the tank was provided so as to maintain the water level in the tank about an inch below the top of the crocks, and the water inlet so arranged as to keep the water in the tank in constant circulation. A translucent cover was provided for the tank which when in place allowed adequate light to reach the plants but at the same time prevented excessive loss of water vapor and so maintained a relatively high humidity. Water flowing through the tank was found to keep the temperature of the air around the plants within the range for optimum development of the fungus.

The crocks were filled with clean white sand and sown with Yellow Mammoth tobacco seed. Nutrient was provided in the form of a nutrient solution developed especially for tobacco by Dr. G. M. Shear of the Virginia Agricultural Experiment Station.

The standard solution contains the following salts:

	p.p.m.	
Nacl	Na 29.94	Cl 46.26
$Ca(NO_3)_2 \cdot 4H_2O$	Ca 245.02	N 222.40
KH_2PO_4	K 37.79	P 29.82
$MgSo_4 \cdot 7H_2O$	Mg 30.14	S 39.98
KNO_3	K 372.74	N 160.83
$Mg(NO_3)_2 \cdot 6H_2O$	Mg 9.90	N 11.38
$NH_4H_2PO_4$	N 12.10	P 26.90
H_3BO_3	B .85	
$MnSO_4 \cdot 4H_2O$	Mn 1.23	S .71
$FeCl_3 \cdot 6H_2O$	Fe .99	Cl 1.892

The nutrient solutions were made up and placed in five-gallon bottles arranged underneath the bench on which rested the tank containing the crocks of sand. The bottles were connected with the crocks so that by forcing compressed air into the bottles the nutrient solution was forced up through glass and rubber tubing into the sand, thus irrigating the plants. When the compressed air was released the excess solution drained back into the bottles. The plants were irrigated once or twice a day, depending on the age and size of the plants. The solutions were renewed periodically.

It was found to be difficult to raise large numbers of plants under the same conditions in the greenhouse, and to keep some free of the disease while others were passing through various stages of

infection. In order to protect plants from infection by air-borne conidia, they were grown under spore-tight covers. A frame one yard square was constructed of six-inch boards and then covered with a double thickness of cellophane. The edges of the cellophane were cemented to the wooden frame. A stream of filtered air from a small aquarium air-pump was forced through the frames continuously while they were in place so as to prevent an accumulation of carbon dioxide. Two large holes were drilled in the side of each frame so that samples of leaf tissue could be removed by means of long forceps without removing the frames. These holes were kept plugged with cotton. Figures 1 and 3 show the frames in place.

Sampling of Leaf Tissue for Analysis

In collecting samples of leaf tissue for analysis an effort was made to collect a representative sample. Leaves were taken from 40 to 100 plants depending upon the size of the plants and the number of plants available. The leaves were washed in tap water, water droplets removed by folding in a turkish towel and the sample placed in a 125 or 150 cc. flask. The samples were then autoclaved at 5 lbs. pressure for five minutes; then dried to constant weight in an oven at 60° to 70° C. as outlined by Loomis and Shull (15). The dry leaf tissue was powered and thoroughly mixed and a sample weighed out analytically for extraction.

Extraction of Sample

To each one gram sample of dried ground leaf tissue was added approximately 0.1 gm. calcium carbonate and 50 cc. of 80% ethyl alcohol. The sample thus prepared was refluxed for 1½ hours. The

liquid was filtered off and the residue washed with 50 cc. of hot 80% alcohol. The filtrate was then heated on a water bath at 65° C. until practically all of the alcohol was removed and the volume of the remaining liquid was not more than 20 cc.

Preparation of Extract

The 20 cc. of sample extract was placed in a 50 cc. volumetric flask, a few drops of phenolphthalein added, and the solution neutralized with sodium hydroxide. One cc. of 33% lead acetate solution was then added and the mixture shaken; then 3 cc. of a 10% solution of sodium phosphate was added to remove the excess lead. If acid or alkaline, the solution was again neutralized, after which it was diluted to exactly 50 cc. and than mixed thoroughly. After the precipitate had settled, an aliquot for analysis was removed by means of a pipette.

Method of Sugar Analysis

Determinations of reducing sugars were made by a modification of Shaffer and Hartmann's micro method described by Stiles, Peterson and Fred (23). This method was found by Orcutt and Wilson (20) to be satisfactory for determinations of sugars in extracts of soybean tissue.

Determinations of total sugars were made by the same method on aliquots of extract which had been hydrolyzed by adding 0.4 m.l. of concentrated hydrochloric acid to 5 cc. of extract and heating on a 70° C. water bath for five minutes after the temperature of the solution reached 67° C. as suggested by Loomis and Shull (15). To check the accuracy of the method, both pure sugar solutions and sample

extract, to which known amounts of sugar were added, were tested and the per cent of recovery calculated. No figures were reported where the percentage of recovery was not between 98.0% and 100.9%. The amount of reducing sugars and the total sugar in the leaf tissue was calculated from these figures and is given as milligrams of reducing sugar per gram of dry tissue.

Nitrate-nitrogen determinations were made on 5 cc. aliquots of the prepared sample by the official A. O. A. C. phenol-di-sulfonic acid method. The results are reported as milligrams nitrate-nitrogen per gram of dried tissue.

Ammonia-nitrogen was also determined by a colorimetric method. A 0.25 gm. sample of dried leaf tissue was weighed out analytically and placed in a 125 cc. flask. To this was added 25 cc. of distilled water and 1 cc. of 40% sodium hydroxide. The flask was then connected to a distilling apparatus and about 20 cc. of liquid distilled off and collected in a flask containing 1 cc. of saturated potassium sulfate solution and enough distilled water to cover the end of the delivery tube. The distillate was washed into a 200 cc. volumetric flask and diluted to about 180 cc. Nessler's compound was then added until no further color change occurred (about 5 to 6 cc.). The solution was then diluted with distilled water to exactly 200 cc. A portion of this solution was immediately placed in the glass cell of a photoelectric colorimeter (Cenco photelometer) and a reading taken. By comparing these readings to a curve plotted on cross section paper prepared from photelometer readings made on standard solutions,

parts per million of ammonia-nitrogen in the diluted sample could be read off directly. From these figures milligrams of ammonia-nitrogen per gram of dried leaf tissue were calculated.

DESCRIPTION OF DOWNY MILDEW OF TOBACCO WITH OBSERVATIONS ON THE DEVELOPMENT AND DURATION OF RESISTANCE FOLLOWING RECOVERY FROM AN INITIAL ATTACK

The first symptoms of downy mildew infection on tobacco plants are a slight yellowing and a downward curling of the margins of the leaves. This is especially true of small plants as shown in figure 5, but on older plants the curling of the leaves may be less pronounced. In a few days following infection, if conditions for the development of the fungus are favorable, bluish or grayish downy masses of conidiophores are produced on the lower side of infected leaves (Fig. 2). At this time yellow blotches appear on the upper side of the leaves above the areas upon which sporulation is taking place. After a few more days these yellow areas become water soaked, then dry and necrotic (Fig. 12, A and B), after which sporulation ceases. On young plants entire leaves may die, but on older plants the tissue is often killed only in spots and the leaves may become deformed or ragged as shown in figure 12, C and figures 9 and 10. Very small plants are more often killed outright by the disease than larger plants, but under some conditions large plants may be killed as a result of the initial infection (Fig. 11).

The length of time between inoculation and the appearance of the first symptoms of the disease may be as short as 24 to 36 hours under favorable conditions. Under greenhouse conditions with a temperature of 65° to 70° F., five to six days is the usual length of time between inoculation and first sporulation. Within the range of tolerance of the fungus, a higher temperature (75° - 80° F.) shortens

Figure 1. Two spore-tight frames in place in the greenhouse. These frames were placed over tobacco seedlings to protect them from air-borne spores of Peronospora tabacina Adam.

Figure 2. Portion of the under side of young diseased tobacco leaf showing a mass of conidiophores of Peronospora tabacina Adam. much enlarged.

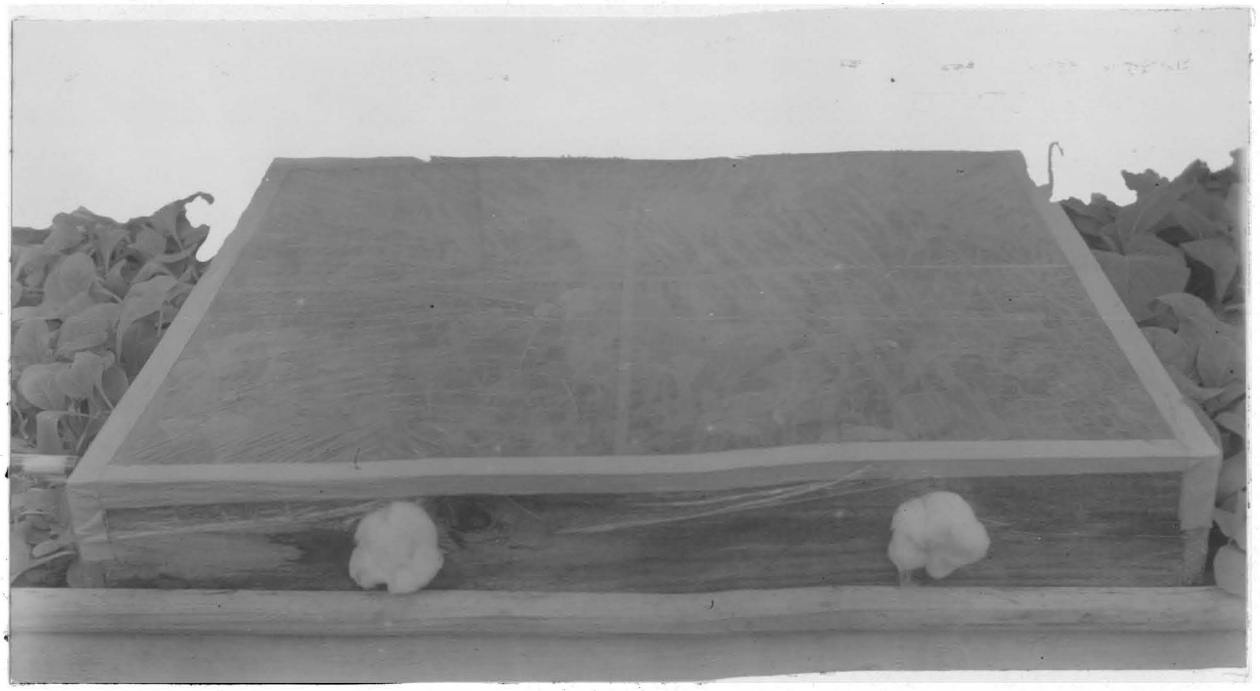

Figure 3. A spore-tight frame under which are growing disease-free seedlings in place in the greenhouse.

the incubation period and a lower temperature (50° - 60° F.) lengthens it. Figure 4 shows a young healthy plant and figure 5 a plant of the same age infected with the downy mildew fungus. In severe cases all of the leaves, the terminal bud and even the stem may become infected.

The percentage of recovery from the disease varies from a few plants in some cases to over 90% in others, depending upon the age of the plants and possibly on several environmental factors. The first sign of recovery is the production of new leaves from the terminal bud which are dark green in color. Chlorotic or necrotic patches of minute flecks may develop on these leaves (Fig. 13, B and C) In some cases only a part of the leaf is so affected, the rest appearing perfectly normal (Fig. 13, A). Microscopic examination of cleared tissue from such leaves reveals the presence of the hyphae of the downy mildew fungus in the chlorotic spots. As recovery proceeds leaves are produced from the terminal bud which, except for their darker green color, appear perfectly normal (Fig. 6, 7, 9, and 10).

Recovered plants are for a time resistant to a second attack by the downy mildew fungus. The duration of the period or resistance, however, is variable. One bed of young plants about three weeks old in the greenhouse developed the active stage of the disease between December 24 and January 1. On January 4 the plants appeared to have recovered. They were exposed to continuous natural inoculation, but reinfection did not occur until some time later, with sporulation occurring on January 12. Allowing the usual incubation period of five to six days, there would remain a minimum of only two or three

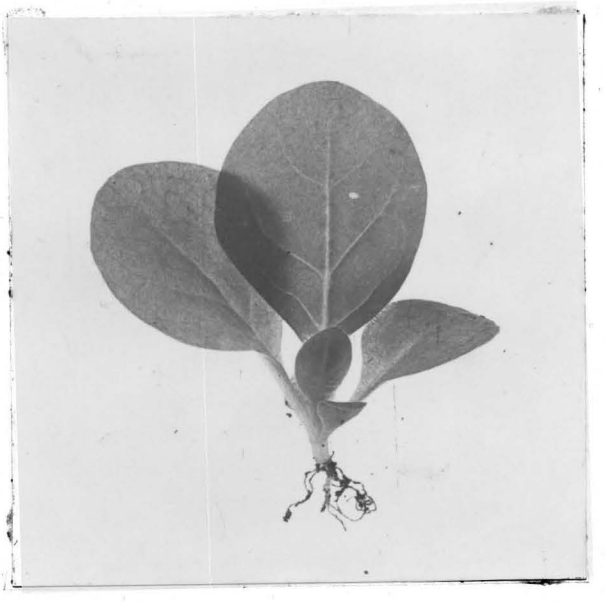

Figure 4. Healthy young tobacco plant. (twice natural size)

Figure 5. A young tobacco plant infected with downy mildew. Notice curling of leaves and chlorosis of the lower one. (twice natural size)

Figure 6. A young diseased tobacco plant beginning to recover. The small leaf at left is apparently healthy. The large leaf at right is curled downward at the tip indicating infection, but otherwise it appears healthy. (twice natural size)

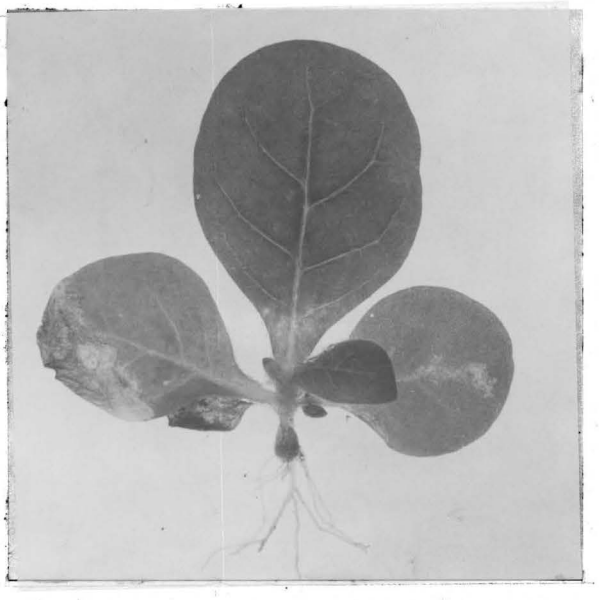

Figure 7. A young recovered tobacco plant. One leaf has been killed and two others badly injured by downy mildew. The large leaf is uninfected except for a small chlorotic area near its base. The small bud leaf is dark green in color and appears to be disease-free. (twice natural size)

days during which the plants were resistant. At other seasons of the year, however, the period of resistance appeared to be longer. On the plants in another bed, similar to the one just described, the fungus first sporulated on June 21. By June 28 the plants had recovered and, although continuously exposed to natural inoculation, did not again show sporulation until July 11. On other plants in the same greenhouse, sporulation was abundant during this time. Again allowing a five or six day incubation period, there were eight days during which the plants were resistant. On another occasion there were plants in four pots upon which sporulation first occurred on March 29 and which had apparently recovered on April 4. On April 10 these plants were artificially reinoculated and on April 17 sporulation occurred on the new leaves. In this case the period of resistance was not longer than six days. Had these plants been inoculated sooner or had there been enough infected plants sporulating in the greenhouse at the time to provide a sufficiently abundant source of natural inoculum, the period of resistance may have proved shorter.

The downy mildew fungus does not always sporulate on tobacco plants undergoing a second attack. In two experiments, described elsewhere in this paper, the infection of recovered plants resulted in a yellowing and a necrosis without sporulation. A group of plants seeded in the greenhouse on May 7 became infected and first sporulation occurred about June 10. By June 21 these plants had recovered. They were exposed to continuous natural inoculation and in addition were sprayed with a suspension of spores on June 21, 23 and 27. On

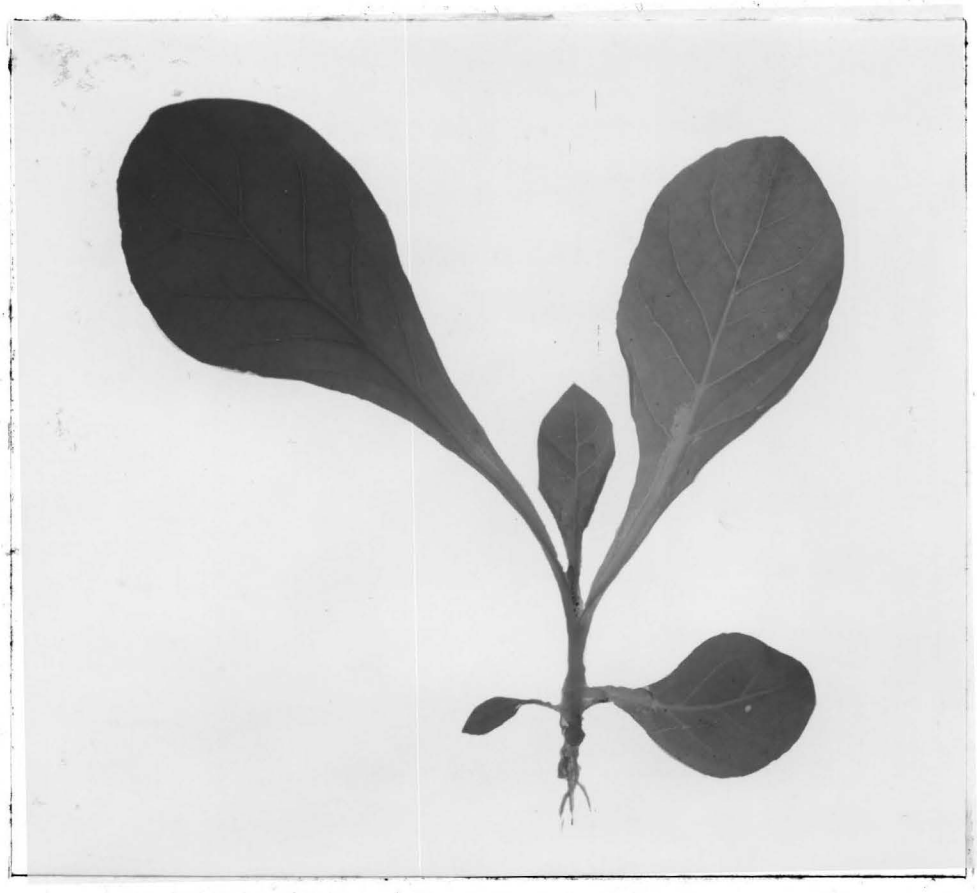

Figure 8. A large healthy tobacco seedling.

Figure 9. Tobacco plant recovering from downy mildew. Three leaves are badly damaged by the disease. One large leaf is infected only at the tip, while the small bud leaf is healthy. This plant was the same age as the plant in figure 8. Growth was apparently stunted by the disease.

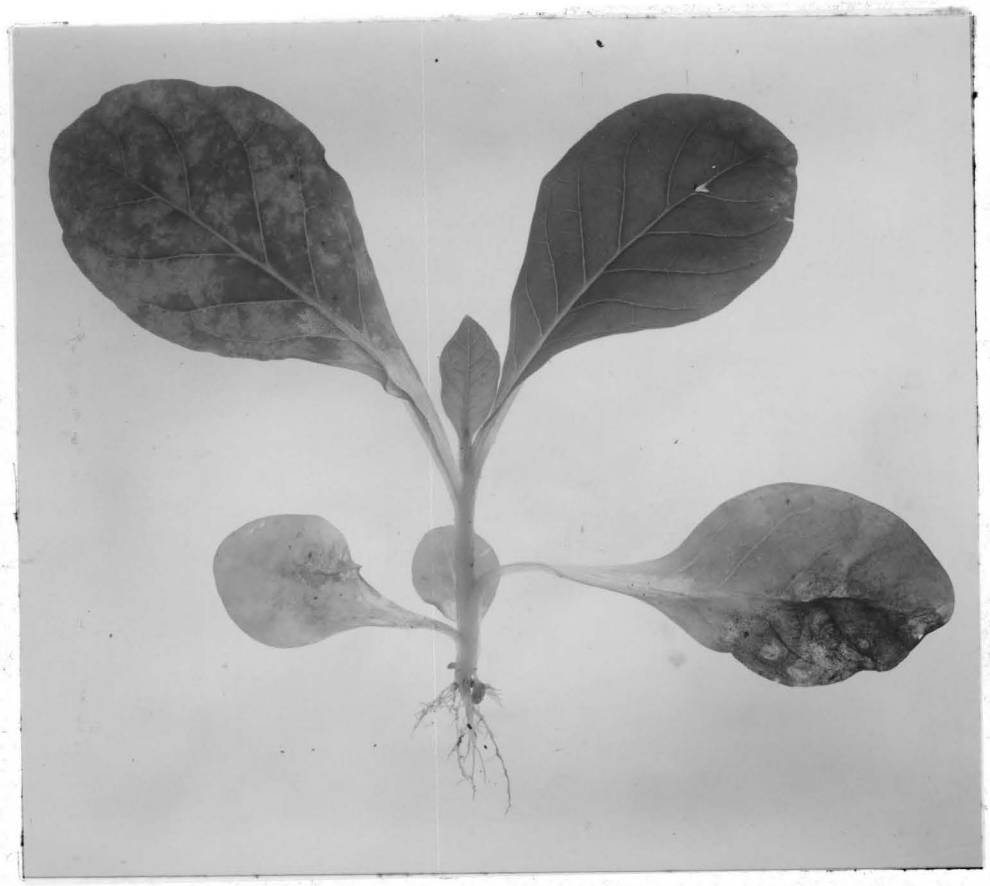

Figure 10. A recovered tobacco plant. The large leaf at the left shows chlorotic spots which are associated with recovery. The large leaf at the right is dark green and healthy except for chlorotic spots at its tip. The small leaf in the center is healthy.

Figure 11. Tobacco plant of the same age as the one in figure 8, which was killed by the initial attack of downy mildew.

July 1 minute necrotic flecks were observed on some of the leaves (Fig. 14). None of the typical symptoms of infection ever developed and sporulation never occurred. Plants of the same age growing beside the recovered plants, but which had not previously been infected, developed normal symptoms of the disease. These recovered plants were further exposed to natural inoculation and two weeks later some of the leaves showed large irregular yellow spots which were typical of the disease as described by Pinckard and Shaw (21) on plants in the field. Observations on this group of plants indicate that they possessed a degree of resistance for a period of fourteen or fifteen days.

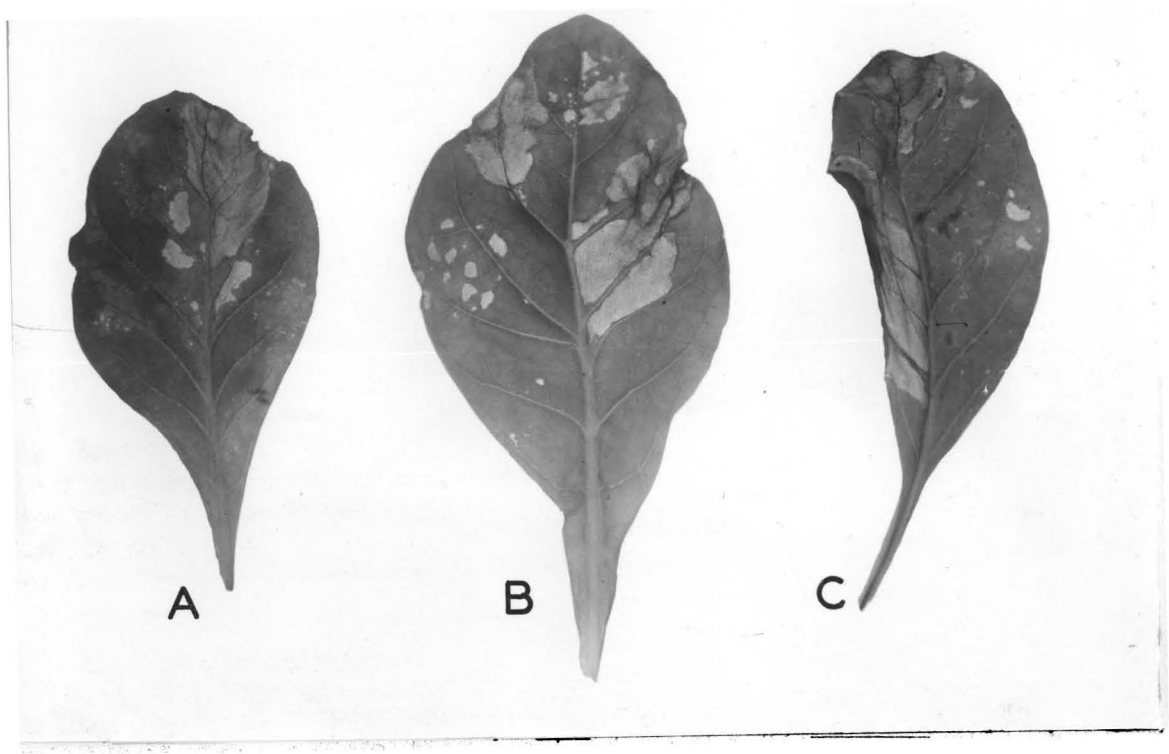

Figure 12. Leaves from tobacco plants infected with downy mildew. A and B show necrotic spots following sporulation. C shows necrosis of most of the tissue on the left side of the midrib. The tissue on the right side of the midrib may continue to grow and result in deformity of the leaf. This type of necrosis without sporulation sometimes follows reinfection of recovered plants.

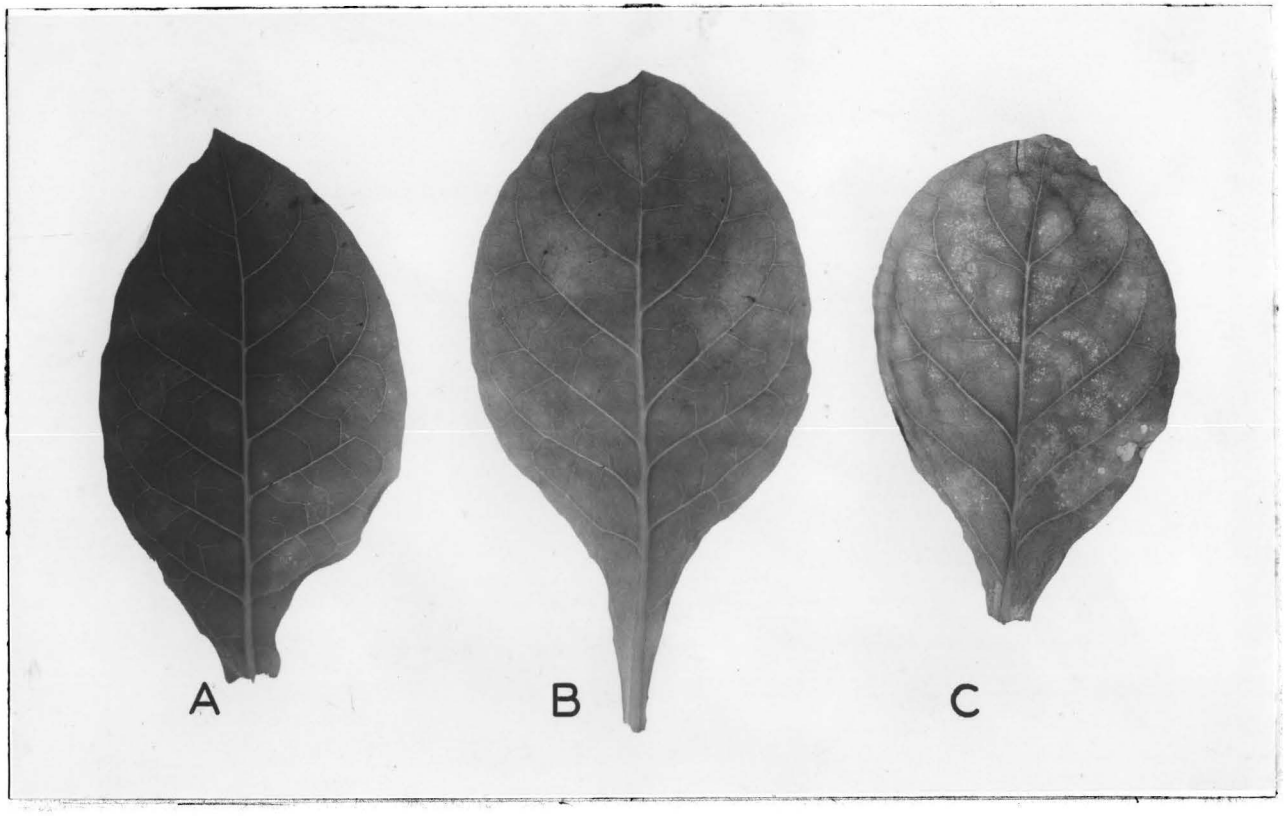

Figure 13. Leaves from tobacco plants recovering from downy mildew. A, dark green leaf with some yellowing at one side. B, leaf showing chlorotic spots. C, leaf showing patches of necrotic flecks.

Figure 14. Diseased and recovered tobacco plants of the same age in the greenhouse. Diseased plants on left; recovered plants on right. Photograph made just after first sporulation occured on infected plants. Note flecks on recovered leaves at A.

Figure 15. Same as figure 14. Photograph made two days later.

Figure 16. Same as figure 14. Photograph made five days later as the disease reached its maximum severity.

Figure 17. Same as figure 14. Photograph made eleven days later showing diseased plants on left beginning to recover.

ANALYSIS OF PLANT TISSUE

This study was made to determine if there was a substantial difference in the sugar, nitrate-nitrogen and ammonia-nitrogen content of susceptible tobacco plants and that of recovered tobacco plants. Samples of leaf tissue for analysis were obtained (1) from susceptible, infected and recovered tobacco plants growing in soil in the greenhouse, and (2) from plants grown in sand cultures to which were added nutrient solutions containing different concentrations of nitrogen and potassium bearing salts; (3) from plants grown in sand-peat mixtures to which was added mixed fertilizers containing varying amounts of nitrogen and potassium.

Analysis of Leaf Tissue from Tobacco Plants Growing in Soil

Approximately five square feet of bed surface in the greenhouse was seeded to a hybrid tobacco. Soon after the seeds germinated, a part of the bed was covered with the spore tight frames described above to protect the seedlings from air-borne spores (Figs. 1 and 3). The plants not protected by these covers contracted downy mildew by the time the largest leaves were about the size of a quarter. Although the attack was severe the majority of the plants recovered. After these unprotected plants had fully recovered, one of the spore tight frames was removed in order to expose the now disease-free plants to infection. The disease-free (susceptible) plants as well as the recovered ones were then sprayed with a suspension of spores. The frame was then replaced so as to cover a part of the healthy plants and a part of the recovered plants. The disease-free plants under

another frame were left uninoculated. The previously uninfected plants which were inoculated developed the disease (Figs. 14, 15, 16 and 17) but the recovered ones showed no signs of infection, indicating that the recovered plants were resistant at that time. When sporulation occurred on the susceptible plants following the inoculation, leaf samples for analysis were collected from the three groups of plants, namely, susceptible, infected and recovered. The first samples were collected at 5 P. M. Results of the analysis of these samples are shown in Table 1. There was more nitrate-nitrogen in the susceptible than in the recovered plants (Fig. 19), while the infected plants in this case contained more than either the susceptible or recovered ones. Total sugars were the same in infected and recovered plants, but were much lower in the susceptible plants. Only the sample from recovered plants gave a test for reducing sugars. Ammonia-nitrogen in all three samples was practically the same. The ratio of total sugar to nitrate-nitrogen was low in susceptible plants, intermediate in infected plants and high in recovered plants.

Samples were taken from the same three groups of plants at 5 A. M. two days later and the same determinations made upon them. Results of these determinations are shown in Table 2. Again the susceptible plants contained more nitrate-nitrogen than the recovered plants, but in this case, the nitrate-nitrogen content of the infected plants was intermediate. None of the samples at this time gave a test for reducing sugars. Total sugars were very low in susceptible plants, intermediate in recovered plants and highest in infected ones. There was not enough sample from the susceptible plants to be tested for

Table 1 - Results of chemical analysis of susceptible, infected and recovered tobacco plants growing in soil in the greenhouse. The samples were taken at 5:00 P. M.

Condition of plants when sampled	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	mgm/gm total sugar	Ratio total sugar/NO_3-N
Recovered	3.5	0.25	3.32	6.72	26.9
Infected	3.5	0.85	0.0	6.72	7.91
Susceptible	3.3	0.34	0.0	1.42	4.24

Table 2 - Results of chemical analysis of susceptible, infected and recovered tobacco plants. These samples are from the same lot of plants as recorded in Table 1, but were taken two days later and at 5:00 A. M.

Condition of plants when sampled	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	mgm/gm total sugar	Ratio total sugar/NO_3-N
Recovered	2.8	0.27	0.0	3.32	12.5
Infected	2.5	0.50	0.0	5.71	11.4
Susceptible	No test	0.61	0.0	0.6	1.0

ammonia-nitrogen, but ammonia-nitrogen in the samples from infected and recovered plants was practically the same. Again in this series of samples the ratio of total sugar to nitrate-nitrogen was found to be low for susceptible plants, intermediate for infected plants and high for recovered plants.

Ten days later another series of samples were taken from the same three groups of plants and analyzed with the results recorded in Table 3. At this sampling the nitrate-nitrogen level as well as the total sugar level of the three samples was above that of the two previous samplings. Nitrate-nitrogen was highest in the infected plants, intermediate in susceptible plants and lowest in the recovered plants. Total sugars were again highest in the recovered plants, intermediate in the infected and lowest in susceptible ones. Reducing sugars were found only in the infected plants, none being present in the susceptible or the resistant ones. There was a greater difference in ammonia-nitrogen in these samples than in the other two series of samples. The sample from infected plants gave no test for ammonia-nitrogen. As in the two previous samplings, the ratio of total sugar to nitrate-nitrogen was low for susceptible, intermediate for infected and high for recovered plants.

A short time after the final sample was taken from the group of recovered plants, symptoms developed upon them which closely resembled those of field infection. This was interpreted as indicating that the resistance acquired as a result of recovery from the initial attack of the parasite had been lost and that the plants had regained susceptibility. If this be true, a comparison of the chemi-

Table 3 - Results of chemical analysis of susceptible, infected and recovered plants. Samples taken ten days after those recorded in Table 2 and at 5:00 P. M.

Condition of plants when sampled	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	mgm/gm total sugar	Ratio total sugar/NO_3-N
Recovered	3.2	1.5	0.0	10.7	7.1
Infected	0.0	3.5	5.1	9.1	2.6
Susceptible	1.9	3.3	0.0	7.3	2.2

Table 4 - A summary of the chemical analysis of recovered plants recorded in Tables 1, 2, and 3.

Condition of Plants when sampled	mgm/gm NO_3-N	mgm/gm total sugar	Ratio total sugar/NO_3-N
Recovered plant			
Recently recovered (5 P.M)	0.25	6.72	26.9
Two days later (5 A.M.)	0.27	3.32	12.5
Twelve days later (5 P.M.)	1.5	10.7	7.1

cal content of the three samples taken at intervals from this lot of plants (and recorded in Tables 1, 2 and 3) should show whether or not there is a change in the nitrate-nitrogen and total sugar content of the leaves, as the plants lose the resistance acquired by recovery from downy mildew. Table 4 shows such a comparison. These data show that nitrate-nitrogen was low at the first sampling, practically the same at the second sampling (two days later) and highest at the third sampling (12 days later). At the third sampling total sugar was considerably higher than at the first sampling 12 days earlier. The ratio of total sugar to nitrate-nitrogen however was highest at the first sampling when the plants had recently recovered and were presumably most resistant, intermediate at the second sampling and lowest at the third sampling when the plants were presumably again approaching a condition of susceptibility.

Data indicating a similar trend were obtained from analysis of another series of plants. A number of very young plants in the greenhouse were selected which had recovered from the disease. Some of the leaves on these plants showed no signs of injury while other leaves showed injury in the form of small necrotic flecks. Samples of both types of leaves were collected for analysis. Six days later most of the plants in this bed had become reinfected and another sample of leaves was collected at that time. These samples were analyzed and the results are shown in Table 5. No significant difference was found in the nitrate-nitrogen content of samples from injured and uninjured leaves taken from the recovered plants, but the nitrate-nitrogen content of infected leaves was considerably higher than that from the

Table 5 - Results of chemical analysis of sample of injured and uninjured leaves from recovered plants, and of sample of leaves of same lot of plants after regaining susceptibility.

Sample	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	mgm/gm total sugar	Ratio total sugar/NO_3-N
Injured leaves from recovered plants	3.2	0.30	11.9	29.2	97.3
Uninjured leaves from recovered plants of the same lot	2.8	0.31	10.9	29.2	94.1
Leaves from plants of the same lot after becoming re-infected	3.2	0.53	12.8	28.6	53.9

recovered ones. The total sugar content of the two samples from recovered leaves was the same while that of the infected leaves was somewhat lower. A comparison of the total sugar to nitrate-nitrogen ratio showed no significant difference between the two lots of recovered leaves, but the ratio for the infected leaves was much lower.

Analysis of Leaf Tissue from Tobacco Plants Raised in Crocks of Quartz Sand Irrigated with Nutrient Solutions

A series of plants were raised in crocks of quartz sand in the tank constructed in the greenhouse for that purpose and described above. The series consisted of 12 crocks divided into six groups of two crocks each. The crocks of sand were seeded to Yellow Mammoth tobacco and kept moist with distilled water until the seed had germinated and the seedlings reached the two-leaf stage after which they were irrigated with nutrient solutions. Groups 1, 4, 5 and 6 received a complete nutrient solution while in Group 2 the potassium content of the solution was reduced from 410 p.p.m. to 224 p.p.m. and in Group 3 it was reduced to 37 p.p.m. The light reaching plants of Groups 4, 5 and 6 was varied as follows: Group 4, shaded with cloth on wire frames for 24 hours before and 24 hours after inoculation; Group 5, regular daylight supplemented by light from a 15 Watt fluorescent tube placed six inches above the plants for 24 hours before and 24 hours after inoculation; and Group 6, kept in darkness for 24 hours before and 24 hours after inoculation by inverting large flower pots over the crocks, plugging the holes in the bottoms with cotton and sealing the cracks where the pots met the crocks with black tape.

After the first 24 hours following inoculation the plants in Group 6 were given three days of daylight followed by three days of darkness and so on until the experiment was discontinued. A summary of the treatments applied to this series of plants is given in Table 6.

When the plants were about a month old samples of leaf tissue for analysis were collected from each group and the entire series immediately inoculated by spraying with a heavy suspension of spores in tap water. At the time of inoculation the plants of Group 3 were showing symptoms of potassium deficiency. Five days after inoculation signs of infection were evident on most of the plants and on the ninth day sporulation of the fungus occurred on plants in all of the groups except Group 6. Sporulation was very light on plants of Group 3. At this time samples of leaf tissue were again collected from plants of each group. The plants were kept under observation for about two weeks after the final samples were taken. Groups 1 and 2 were severely infected, but the percentage of recovery was high. Groups 4 and 5 were about as severely attacked as Groups 1 and 2, but very few of the plants in these groups survived. Plants in Group 3 were never very severely infected and the percentage of recovery was higher than in any other group. The disease was slow in developing on plants of Group 6, but it was eventually severe and practically all of them were destroyed.

The results of the analyses are recorded in Table 7. Ammonia-nitrogen both before and after infection was higher in samples from Group 3 than in other samples of the series. This may be explained by the fact that in reducing the potassium content of the nutrient

Table 6 - Nutrient solution and light treatment used on first series of plants grown in crocks of quartz sand.

Group No.	Nutrient Solution*	Light Treatment
1.	Complete	Normal daylight
2.	K reduced to 224 ppm	Normal daylight
3.	K reduced to 37 ppm	Normal daylight
4.	Complete	Shaded with cloth on wire frame 24 hrs. before and several days after inoculation.
5.	Complete	Daylight supplemented with 15 Watt cold florescent light 24 hrs. before and several days after inoculation.
6.	Complete	Kept in complete darkness 24 hrs. before and 24 hrs. after inoculation. Then 3 days of normal light followed by 3 days of darkness.

*The composition of the complete or standard nutrient solution used in this study is given above under materials and methods.

Table 7 - Results of chemical analysis of samples taken before and during infection of first series of plants grown in crocks of quartz sand.

Group No.	Samples taken previous to infection				Samples taken during infection			
	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	Ratio sugar/ NO_3-N	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	Ratio sugar/ NO_3-N
1.	2.1	3.4	32.7	9.6	3.3	3.4	6.64	1.9
2.	3.1	1.7	33.2	19.5	3.3	2.1	6.12	2.9
3.	7.5	1.5	27.4	18.2	6.8	1.2	49.2	40.9
4.	3.8	4.8	29.7	6.2	4.4	3.9	23.7	6.2
5.	3.0	2.8	28.4	10.2	1.7	2.2	11.4	5.2
6.	5.8	4.2	28.4	6.8	6.3	4.5	28.1	6.2

solution, potassium nitrate was eliminated and ammonium nitrate added to maintain the nitrogen level. An increase in ammonia-nitrogen under such conditions is in accord with results obtained by Sessions and Shive working with oats (24).

Samples of tissue from Group 6 also showed a higher concentration of ammonia-nitrogen than the other samples, but no explanation of this fact can be offered. Changes in the amount of nitrate-nitrogen in samples taken before and after infection were small. The sample from Group 1 collected before infection contained the same amount of nitrate-nitrogen as the sample collected after infection. There was a slight increase in the nitrate-nitrogen in samples from Group 2 and 6 and a slight decrease in samples from Groups 3, 4, and 5 collected after infection. Changes with respect to reducing sugars were quite pronounced. Samples taken from Groups 1 and 2 after infection showed a great reduction in the quantity of reducing sugars as compared to that in similar samples collected before inoculation. There was a similar reduction of reducing sugars in samples from Groups 4 and 5 although less pronounced, while in samples from Group 6 they remained practically unchanged. In Group 3, however, the sample collected after infection had taken place contained almost twice as much reducing sugar as the sample collected previous to inoculation. In the samples taken just before inoculation the ratio of reducing sugar to nitrate-nitrogen was highest for Group 2, almost as high for Group 3 and lowest for Group 1. After the plants had become infected the ratio had changed so that for Groups 1 and 2 it was very low and for Group 3 it was very high. This correlates with the severity of in-

fection, those with the low ratio being severely infected and the one with the high ratio being very mildly infected. In Group 4 there was no difference in the reducing sugar to nitrate-nitrogen ratio in samples taken before and after infection and practically no change in samples from Group 6. In Group 5, however, there was a reduction in the ratio following infection. In these three groups of plants there is no correlation between the reducing sugar to nitrate-nitrogen ratio and the severity of infection.

A second series of plants were raised in crocks of sand in the same manner as the first series just described. The treatment of this series was the same as that of the first series except that in Group 5 chlorine was increased from 48 p.p.m. to 146 p.p.m. and no artificial light was used. This series was seeded in December and the plants grew slowly so that they were two months old before the first samples were collected. Twenty-four hours before collecting the first samples Group 4 was shaded and Group 6 placed in darkness in the same manner as in the previous series. When the samples were taken, it was discovered that plants of Groups 1, 2, and 4 had already developed infection and sporulation was taking place upon them. No sporulation had occurred on plants of Groups 3, 5 and 6 but other symptoms indicated that they also were infected. In as much as the plants of this series had become accidentally infected before samples were taken, it was decided to collect no further samples from them, but to keep them under observation for a period of time and note any differences which might occur in the development of the disease on the different groups of plants.

The progress of the disease on plants of Groups 1, 2 and 4 was typical and signs of recovery were evident when the observations were discontinued. The disease was slow in developing on plants of Group 6, but finally became severe and most of the plants were killed. It also developed slowly on plants of Group 3 and sporulation was confined to small patches near the base of the infected leaves. The plants in this group showed signs of potassium deficiency. The leaves of plants of Group 5 were somewhat more rigid and darker green in color than leaves of the check plants (Group 1). The disease was very slow in developing on these plants and when observations were discontinued it had not become severe.

The samples of leaf tissue collected from this series of plants were analyzed and the results are recorded in Table 8. As in the previous series, the amount of ammonia-nitrogen was higher in the sample from plants of Group 3 than in samples from any of the other groups. There was practically no difference in ammonia-nitrogen in samples from the other five groups. Nitrate-nitrogen in samples from the first four groups was about the same, but only about 3/5 as much was found in the sample from Group 5. The amount of reducing sugars for Group 3 was very high, intermediate for Groups 2 and 4 and quite low for Group 1. No reducing sugars were found in the sample from Group 5 but a small amount of non-reducing sugar was present in the hydrolyzed sample. The ratio of reducing sugar to nitrate-nitrogen was highest in plants of Group 3 and these plants were the least severely attacked by the fungus. In both Group 1 and Group 2 the disease was severe. In

Table 8 - Chemical analysis of second series of plants grown in crocks of quartz sand.

Group No.	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	Ratio reducing sugar/NO_3-N	mgm/gm total sugar	Ratio total sugar/NO_3-N
1.	2.5	2.3	2.63	1.14	9.40	4.13
2.	2.8	2.4	26.0	10.8	34.9	14.7
3.	4.1	2.6	50.5	19.4	62.5	24.3
4.	2.8	2.6	10.9	4.2	17.6	6.81
5.	3.0	1.5	0.0	-	4.34	2.99
6.	2.5	-	-	-	-	-

Group 2 the reducing sugar to nitrate-nitrogen ratio was intermediate, while in Group 1 it was very low.

Analysis of Leaf Tissue from Tobacco Plants Grown in a Sand-Peat Mixture to Which Mixed Fertilizers Were Added

As was pointed out previously it has been reported (13) that tobacco seedlings supplied with high amounts of nitrogen or low amounts of potassium are more resistant to an attack of downy mildew than plants receiving low nitrogen or high potassium. Since certain experiments described above seem to verify those results it was decided to test the effect of such treatment upon the ammonia-nitrogen, nitrate nitrogen, and sugar content of tobacco seedlings. For this purpose tobacco plants were grown in two-gallon earthenware crocks of sand-peat mixture. Before the crocks were seeded, 25 grams of a mixed fertilizer were added to each crock as indicated in Table 9.

When the plants growing in these crocks were about one month old, they were exposed to natural inoculation with P. tabacina and developed downy mildew. As soon as plants in all of the crocks showed symptoms of infection, samples of leaf tissue were collected for analysis. At this time only plants in crocks 7 and 8 (3-8-3 & Vit.) and 1 and 2 (5-8-0) were sporulating. Subsequently sporulation occurred on plants in all of the crocks but the disease never became severe. No differences could be observed in the severity of the disease or in recovery as a result of the various fertilizer treatments.

Results of the analysis of these samples appear in Table 9. The results did not show any correlation between the fertilizer treat-

ment and the amount of reducing sugars or total sugars in the samples. The ratio of total sugar to nitrate-nitrogen varied from 2.55 to 8.66 within this series of samples, but was not correlated with fertilizer treatment or with the severity of the disease.

The results of this experiment are not consistent with those reported by Henderson (13) or with results obtained from the sand culture studies in which the nutrients were supplied as c. p. chemicals. An analysis of the peat used in preparing the medium on which the plants were grown was not available and it is possible that it carried certain nutrients that may have influenced the results.

Table 9 - Results of chemical analysis of tobacco plants grown in crocks of sand-peat mixture.

Crock No.	Fertilizer applied*	mgm/gm NH_3-N	mgm/gm NO_3-N	mgm/gm reducing sugar	mgm/gm total sugar	Ratio total sugar/NO_3-N
1 & 2	5-8-0	2.6	2.3	3.82	5.88	2.55
3 & 4	5-8-0**	3.8	1.8	8.57	15.6	8.66
5 & 6	3-8-3	2.3	1.9	6.12	10.9	5.74
7 & 8	3-8-3**	2.5	2.6	5.88	10.6	4.08
9 & 10	2-8-5	2.2	1.1	2.84	2.84	2.76
11 & 12	2-8-5**	2.3	1.6	5.26	5.26	3.28

*The fertilizer mixture indicated was applied at the rate of 25 grams per crock.

**One-fourth of the nitrogen was obtained from "vitamin meal," a proprietary product which is supposed to carry certain vitamins beneficial for plant growth.

IMMEDIATE EFFECT OF CERTAIN NUTRIENT SALTS AND OF SUGAR ON SPORULATION OF P. TABACINA ON INFECTED TOBACCO PLANTS

From the analysis of leaf tissue from plants grown in sand cultures and in soil it appeared that: (1) Plants grown under conditions of very reduced potassium were less severely affected by downy mildew than plants receiving a greater amount of potassium; (2) Plants which had recovered from the disease or plants which were not severely affected by it contained a greater amount of sugar per unit of nitrate-nitrogen than did susceptible plants or severely diseased plants.

If reduced potassium decreased the severity of disease, it seemed possible that increased potassium might stimulate the development of the fungus. It was thought that if tobacco plants were placed in a flask containing a solution of a potassium salt, the plant would absorb enough of the potassium to affect the growth of the fungus. The same method could be used to test the effect of nitrate or sugar or other material in solution. The effect of such treatment could be detected by comparing the abundance of sporulation on the treated plants with that on check plants in flasks of distilled water.

Seven experiments were performed to test the effect of various solutions upon sporulation of the fungus. In brief the procedure followed in these tests was as follows: Plants of uniform size were pulled from a plant bed; the roots cut off under water (except in Experiment 3); then divided into groups of three or five plants and each group placed in flasks so that the base of the stem was immersed in the solution to be tested. The flasks containing the plants were then placed in an environment suitable for sporulation of the fungus. The plants were

examined at intervals and notes taken on the abundance of sporulation. Except in Experiment 1, infected plants from diseased plant beds were used. In the case where healthy plants were used, they were inoculated immediately after being placed in the flasks of solutions. Details of each experiment together with the results are given in Tables 10 to 16.

The data show that in every case sporulation was more abundant on plants in potassium nitrate solution than on the check plants or on plants in any of the other solutions tested. Plants in potassium sulfate and potassium phosphate solutions showed much less sporulation than those in potassium nitrate solution and less than those in distilled water except in one instance when potassium sulfate solution was used. In all except one case sporulation was lighter on plants in calcium nitrate solution and in calcium chloride solution than on the checks. In Experiment 3 in which plants without their roots removed were used, sporulation was more abundant on plants in the solutions of calcium salts than on the check plants. Sporulation on plants in sodium nitrate solution was as light or lighter than that on the check plants. Magnesium nitrate and ammonium nitrate were used in only two cases. No sporulation occurred on plants in magnesium nitrate solution, but on plants with roots removed in ammonium nitrate solution sporulation was more abundant than on the check. In all but one case sporulation was less aboundant on plants in 2% and 3% sucrose solutions than on the check, but in two out of three cases the opposite is true for plants in a 1.5% solution of sucrose. On plants in solutions containing both sugar and potassium nitrate sporulation was less abundant than when potassium nitrate solution alone was used. Sporulation was more abundant on

plants in solutions containing both sugar and calcium nitrate than on plants in calcium nitrate solution alone.

Table 10 - Experiment 1 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used	Sporulation:* after 5 days
Nicotinic Acid ½% sol.	L
Asparagine ½% sol.	Plants killed
$Ca(NO_3)_2$ (N = 222.4 p.p.m.)	O
KNO_3 (N = 160.83 p.p.m.)	VH
Sucrose 3% sol.	M
Dist. water (check)	H

*The following symbols were used in recording the abundance of sporulation: O - no sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

Note: Five plants with roots removed were placed in each solution. All plants were kept under greenhouse conditions.

Table 11 - Experiment 2 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used	Sporulation:* after 36 hrs.	Sporulation:* after 60 hrs.
$Ca(NO_3)_2$ (N = 222.4 p.p.m.)	0	0
KNO_3 (N = 160.83 p.p.m.)	H	VH
Sucrose 3% sol.	0	0
Dist. water (check)	L	M-H

*The following symbols were used in recording the abundance of sporulation: 0 - no sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

Note: Five infected plants with roots removed were placed in each solution. All plants were kept under greenhouse conditions.

Table 12 - Experiment 3 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used		Sporulation:* after 14 hrs.
$Ca(NO_3)_2$	(N = 166 ppm) (Ca = 183.75 ppm)	VL
KNO_3	(N = 160 ppm) (K = 372 ppm)	L
NH_4NO_3	(N = 161 ppm)	0
$Mg(NO_3)_2$	(N = 159 ppm)	0
$NaNO_3$	(N = 160 ppm)	T
K_2SO_4	(K = 372 ppm)	0
$CaCl_2$	(Ca = 183 ppm)	L-M
Sucrose 1½% & $Ca(NO_3)_2$	(N = 166 ppm)	T
Sucrose 1½% sol.		0
Dextrose 1% sol.		VL
Dist. water (check)		T
Tap water (check)		T

*The following symbols were used in recording the abundance of sporulation: 0 - No sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

Note: Three infected plants with roots intact were placed in each solution. All plants were kept in a refrigerator at approximately 65° F.

Table 13 - Experiment 4 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used		Sporulation:* after 14 hrs.	after 38 hrs.	after 62 hrs.
$Ca(NO_3)_2$	(N = 166 ppm) (Ca = 183.75 ppm)	0	0	0
KNO_3	(N = 160 ppm) (K = 372 ppm)	M-H	M-H	M
NH_4NO_3	(N = 161 ppm)	L-M	L-M	L
$Mg(NO_3)_2$	(N = 159 ppm)	0	0	0
$NaNO_3$	(N = 160 ppm)	0	0	0
K_2SO_4	(K = 372 ppm)	0	0	0
$CaCl_2$	(Ca = 183 ppm)	0	0	T
Sucrose 1½% & $Ca(NO_3)_2$	(N = 166 ppm)	VL	0	T
Sucrose 1½% sol.		0	0	T
Dextrose 1% sol.		0	0	T
Dist. water (check)		0	0	0
Tap water (check)		0	0	0

*The following symbols were used in recording the abundance of sporulation; O - no sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

Note: Three infected plants with roots intact were placed in each solution. All plants were kept in a refrigerator at approximately 65° F. No further sporulation occurred after 62 hrs.

Table 14 - Experiment 5 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Treatment		Sporulation* after: 36 hrs.	60 hrs.	84 hrs.	108 hrs.	132 hrs.	156 hrs.
$Ca(NO_3)_2$	(N = 166 ppm) (Ca = 183.75 ppm)	0	0	0	0	T	T
$CaCl_2$	(Ca = 183 ppm)	0	0	0	0	0	0
KNO_3	(N = 160 ppm) (K = 372 ppm)	L-M	H	T	0	T	0
K_2SO_4	(K = 372 ppm)	0	L-M	T	0	0	0
$NaNO_3$	(N = 160 ppm)	0	0	0	0	0	0
Sucrose 1½% & $Ca(NO_3)_2$	(N = 166 ppm)	0	0	0	0	0	0
Sucrose 1½% & KNO_3	(N = 160 ppm)	0	L	0	0	0	0
Sucrose 1½%		0	VL	L-M	M-H	T	L
Dist. water (check)		0	T	0	0	0	0

*The following symbols were used in recording the abundance of sporulation: 0 - no sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

Note: Five infected plants with roots removed were placed in each solution. All plants kept outdoors until after the 60 hour observation then placed in a moist chamber for the duration of the experiment. At last observation all plants except those in solutions containing sucrose or sucrose and $Ca(NO_3)_2$ or KNO_3 showed signs of recovery.

Table 15 - Experiment 6 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used		Sporulation* after: 14 hrs.	38 hrs.	62 hrs.	86 hrs.	110 hrs.
$Ca(NO_3)_2$	(N = 166 ppm) (Ca = 183.75 ppm)	0	0	T	0	T
$CaCl_2$	(Ca = 183 ppm)	0	0	T**	0	T
KNO_3	(N = 160 ppm) (K = 372 ppm)	T	0	M-H	0	M-H
K_2SO_4	(K = 372 ppm)	T	T	T	0	0
KH_2PO_4	(K = 372 ppm)	0	0	0***	0	0
$NaNO_3$	(N = 160 ppm)	T	0	L	0	0
Sucrose 2% & KNO_3	(N = 160 ppm)	L	M-H	M	0	0
Sucrose 2% & KH_2PO_4	(K = 372 ppm)	0	0	0	0	0
Sucrose 2%		0	0	0	0	0
Dist. water (check)		0	L	0	0	0

*The following symbols were used in recording the abundance of sporulation: 0 - no sporulation; T - trace of sporulation; VL - very little sporulation; L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

**Leaves becoming brittle and erect.

***Leaves becoming water soaked.

Note: Five infected plants with roots removed were placed in each solution. Plants were left outside the first night; thereafter kept in a moist chamber.

Table 16 - Experiment 7 - Immediate effect of certain nutrient salts and sugar on sporulation of P. tabacina on infected tobacco plants.

Solution used	Sporulation* after: 14 hrs.	38 hrs.	62 hrs.	86 hrs.	110 hrs.
$Ca(NO_3)_2$ (N = 166 ppm) (Ca = 183.75 ppm)	O	T	T	O	O
$CaCl_2$ (Ca = 183 ppm)	O	O	O	T	O
KNO_3 (N = 160 ppm) (K = 372 ppm)	L	L	M	M-H	O
K_2SO_4 (K = 372 ppm)	T	T	O	O	O
KH_2PO_4 (K = 372 ppm)	O	O**	O	O	O***
$NaNO_3$ (N = 160 ppm)	O	T	L	T	O
Sucrose 2% & KNO_3 (N = 160 ppm)	O	L	L	O	O
Sucrose 2% & K_2SO_4 (K = 372 ppm)	O	T	T	O	O
Sucrose 2% & KH_2PO_4 (K = 372 ppm)	O	O	O	O	O
Sucrose 2%	O	O	O	O	T
Dist. water (check)	O	O	L	O	O

*The following symbols were used in recording the abundance of sporulation: O - no sporulation; T - trace of sporulation; VL - very little sporulation; - L - little sporulation; M - moderate sporulation; H - heavy sporulation; VH - very heavy sporulation.

**Leaves becoming water soaked.

***Leaves badly rotted.

Note: Five infected plants with roots removed were placed in each solution and kept in a moist chamber during the entire experiment.

APPLICATION OF CHLORIDE SOLUTION TO THE SOIL

Plants growing in crocks of sand irrigated with a nutrient solution high in chlorine were less severely affected by downy mildew than check plants in the same series. This suggested that the chlorine may have been responsible for the reduction in the severity of the attack. It was decided to test the effect of applying solutions of sodium chloride and calcium chloride to the soil in which tobacco seedlings were growing. Sodium chloride was applied to small seedlings in the greenhouse at the rate of 100 lb. per acre and calcium chloride at the rate of 138 lb. per acre. These salts were dissolved in water and the solutions sprinkled over the surface of the plant bed by means of a watering can. The bed was well watered after application of the solutions. The plants in the treated bed were showing first symptoms of infection when the treatments were made. The plants were examined at intervals following the treatment, but no noticeable effect on the severity of the disease or on the subsequent recovery of the plants could be detected.

DEFOLIATION EXPERIMENTS

It was postulated that the death of the lower leaves of infected plants might result in an accumulation of absorbed materials in the remaining leaves and that this higher concentration of salts in the leaves might be responsible for the resistance demonstrated by recovered plants. It seemed possible therefore that by clipping off the lower leaves of uninfected plants conditions within the remaining leaves might simulate those in the leaves of recovered plants.

To test this idea twelve six-inch pots were seeded with Yellow Mammoth tobacco seed and placed in a 80° F. incubator. When the plants were 35 days old and were about the size of a half-dollar all except the small terminal bud leaves were removed from plants in six of the pots. After four days all 12 pots were removed to the greenhouse and the plants inoculated with downy mildew by atomizing with a heavy suspension of spores. Five days later sporulation occurred on plants in all of the pots. No difference in the severity of the disease could be detected on the treated and untreated plants.

Feeling that possibly insufficient time had been allowed between removal of the leaves and inoculation to allow changes to take place in the composition of the remaining leaves, another test was set up using a series of eight pots containing plants 31 days old and of about the same size as those used in the test described above. The plants in four of these pots were inoculated by atomizing with a suspension of spores and sporulation occurred on them eight days later. The other four pots were removed to another building to avoid

Table 17 - Details and results of the third defoliation experiment.

Group No.	Record of Treatment and Results					
	Feb. 12	Feb. 13	Feb. 19	March 10	March 11	March 20
1	Removed to greenhouse	Inoculated	Heavy sporulation left in greenhouse	Recovery poor	Inoculated	Severe infection
2	Removed to greenhouse	Inoculated	Heavy sporulation placed in 80° incubator	Recovery very good	Removed to greenhouse Inoculated	Very severe infection
3	Removed to greenhouse	Not inoculated	No infection. Left in greenhouse	Slight accidental infection*	Inoculated	Very light infection
4	Removed to greenhouse	Leaves removed. Not inoculated	No infection. Left in greenhouse	New leaves appearing	Inoculated	Light infection
5	Left in 80° incubator	Leaves removed. Not inoculated	No infection. Left in 80° incubator	New leaves large and succulent	Removed to greenhouse. Inoculated	Very severe infection
6	Removed to greenhouse	Inoculated	Heavy sporulation*	Considerable P. D. B. injury	Inoculated	Infection

*Fumigated with P. D. B. to eradicate the fungus.

natural infection and there the larger leaves were removed, and as new leaves developed they also were removed. Recovery in the inoculated pots was poor but 10 days after the first sporulation appeared, the surviving plants were developing new small uninfected leaves. At this time the whole series of eight pots were placed together in the greenhouse and all the plants were inoculated by atomizing with a spore suspension. At the end of a week all plants showed symptoms of infection. Heavy sporulation occurred on the plants which had not been previously infected and most of the plants were killed in a few days, while the plants which had recovered from the previous attack no sporulation occurred although necrotic areas appeared on the leaves. None of these recovered plants was killed by the second attack of the disease.

Another series consisting of plants growing in 12 six-inch pots was used to test the effect of temperature as well as removal of leaves upon recovery. The pots had been seeded with Yellow Mammoth tobacco on January 6, and kept in a 80° incubator until the beginning of the experiment on February 2. At that time the pots were divided into six groups of two pots each and treated as shown in Table 17 with the results indicated.

Following the inoculation on March 11 plants of each of the groups developed symptoms of infection such as yellowing and curling of the leaves. By March 20 necrotic areas but no sporulation had developed on the plants in this series. The severity of the infection was judged by the degree of necrosis of the leaves.

Results of these experiments indicate that removal of leaves from

healthy tobacco plants does not bring about conditions within the plants which render them resistant to infection by Peronospora tabacina.

EXPERIMENTS WITH INDUCED NECROSIS

It has been observed both in the greenhouse and in the seed bed that after sporulation has progressed for several days, dry necrotic areas (Fig. 12) usually appear on leaves upon which conidiophores have been borne. After these necrotic areas appear, further sporulation on the plant soon ceases, even though microsopic examination of the killed and cleared leaf tissue shows apparently normal hyphae of the fungus to be present in some of the leaves. It seemed possible that some decomposition product developing in these necrotic areas upon being translocated to other parts of the plant might inhibit sporulation. In order to test this possibility necrotic areas were induced on the leaves of several plants by touching them with a hot metal rod. On others, spots were caused by placing drops of boiling water on the leaves, and on another series, by placing small drops of dilute sulphuric acid on the leaves. Three days later all of the plants were inoculated by atomizing with a heavy suspension of spores and at the end of the usual incubation period all of the plants developed typical symptoms of infection and sporulation was heavy.

In another test plants which had been inoculated three or four days previously but upon which spores had not yet appeared, were treated in a similar manner to that described above to produce necrotic areas. The progress of the development of infection proceeded normally and sporulation was abundant, indicating that the artificially induced necrosis did not bring about resistance.

EXPERIMENTS ON THE INOCULATION OF A SINGLE LEAF OF A TOBACCO PLANT

As described previously in this paper leaves developed during or soon after the recovery of a tobacco seedling from downy mildew are for a time at least immune to a second attack. This would indicate that if the resistance is due to a spedific substance produced, either by the plant or by the fungus that this specific substance must be translocatable. If such a translocatable substance exists it seems reasonable to suppose that the infection and recovery of a single leaf of a tobacco plant might confer immunity to a second attack of the fungus upon the entire plant. To test this possibility an experiment was set up in the greenhouse in the spring of 1939. For this experiment plants about three to four inches high, which were known never to have been infected with downy mildew were removed from a seed flat and transplanted into a bed in the greenhouse. A narrow slit was made in the metal cover of a half gallon fruit jar. One leaf of the plant was inserted through this slit and the space around the petiole plugged with cotton. A small hole had been drilled in each jar, near the bottom, and the jar was then screwed into the metal cover so that the jar lay on the bed beside the plant with the leaf inside it (Fig. 18). Eighteen plants were fitted with jars in this fashion. The leaf inside 12 of the jars was inoculated with downy mildew by inserting the tube of an atomizer through the hole in the base of the jar and spraying the leaf with a heavy suspension of spores. The leaves in the six remaining jars were sprayed with sterile water. The hole at the base of each jar was then plugged with cotton and all of the jars shaded for a few days by

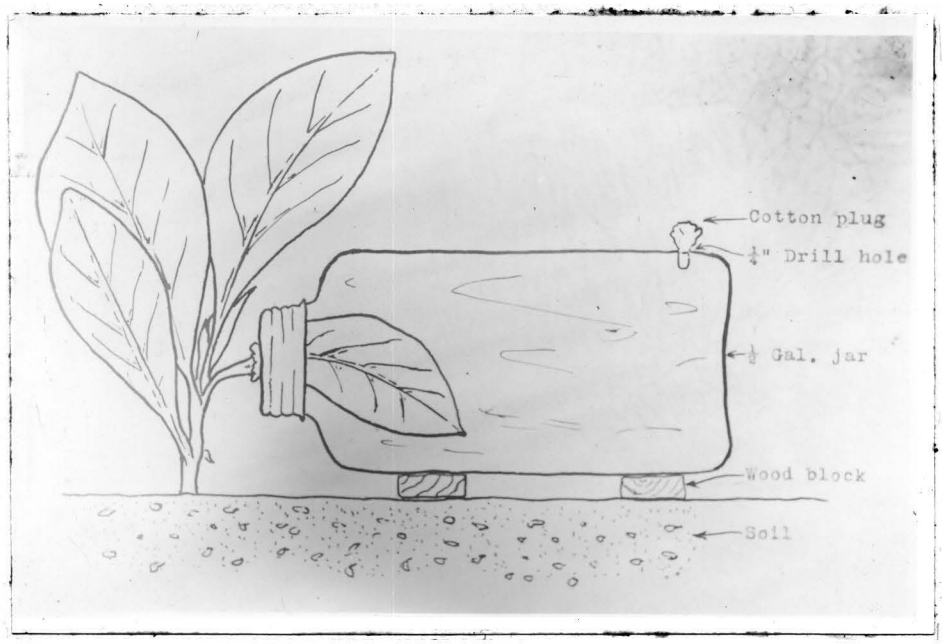

Figure 18. Sketch of apparatus used in attempts to inoculate a single leaf of a plant with downy mildew.

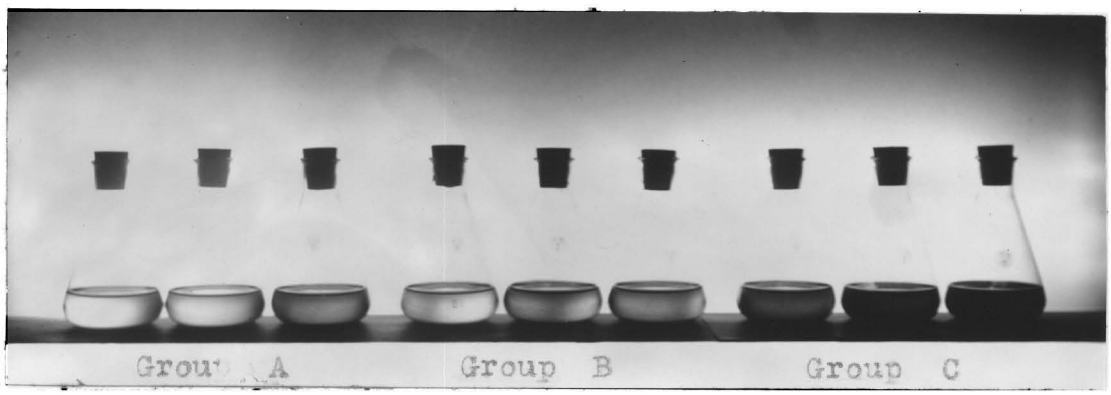

Figure 19. Nitrate-nitrogen tests on extracts from three series of samples from recovered, infected and susceptible tobacco plants. In each group the order of the flasks is: recovered, infected and susceptible. Increasing depth of color indicates increasing amounts of nitrate-nitrogen.
Group A - samples taken June 21, 5:00 P. M.
Group B - " " June 23, 5:00 A. M.
Group C - " " July 1, 5:00 P. M.

covering them with newspaper. High humidity was maintained within the jars by occasionally spraying the enclosed leaves with sterile water.

After three or four days the inoculated leaves began to curl downward on the margins and became somewhat lighter in color. After seven days necrotic areas developed on the leaves, but no sporulation was observed. The leaves in some of the jars died altogether. The six uninoculated leaves turned yellow but no curling of the margins occurred and no necrotic areas developed upon them.

At the end of two weeks all of the plants were inoculated by spraying with a suspension of spores. All 18 of the plants showed symptoms of infection after the usual incubation period but no sporulation took place on any of them. None of the plants was killed by the disease, but plants of the size used in this experiment are seldom killed by downy mildew.

The experiment was repeated with similar results, but as it was impossible to obtain sporulation on the leaves within the jars, the experiment was discontinued. The indication is that infection of a single leaf does not confer immunity from a second attack upon the rest of the plant

DISCUSSION

Analyses of leaves from tobacco plants which had recovered from downy mildew and as a result were resistant to a second attack showed that the ratio of total sugar to nitrate-nitrogen in such leaves was higher than in leaves collected at the same time from plants of the same age, and growing under the same conditions, but which had never had the disease. In leaves collected while the disease was still active this ratio was intermediate between that of the recovered and of the susceptible plants. Analyses further showed that as recovered plants regained susceptibility this ratio decreased, that is, it was higher in leaves collected soon after recovery than it was in leaves collected some time after the plants had recovered. In leaves from recovered plants that had regained susceptibility, as shown by inoculation, the ratio was again low. Also, the disease was more severe on plants supplied with a balance of nutrients that would produce in the leaves a low ratio of total sugar to nitrate-nitrogen than on plants supplied with a balance of nutrients that would produce a high ratio of total sugar to nitrate-nitrogen. And again, sporulation was less abundant on plants placed in 2% and 3% solutions of sucrose than on plants placed in distilled water. This information suggests that a low ratio of total sugar to nitrate-nitrogen is an indication of susceptibility, while a high ratio is an indication of resistance. If resistance and susceptibility are governed by the ratio of these two materials in the plant, then it would appear that the recovery of plants from an attack of downy mildew is due to the change in this ratio brought about as an effect of the

disease on the plant. The data available at present, however, are not sufficient to prove or disprove such an assumption.

It appears from the data available that a given ratio of total sugar to nitrate-nitrogen may not always indicate resistance. For example, in one test a ratio of 53.9 to 1 was found to be present in leaves of young susceptible plants, while in another group of older recovered (resistant) plants the ratio was only 7.1 to 1. The indication is that for a series of samples collected at the same time from comparable plants the relationship between this ratio and resistance holds true, but that a comparison cannot be made between plants of different ages or plants growing under different environmental conditions.

That some factor or factors distinct from or supplemental to the total sugar to nitrate-nitrogen ratio is involved in this problem is indicated by the fact that sporulation was more abundant on infected tobacco plants placed in flasks of potassium nitrate than it was on check plants in distilled water, while this was not the case for plants in calcium nitrate, magnesium nitrate or sodium nitrate solutions. The NO_3 ion is absorbed more rapidly from calcium nitrate than from potassium nitrate solutions (16). If only the total sugar nitrate-nitrogen ratio were involved sporulation should have been more abundant on the plants in the calcium nitrate solution.

The published reports on the effect of nutrients upon susceptibility of various plants to disease have indicated, in general, that increased nitrogen tends to increase susceptibility and increased potassium tends to decrease susceptibility. In this investigation,

however, plants raised in crocks of sand and irrigated with a nutrient solution low in potassium were less severely affected by the fungus than plants receiving a high concentration of potassium. This is in accord with results reported by Henderson (13). Analyses of samples from the low-potassium plants showed them to contain a high concentration of sugars and to have a higher ratio of sugar to nitrate-nitrogen than samples from plants receiving a normal supply of potassium. The accumulation of carbohydrates in potassium deficient plants is not uncommon (17). Day 1940 (10) reported that potassium deficient tobacco plants stored less starch than control plants. If the carbohydrates are not stored as starch, then it would be expected that there might be an accumulation of sugars in the plants.

Henderson (13) suggests that resistance of tobacco to downy mildew may be directly associated with a wide nitrogen-potassium ratio. In this investigation sporulation was more abundant on infected plants placed in flasks of potassium nitrate solution than on similar plants in distilled water, but was not more abundant on infected plants placed in solutions of potassium sulfate or potassium phosphate. Plant tissue will absorb practically twice as much potassium in the same length of time from the nitrate solution as from the phosphate or sulfate solution (16). This would indicate that a narrowing of the nitrogen-potassium ratio was responsible for the increased sporulation and is in accord with Henderson's suggestion.

SUMMARY AND CONCLUSIONS

This study is concerned with the resistance of tobacco seedlings to a second attack of downy mildew caused by Peronospora tabacina Adam.

Observations were made on the development and duration of resistance following recovery of tobacco seedlings from downy mildew. The duration of the period of resistance is variable and the observations indicate that plants in the greenhouse recovering during the summer remain resistant longer than do plants recovering from an initial attack during the winter.

Samples of leaf tissue from recovered (resistant), infected and previously uninfected (susceptible) plants grown in soil in the greenhouse were analyzed for ammonia-nitrogen, nitrate-nitrogen, reducing sugars and total sugars. Similar analyses were made of samples of leaf tissue from tobacco plants grown in crocks of quartz sand and irrigated with nutrient solutions and from plants grown in crocks of sand-peat mixture to which various mixed fertilizers were added. There was no correlation between the ammonia-nitrogen content of tobacco plants, as determined in these experiments, and the response of the tobacco seedling to an attack by P. tabacina. The volume of evidence obtained in this investigation is not sufficient to warrant the drawing of any definite conclusions concerning the relationship between nitrate-nitrogen and sugar content of the tobacco plant and its susceptibility to attack by P. tabacina, but there was a strong indication that such a relationship exists. Recovered plants contained

a higher ratio of total sugar to nitrate-nitrogen than did comparable plants which had never been infected. Furthermore the ratio of total sugar to nitrate-nitrogen was lower in plants infected for a second time than it was in plants from the same lot shortly after they had recovered from the initial attack.

It seems probable however that the changes in the nitrate-nitrogen and sugar content in recovered tobacco leaves are an indication of recovery and are not responsible for the resistance possessed by such plants.

A series of experiments were performed to test the immediate effect of several nutrient salts and of sugar upon the sporulation of the fungus on the leaves of diseased tobacco plants. There was a very definite response in sporulation to some of these treatments, especially to potassium nitrate, calcium nitrate, and sucrose, but it is not known whether the response of sporulation of the fungus to such treatment is related to the type of resistance possessed by recovered plants.

Sodium chloride and calcium chloride were dissolved in water and applied to the soil in which young tobacco plants were growing in the greenhouse. Although increased chlorine in one of the sand cultural experiments seemed to protect the plants somewhat against downy mildew, application of solutions of these salts to the soil at the rates used (100 lbs. and 138 lbs. per acre respectively) produced no noticable effect on the severity of the disease or subsequent recovery of the plants.

The leaves of recovered (resistant) tobacco plants are usually reduced in number, the lower ones having been killed by the initial

attack of the fungus. Removal of the leaves from healthy tobacco plants in imitation of this condition did not produce resistance to an attack by P. tabacina.

The artificial production of necrotic areas on healthy tobacco leaves in simulation of those usually following sporulation of P. tabacina on diseased leaves had no effect either on the susceptibility of the plants to infection or upon sporulation of the fungus on leaves already infected at the time the necrotic areas were induced.

Results of attempts to confer immunity from downy mildew upon an entire plant by infection of a single leaf of the plant were inconclusive, since under the conditions of the experiment it was impossible to be sure that the single leaf was infected. However, the indication is that infection of a single leaf will not render the entire plant immune from a second attack by the fungus.

LIST OF TABLES

Table Page

LIST OF FIGURES

LITERATURE CITED

1. Adam, D. B.

Blue Mold of Tobacco. Jour. Dept. of Agr.,Victoria, Australia. 1933.

2. Anderson, P. J.

Downy Mildew of Tobacco. Conn. Agr. Exp. Sta., Bul. 405. 1937.

3. _____

Diseases and Decays of Connecticut Tobacco. Conn. Agr. Exp. Sta. Bul. 432. 1940.

4. Angell, H. R. and Hill, A. V.

Downy Mildew (Blue Mold) of Tobacco in Australia. Counc. Sci. Ind. Res. Bul. 65. 1932.

5. Armstrong, G. M. and Sumner, C. B.

Investigations on Downy Mildew of Tobacco. S. C. Agr. Exp. Sta. Bul. 303. 1935.

6. Blattny, C.

Beitrag zur Frage der Dungung des Hopfens als Mittel gegen Hopfenperonospora (Pseudoperonospora humuli). Ernähr. Pflanze 24:140-142, illus. 1912. (Original reference not examined.)

7. Clayton, E. E. and Gaines, J. G.

Downy Mildew of Tobacco. U. S. Dept. of Agr. Circ. 263. 1933.

8. _____

Blue Mold (Downy Mildew) Disease of Tobacco. U. S. Dept. of Agr. Farmers' Bul. 1799. 1938.

9. Chupp, C.

The Effects of Potash and Phosphorus on Tip Burn and Mildew of Cabbage. Phytopath. 20:307-318. 1930.

10. Day, Dorothy.

Starch Formation in Tobacco Plants Deficient in Potassium. Plant Phys. 15:367-375. 1940.

11. Dickson, B. T.

Downy Mildew (Blue Mold) of Tobacco. Australian Tobacco Investigation. Pamphlet 1. 1932.

12. Doran, W. L.

Downy Mildew of Cucumber. Mass. Agr. Exp. Sta. Bul. 283. 1932.

13. Henderson, R. G.

Studies of Tobacco Downy Mildew in Virginia. Va. Agr. Exp. Sta. Technical Bul. 62. 1937.

14. Kincaid, R. R. and Tisdale, W. B.

Downy Mildew (Blue Mold) of Tobacco. Univ. of Florida Agr. Exp. Sta. Bul. 330. 1939.

15. Loomis, W. E. and Shull, C. A.

Methods in Plant Physiology. Mcgraw Hill. 1937.

16. Miller, E. C.

Plant Physiology. Mcgraw Hill Book Company. 1931. pp 174-307.

17. Nightingale, G. T., Schermerhorm, L. G. and Robbins, W. R.

Some Effects of Potassium Deficiency on the Histological Structure and Nitrogenous and Carbohydrate Constituents of Plants, N. J. Agr. Exp. Sta. Bul. 499. 1930.

18. Phillips, T. G., Smith, T. O. and Hepler, J. R.

Some Effects of Potassium and Nitrogen on the Composition of the Tomato Plant. New Hampshire Agr. Exp. Sta. Tech. Bul. 73. 1939.

19. N. C. State College of Agr. and Eng. of Univ. of N. C.

Blue Mold of Tobacco and Its Control. Ext. Circ. 229. 1938.

20. Orcutt, F. S. and Wilson, P. W.

The Effect of Nitrate-Nitrogen on the Carbohydrate Metabolism of Inoculated Soybeans. Soil Sci. Vol. 39:289-296. 1935.

21. Pinckard, J. A. and Shaw, Luther.

Downy Mildew Infection of Flue-cured Tobacco in the Field. Phytopath. 29:79-83. 1939.

22. Quanjer, H. M.

De irvoloed van kaligebrek op de vat baarkeid bloemkool voor Peronospora parasitica. Tijdschr. Plantenziekten. 34:254-256. 1928. (Original reference not examined.)

23. Stiles, H. R., Peterson, W. H. and Fred, C. B.
A Rapid Method for the Determination of Sugar in Bacterial Cultures. Jour. Bact. 12:427-439. 1926.

24. Sessions, A. C. and Shive, J. W.
The Effect of Culture Solution on Growth and Nitrogen Fractions of Oat Plants at Various Stages of Growth.
Soil Sci., 35:355-374. 1933.

25. Va. Agr. Exp. Sta.
Blue Mold (Downy Mildew) of Tobacco and Its Control. Va. Agr. Exp. Sta. Bul. 318. 1938.

26. Wingard, S. A. and Henderson, R. G.
Control of Tobacco Blue Mold (Downy Mildew). Va. Agr. Exp. Sta. Bul. 313. pp1-10. 1937.

27. Wolf, F. A.
Tobacco Diseases and Decay. Duke Univ. Press, Durham, N. C. pp 280-300. 1935.

28. _____
Status of Investigations of Tobacco Downy Mildew. Phytopath. 29:194-199. 1939.

29. _____, Dixon, L. F., McLean, R. A., and Darkis, F. R.
Downy Mildew of Tobacco. Phytopath. 24:337-363. 1934.

30. _____, McLean R. A., and Dixon, L. F.
Further Studies on Downy Mildew of Tobacco. Phytopath. 26:760-777. 1936.