# FIELD STUDIES ON THE RINGSPOT DISEASE OF BURLEY TOBACCO IN WASHINGTON COUNTY, VIRGINIA

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# FIELD STUDIES ON THE RINGSPOT DISEASE OF BURLEY TOBACCO IN WASHINGTON COUNTY, VIRGINIA,

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## INTRODUCTION.

Tobacco ringspot is a virus disease which is becoming more and more prevalent in the State every year. In 1917 when the disease was first observed by Fromme and Wingard ('22) it was considered of minor importance. Infection at that time was observed only on an occasional plant in a field and the crop loss was negligible. However, the percentage of infection has increased from year to year, and now it is not uncommon to find fields in which 90 per cent of the plants are infected. The ringspot virus has been found by Wingard ('28) to be infectious to a large number of plants other than tobacco. This, together with the fact that the infection on tobacco is becoming more and more severe every year, clearly demonstrates that ringspot is a disease of major economic importance.

My interest was first attracted to the ringspot disease in 1927 when serving as temporary Extension Plant Pathologist of the V. P. I. Agricultural Extension Division. I had a splendid opportunity to study this disease under field conditions in ten counties of the State during the summer of 1927, and have made a special study of it on Burley tobacco in Washington County during the season of 1928 and 1929. My studies in 1927 were limited to field and plant bed inspections, location of infection centers in the field with reference to old garden and building sites, and attempts to find weed hosts and other carriers of infection. In 1928 and 1929 I continued the work of 1927, and also made a special study of the transmission of infection by insects, the rate of spread of infection in the field, the source of field infection, weed hosts, the percentage of infection, crop loss, and methods of control.

## REVIEW OF LITERATURE.

Selby ('04) referred to a figure, plate 1, p. 89, which in all probability is ringspot; however, he labeled it tobacco mosaic. There is no description in Selby's bulletin which would indicate that a distinct ringspot type of mosaic had been recognized. The disease was first described and illustrated as ringspot by Fromme and Wingard ('22). They state that ringspot was observed very commonly in Virginia tobacco fields during the period of 1917. They were not successful, however, in isolating a pathogenic organism from the ringspot material that they were studying at this time.

Johnson ('24) published figures of a leaf spot which he regarded as non-parasitic, which is very similar in appearance to ringspot. He attributed this trouble to the effect of unfavorable soil and weather conditions. Wingard and Godkin ('24) discussed ringspot in detail. It was at that time considered a non-parasitic disease affecting only a small percentage of the plants and it was classed as a form of mosaic, or the work of thrips. Nutritional disturbance was also advanced as a probable cause of ringspot.

According to Fromme, Wingard, and Priode ('27) ringspot was found in eleven counties in Virginia. As a rule only a small percentage of plants were affected, but they reported a high incidence of infection in rare cases. They proved that the disease was infectious, but were unable to isolate any pathogenic organism from the infected tobacco material. They concluded, therefore, that ringspot should be classed with the virus diseases. The natural modes of transfer had not been determined at that time. Wingard ('28) reported that the ringspot virus had a very wide host range, although it is very specific in its infective properties, since many plants that were inoculated failed to develop the disease. He was the first to find natural ringspot infection on any plant other than tobacco. Repeated inoculations on tobacco with expressed juice from sweet clover produced typical ringspot. He further stated that the disease must overwinter in either biennial or perennial plants or in the embryo of infected seed. He found too, that plants seem to develop an immunity to the ringspot disease after a certain length of time, but the expressed juice from such plants continued to be highly infectious. Priode ('28) produced ringspot infection on beet (Beta Vulgaris L.),pokeweed (Phytolacca decandra L.), petunia (Petunia hybrida Vilm), and New Zealand Spinach (Tetragonia expansa Murr), He found that virulence was lost rapidly when the virus was stored at a temperature above 5° C., and that dried material is not infectious. His experiments in filtering showed that the virus would not pass a Berkfeldt filter of grade "N".

Johnson ('29) transmitted three types of mosaic to tobacco from apparently healthy potatoes. One of these types he classes as ringspot. This type did not produce symptoms on potatoes. He concluded that potatoes are either true carriers of viruses or that potato protoplasm is the infective agency. Smith ('29) transmitted ringspot to tobacco from mosaic affected potatoes by needle prick and by aphis (Myzus persicae). When ringspot was inoculated back to the potato it produced the original symptoms of mosaic. He found that ringspot could be spread from tobacco to tobacco by needle or aphis, but that when transmitted by aphis the symptoms were different. Smith was able to filter the ringspot virus and infect plants with the resulting filtrate. However, he did not get any symptoms when he inoculated into tobacco the expressed juice from known healthy potatoes.

## MATERIALS AND METHODS.

Throughout this study the disease was observed under actual field conditions. Actual plant beds (Fig. 5), and fields, as prepared by farmers in the eleven counties where this work was conducted, were used.

In the seed bed studies a steam sterilizer was used as illustrated in Fig. 3. The soil was first well pulverized and then thoroughly sterilized. A steam tractor furnished the necessary heat. Suspicious plants growing in or around the plant beds and fields were sent to Dr. S. A. Wingard of the Department of Botany and Plant Pathology of the Virginia Agricultural Experiment Station for inoculation tests with healthy tobacco, to determine whether or not they were harboring the ringspot virus. Tobacco fields selected, in many instances, contained old garden and building sites.

The insect studies were conducted in my home vegetable garden. In order to get ringspot early in the season, inoculum was sent from the Department of Botany and Plant Pathology of the Virginia Experiment Station. Eight healthy tobacco plants were inoculated with this material and placed in an insect proof cage. All subsequent insect work was conducted from this cage. The insect proof cages were of two types (Figures 1 and 2). The cages in Fig. 1 were very simply made and used over field plants. These cages were three and one-half feet high, two feet wide and two feet long. The sides and top were carefully covered with muslin. The legs were driven securely into the soil with dirt banked well against the sides, forming an insect proof bottom to the cage. The second type of cage was made for use on potted plants. A muslin bag slipped over the pot was held erect by a small stick driven into the center of the pot and tied securely at the sides.

All insects were secured from my garden and placed on diseased plants in cages and left for different periods of time, then transferred to caged healthy plants. Tobacco flea beetles (*Epitrix parvula*) were secured from tobacco plants. Potato or cucumber flea beetles (*Epitrix cucumeris*) and leaf hoppers (*Empoasca fabea*) were secured from potatoes. Aphis (*Macrosiphum solanifolii*) were secured from potatoes and clover. The tobacco worm (*Phlegethontius quinquem oculata*) and the lightning bug (*Photinus scintillans*) were found on tobacco plants. The insect catcher described by Kunkel ('26) was used. It consisted of a piece of rubber tubing about twenty inches long fitted over one end of a small piece of glass tubing. A piece of cheese cloth was placed over the other end. A smaller tube which had been drawn out, was fitted snugly within the larger tube. The insect was quickly caught by placing the small glass tube close to the insect and sucking air quickly through the tube.

In determining the rate of spread, eight fields were used. One plant in a marked area in each field was inoculated with the ringspot virus. The new plants affected were observed at ten-day intervals.

The extent of injury to affected plants was determined by measuring the size of leaves on diseased and healthy plants and by counting the number of leaves, at topping time. The quality of the leaf was also taken in to consideration. Numerous inspections and field counts of affected plants gave the percentage of plants diseased. The total loss to the County was estimated by multiplying the average extent of injury to the individual plant by the total percentage of affected plants. Judy's Pride, Kelleys and Lockwood varieties of tobacco were used in all experiments conducted in Washington County.

In reviewing the literature in connection with the ringspot disease, all bulletins, references, etc., on all types of virus diseases were carefully read. Special study of the papers dealing closely with my problem was made and a review written of each. Twenty-five such papers were reviewed.

## EXPERIMENTAL RESULTS.

Since ringspot is becoming of such great economic importance and so little is known regarding it, experiments were undertaken to determine, if possible, the source of infection, means of dissemination of infection, the rate of spread of infection in the field, the extent of injury to affected plants, the percentage of plants affected, the loss to the County, and the method of control. However, these experiments were not undertaken until a thorough study had been made of the literature of virus diseases of plants in general and ringspot in particular. The results of my experiments are given below.

## SOURCE OF INFECTION.

Ringspot infection appears every spring in a high percentage of tobacco fields and yet the sources of infection have not been definitely determined. There are three possible sources of infection: (1) seed, (2) insect carriers, and (3) weed hosts.

SEED: In studying the possibility of ringspot infection being transmitted by tobacco seed, twenty-five plant beds in Washington County were visited at short intervals until transplanting time. Not a single infected plant was found in any of these plant beds. An experiment was conducted on a large scale on one farm to determine if the plant bed soil could transmit the disease. Five hundred square yards of plant bed were steam sterilized to such an extent that all soil organisms should have been destroyed. The sterilizing apparatus is illustrated in Fig. 3. As a result the plant beds were very clean and free from weeds. The plants were very thrifty and vigorous as shown in Fig. 4. However, ringspot developed quite generally in the fields in which these plants were set. This seems to prove conclusively that the ringspot infection in these cases did not originate in the plant bed. Plant beds were also inspected in Appomattox, Nelson, Pittsylvania, Charlotte and Brunswick Counties without finding ringspot infection. However, three plant beds observed in Halifax County were found to have a scattered infection of the disease, but this was late in the season after all transplanting had been completed. Therefore it is safe to assume that the infection did not necessarily originate within the plant bed.

INSECT STUDIES: Because of the similarity between ringspot and certain insect-borne virus diseases, such as curley top of beet, Severin ('29) and Carsner ('24 and '26); and aster yellows, Kunkel ('26), it was thought advisable to study certain insects as possible carriers of the ringspot virus. Experiments were begun, therefore, to check up on insects that are commonly found feeding on tobacco plants. These experiments, however, were not restricted to insects that are common to tobacco. Tobacco flea beetle, (*Epitrix parvula*) attacks tobacco plants both in the plant bed and in the field, and for this reason was looked upon as a possible disseminator of ringspot infection. On June 11th, seventy-five flea beetles were collected from healthy tobacco plants and placed in cages on ringspot affected tobacco plants. On June 22nd, fifteen of these beetles were transferred to two healthy tobacco plants in an insect proof cage. On June 24th, thirty more of these beetles were removed and placed on healthy tobacco plants in insect proof cages. In this case fifteen beetles were placed on each of two plants in separate cages. The plants in these tests were inspected at regular intervals in order to check up on the condition of the beetles and also to determine whether or not infection had developed on the plants. On July 6th, the beetles were alive and thrifty but no ringspot infection had developed. On August 2nd, the plants were normal and free from ringspot.

On July 4th, fifteen flea beetles were transferred from ringspot infected tobacco plants in the field to a healthy tobacco plant in an insect proof cage. This plant was examined on August 24th, and found to be normal, no ringspot had developed. On July 5th, fifteen flea beetles which had fed on diseased tobacco plants for twenty-five days were transferred to a caged healthy tobacco plant. These insects came from the same cage as those in the first two tests. On August 24th no ringspot had developed. These experiments with the tobacco flea beetle seem to show that ringspot is not transmitted by tobacco flea beetles.

On June 11th, seventy-five potato flea beetles, (Epitrix cucumeris) were collected from potato plants and placed in an insect proof cage on ringspot infected tobacco plants. Upon examination, eight days later, all of these beetles were dead. This shows that tobacco is an unfavorable host for this insect.

On June 19th, fifty adult and nymph aphids (macrosiphum solanifolii) were placed on diseased tobacco plants in an insect proof cage. Seven days later all aphids were dead. Apparently tobacco is not a favorable host for aphids.

On June 11th, five tobacco worms (Phlegethontius quinquemoculata) were placed in a cage on an infected plant. On July 4th, these worms were transferred to a healthy caged plant. On August 24th this plant was normal and healthy, no ringspot had developed.

On June 24th, fifty leaf hoppers (*Empoasca fabea*) were placed on diseased tobacco plants. On July 4th, ten days later, all leaf hoppers were dead. On July 4th, fifteen leaf hoppers were transferred from potatoes in the field to a healthy tobacco plant in a cage. On August 2nd these leaf hoppers were also dead and no infection resulted. Leaf hoppers did not survive on tobacco, it being an unfavorable host for this insect.

One hundred and twenty-five tobacco plants were used in my garden as checks for the insect studies described above. On June 20th, ringspot infection was found on three of these check plants. On July 1st, five per cent of all the plants were affected, and on July 6th, twenty per cent showed ringspot. A great variety of weeds completely surrounded the field. Tobacco flea beetles and the common lightning bug were the only insects observed on the tobacco plants.

WEED HOSTS: Many perennial weeds have been found to be succeptible to the ringspot disease and many of them such as the horse nettle and pokeweed are common in and around tobacco fields. In order to determine if such plants were hosts to ringspot, suspicious specimens were sent to Dr. S. A. Wingard of the Department of Botany and Plant Pathology of the Virginia Agricultural Experiment Station to be used as inoculum on healthy tobacco plants to determine whether or not they were harboring the ringspot virus.

Sweet clover (Melilotus alba) on several occasions produced typical ringspot infection when inoculated on healthy tobacco plants. In 1928, Dr. S. A. Wingard of the Virginia Agricultural Experiment Station made successful inoculations from sweet clover found near the Blacksburg Experiment Station barns. In 1929 Mr. R. G. Henderson of the same station produced typical ringspot infection on tobacco from sweet clover received from Staunton, Virginia. He also obtained infection from sweet clover grown on the Experiment Station plats at Blacksburg. Affected sweet clover usually shows dwarfing and yellowing, very often the leaves curl slightly. On July 5th, I sent Dr. S. A. Wingard several suspicious weeds in which were included stick weed or yellow crown beard (Verbesina alternifolia). Healthy tobacco plants were inoculated with the expressed juice from this plant on July 6th. Nine days later, July 15th, very distinct ringspot infection was found on the inoculated plants. The stick weed was slightly yellowish and the upper leaves distorted and purple, rosette like. No unusual spotting on the leaves was noticeable. Stickweed has since been found by me to be a common carrier of the ringspot virus.

Other plants sent to Dr. Wingard and Mr. Henderson were: prickly lettuce (Lactuca viro sa); broad leaf plantain, (Plantago major), burdock (Arctium lappa), pokeweed (Phytolacca decandra), milkweed (Asclepas sp.), bittersweet (*Solanum dulcamara*), smart weed (*Polygonum sp.*), lambs quabter (*Chenepodium album*), broad leaf dock (*Rumex obstisfolia*). button weed (*Malva rotundifolia*), Jimson weed (*Datura stramonium*), red clover (*Trifolium pratense*), mamoth clover(*Trifolium medium*), alsike clover (*Trifolium hybridum*), and white clover (*Trifolium repens*).

These plants were sent in at different periods of the year, and some species several times. Suspicious plants, not only from Washington County but also from Halifax, Amherst, Nelson, Appamattox, Pittsylvania, Mecklenburg, Charlotte, Brunswick, Dinwiddie, and Montgomery Counties were used.

## RATE OF SPREAD IN THE FIELD.

After tobacco has been set in the field it has been noticed that in some instances, a very rapid spread of ringspot occurs. In order to study the rate of spread in the field, inoculations were made in seven fields and observations made of the rate of spread of infection, at ten-day intervals. The rate of spread in each field is shown in the graphs below.

Field 1

= Original plant that was inoculated June 10

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.

x Plant inoculated on June 10

There was no spread of infection in this field.

Field 2.

X = Original plant that was inoculated June 10.

O = Plant showing infection July 6.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.

X Plant inoculated June 10.

Arrow points to plant that showed infection on July 6. There was no further spread of infection in this field.

O

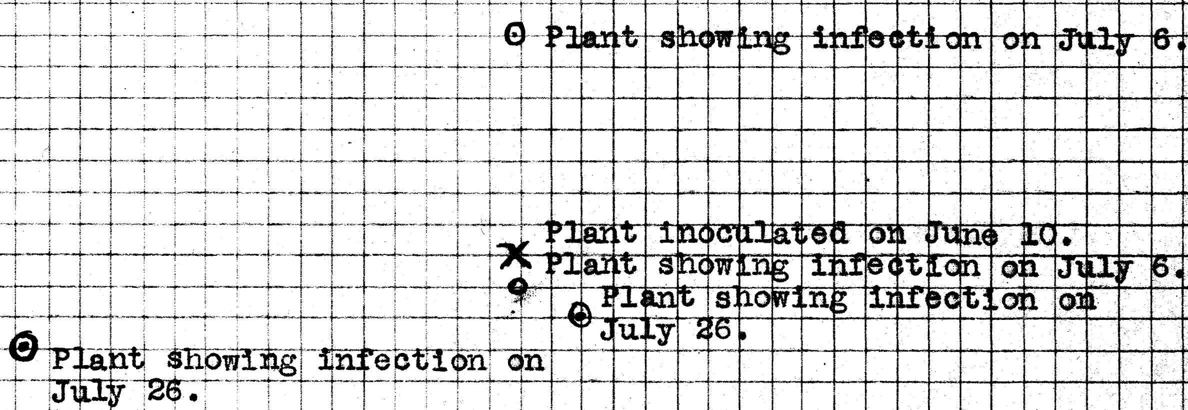
Field 3.

X = Original plant that was inoculated on June 10.

O = Plants showing infection on July 6.

⊙ Plants showing infection on July 26.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.




There was no further spread of infection in this field during the remainder of the season.

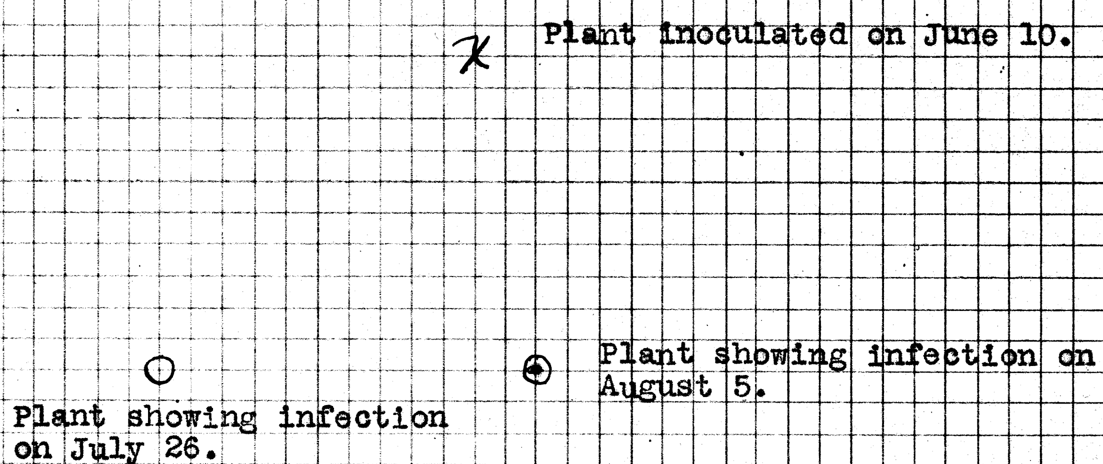
# Field 4.

X = Original plant that was inoculated on June 10.

O = Plants that were showing infection on July 26.

⊙ = Plants showing infection on August 5.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.




No further spread of ringspot was observed during the season.

Field 5.

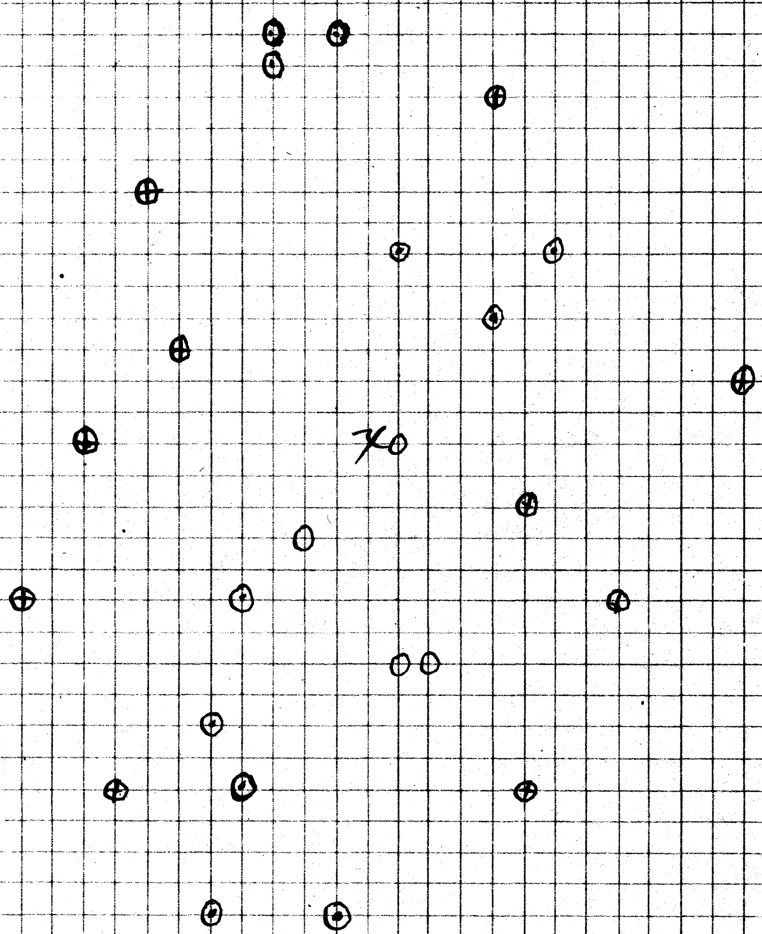
X = Original plant that was inoculated on June 10.

O = Plants that were showing infection on June 26.

⊙ = Plants that were showing infection on July 16.

⊕ = General infection showing on July 26.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.



At cutting time the ringspot symptoms had become masked to such an extent that only 15 per cent of the plants showed evidence of infection.

Field 6

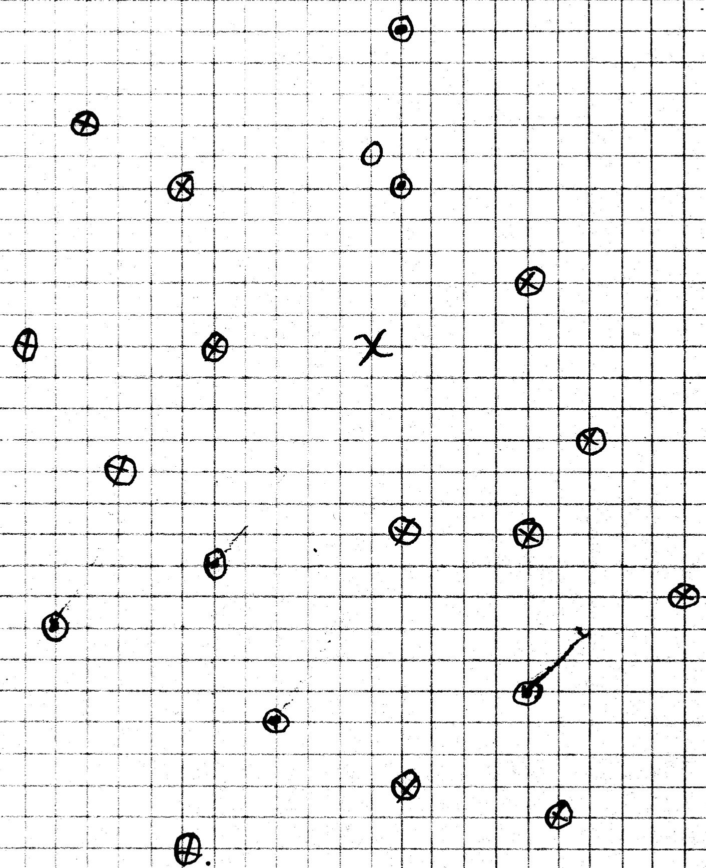
x = Original plant that was inoculated on July 10.

O = Plants that were showing infection on July 6.

⊙ = Plants that were showing infection on July 26.

⊕ = General infection showing on August 5.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.



At cutting time there were only 10 per cent of the plants showing ringspot symptoms.

## Field 7.

X = Original plant that was inoculated on July 16.

The distance between plants in the row is represented by two of the smallest divisions on the coordinate paper, and the distance between the rows is assumed to be the same.

There was no spread of infection in this field, although the inoculated plant remained conspicuous through the entire season. The leaves were narrow, dwarfed and considerably injured.

FIELD 1.

In this study a tobacco plant was inoculated with the ringspot virus received from the Virginia Agricultural Experiment Station on June 10. Typical ringspot lesions appeared on June 16. In a few days the infection became systemic, all leaves showing symptoms of the disease. This plat was observed at ten-day intervals throughout the growing season, but no spread occurred in the area under observation.

FIELD 2.

This plat was selected from a part of the same field as number 1. The plants were observed at ten-day intervals. On July 6, one plant in the far corner of the field had developed ringspot. No further spread of the disease occurred during the year.

FIELD 3.

One tobacco plant was inoculated with the expressed juice of ringspot infected tobacco on June 10. Six days later, ringspot developed on the inoculated plant. Observations were made at ten-day intervals throughout the growing season. On July 6, two additional plants, one of which was next to the inoculated plant and the other seven hills distant in the same row, were affected. On July 26, ringspot was found on two more plants in the area under observation. One of these was the first plant in the second row to the right, and the other was the fifteenth plant in the twelfth row to the left. No further spread occurred during the year. Two of the affected plants had completely masked all symptoms of ringspot by topping time.

## FIELD 4.

This plat was a part of a large field in which Plat 3 was also located. The inoculation of a tobacco plant in a marked location was made on June 10th, as in the former studies. In a few days ringspot developed. During the following ten days no additional ringspot occurred within the marked area. On June 26th, the tenth plant in the tenth row to the left had developed infection. Ten days later the tenth plant in the second row to the right was found to be affected. No further spread was observed during the rest of the season.

## FIELD 5.

This plat was located on one end of a former garden, and a dairy barn adjoined the other end of the field. Numerous weeds of many varieties grew along the fence rows and within the field. A tobacco plant was inoculated with the ringspot virus on June 10th, and in a few days it developed typical ringspot. On June 26th, four new plants were affected in close proximity. On July 16th, the infection was found to be quite general throughout the field, about 25 per cent of the plants in the area under observation being affected. At topping time the ringspot symptoms had become masked to such an extent that only 15 per cent of the plants showed evidence of infection.

FIELD 6.

This plat was located under the same conditions as Plat 5, and the same method of inoculating was also used. On July 6th, the first spread of disease was noted, the sixth plant in the same row developed ringspot symptoms. The next spread was observed on July 26th, when the fourth, eighth, and eleventh plants in the fifth row to the right, and the sixth, eighth, and twelfth plants, left, were found to be affected. On August 5th, the infection was found quite general, about 20 per cent of the plants in the field had developed the disease. As reported in the previous plat, the visible infection began to decrease after this date and at cutting time there were only 10 per cent of the plants that showed ringspot symptoms.

FIELD 7.

A plant was selected in the center of a small field, in which rye had been grown as the previous crop. The weeds were kept down in the early part of the tobacco season. On July 16th, the tobacco plant was inoculated with ringspot virus, six days later it developed a systemic infection and remained very conspicious throughout the entire season. The leaves were narrow, dwarfed and considerably injured, but no infection developed on other plants during the remainder of the season.

It is apparent that the results of the studies on the rate of spread of ringspot infection, reported above, are not consistent. It does not appear that the infection that developed on additional plants in these plats came from the inoculated plants, because, at times, additional plants became infected before ringspot developed on these inoculated, and some plants in the far side of the field became affected before those in the vicinity of the inoculated plant. It, therefore, seems that the infection came from some other source, such as weed hosts. Various kinds of weeds were found in great abundance, surrounding every field where a spread of infection was noted. Just how the disease was spread has not been determined. The common tobacco flea beetle was present in considerable numbers throughout the entire season and it seems that this insect should be an ideal carrier of ringspot virus; however, my insect studies did not prove this to be the case.

## EXTENT OF INJURY TO THE AFFECTED PLANTS.

It is very important to know the extent of injury to affected plants by ringspot; therefore, forty healthy and forty diseased plants were measured at cutting time. The number of leaves on the plant, the extent of tissue injury, and quality of leaf were determined. The results of this study are shown in Tables 1 and 2.

TABLE 1. - Extent of injury to ringspot affected plants.

| Healthy plants | | | Affected plants. | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimension of leaf in inches |
| 1 | 1 | 17 x 9 | 1 | 1 | 17 x 7 |
| | 2 | 21 x 11 | | 2 | 13 x 5 |
| | 3 | 17 x 9 | | 3 | 16 x 7 |
| | 4 | 20 x 10 | | 4 | 20 x 9 |
| | 5 | 22 x 13 | | 5 | 21 x 10 |
| | 6 | 24 x 13 | | 6 | 21 x 11 |
| | 7 | 23 x 13 | | 7 | 18 x 7 |
| | 8 | 23 x 13 | | 8 | 21 x 9 |
| | 9 | 24 x 14 | | 9 | 16 x 9 |
| | 10 | 19 x 9 | | 10 | 17 x 7 |
| | 11 | 23 x 12 | | 11 | 22 x 11 |
| | 12 | 22 x 12 | | 12 | 18 x 7 |
| | 13 | 21 x 12 | | 13 | 24 x 11 |
| | 14 | 19 x 11 | | 14 | 21 x 10 |
| | | | | 15 | 20 x 9 |
| | | | | 16 | 20 x 8 |
| | Average - | 21 x 12 | | Average - | 19 x 8 |
| 2 | 1 | 24 x 2 | 2 | 1 | 22 x 5 |
| | 2 | 26 x 12 | | 2 | 19 x 7 |
| | 3 | 24 x 13 | | 3 | 22 x 5 |
| | 4 | 27 x 14 | | 4 | 18 x 7 |
| | 5 | 29 x 15 | | 5 | 20 x 8 |
| | 6 | 26 x 12 | | 6 | 21 x 8 |
| | 7 | 27 x 13 | | 7 | 21 x 9 |
| | 8 | 27 x 11 | | 8 | 21 x 10 |
| | 9 | 25 x 10 | | 9 | 24 x 9 |
| | 10 | 21 x 9 | | 10 | 20 x 9 |
| | 11 | 27 x 12 | | 11 | 19 x 9 |
| | 12 | 24 x 10 | | 12 | 21 x 9 |
| | 13 | 27 x 11 | | 13 | 21 x 9 |
| | | | | 14 | 20 x 10 |
| | | | | 15 | 19 x 8 |
| | Average - | 20 x 9 | | Average - | 20 x 9 |

TABLE 1, Continued.

| Healthy plants: Plant No. | Leaf No. | Dimensions of leaf in inches | Affected plants: Plant No. | Leaf No. | Dimensions of leaf in inches |
|---|---|---|---|---|---|
| 3. | 1 | 24 x 11 | 3 | 1 | 12 x 4 |
| | 2 | 22 x 9 | | 2 | 18 x 7 |
| | 3 | 33 x 14 | | 3 | 18 x 7 |
| | 4 | 30 x 14 | | 4 | 20 x 13 |
| | 5 | 31 x 15 | | 5 | 19 x 13 |
| | 6 | 31 x 13 | | 6 | 16 x 10 |
| | 7 | 33 x 15 | | 7 | 16 x 9 |
| | 8 | 30 x 13 | | 8 | 20 x 13 |
| | 9 | 31 x 14 | | 9 | 18 x 9 |
| | 10 | 31 x 13 | | | |
| | 11 | 32 x 14 | | | |
| | 12 | 28 x 12 | | | |
| | 13 | 31 x 15 | | | |
| | 14 | 28 x 13 | | | |
| | 15 | 23 x 10 | | | |
| | 16 | 24 x 11 | | | |
| | 17 | 22 x 10 | | | |
| | 18 | 26 x 13 | | | |
| | 19 | 19 x 9 | | | |
| Average - | | 28 x 13 | Average - | | 17 x 9 |

| Healthy plants: Plant No. | Leaf No. | Dimensions of leaf in inches | Affected plants: Plant No. | Leaf No. | Dimensions of leaf in inches |
|---|---|---|---|---|---|
| 4. | 1 | 21 x 9 | 4 | 1 | 17 x 7 |
| | 2 | 18 x 7 | | 2 | 20 x 9 |
| | 3 | 22 x 18 | | 3 | 22 x 9 |
| | 4 | 24 x 10 | | 4 | 24 x 11 |
| | 5 | 24 x 10 | | 5 | 26 x 10 |
| | 6 | 27 x 12 | | 6 | 28 x 13 |
| | 7 | 24 x 9 | | 7 | 28 x 12 |
| | 8 | 26 x 12 | | 8 | 28 x 13 |
| | 9 | 25 x 10 | | 9 | 28 x 12 |
| | 10 | 25 x 11 | | 10 | 30 x 14 |
| | 11 | 23 x 12 | | 11 | 29 x 14 |
| | 12 | 23 x 11 | | 12 | 28 x 14 |
| | 13 | 28 x 9 | | 13 | 28 x 14 |
| | 14 | 24 x 10 | | 14 | 21 x 13 |
| | 15 | 22 x 9 | | 15 | 24 x 13 |
| | 16 | 18 x 8 | | 16 | 24 x 10 |
| Average - | | 24 x 10 | Average - | | 25 x 12 |

TABLE 1, Continued

| Healthy plants: Plant No. | Leaf No. | Dimensions of leaf in inches. | Affected plants: Plant No. | Leaf No. | Dimensions of leaf in inches |
|---|---|---|---|---|---|
| 5 | 1 | 16 x 8 | 5 | 1 | 25 x 10 |
| | 2 | 20 x 8 | | 2 | 21 x 6 |
| | 3 | 28 x 11 | | 3 | 29 x 11 |
| | 4 | 27 x 13 | | 4 | 33 x 13 |
| | 5 | 30 x 13 | | 5 | 32 x 12 |
| | 6 | 30 x 13 | | 6 | 32 x 12 |
| | 7 | 30 x 14 | | 7 | 28 x 11 |
| | 8 | 30 x 13 | | 8 | 29 x 13 |
| | 9 | 27 x 12 | | 9 | 31 x 12 |
| | 10 | 27 x 13 | | 10 | 28 x 12 |
| | 11 | 26 x 12 | | 11 | 27 x 11 |
| | 12 | 23 x 10 | | 12 | 31 x 12 |
| | 13 | 23 x 13 | | 13 | 30 x 14 |
| | 14 | 22 x 10 | | 14 | 24 x 12 |
| | 15 | 16 x 7 | | 15 | 25 x 11 |
| | | | | 16 | 21 x 5 |
| | Average - | 25 x 12 | | Average - | 28 x 11 |
| 6 | 1 | 14 x 6 | 6 | 1 | 17 x 6 |
| | 2 | 10 x 4 | | 2 | 14 x 5 |
| | 3 | 13 x 5 | | 3 | 21 x 9 |
| | 4 | 18 x 7 | | 4 | 21 x 10 |
| | 5 | 24 x 11 | | 5 | 25 x 11 |
| | 6 | 26 x 12 | | 6 | 24 x 10 |
| | 7 | 24 x 11 | | 7 | 26 x 12 |
| | 8 | 25 x 13 | | 8 | 27 x 13 |
| | 9 | 26 x 13 | | 9 | 27 x 14 |
| | 10 | 26 x 14 | | 10 | 27 x 15 |
| | 11 | 25 x 14 | | 11 | 24 x 14 |
| | 12 | 26 x 14 | | 12 | 23 x 12 |
| | 13 | 25 x 14 | | 13 | 26 x 14 |
| | 14 | 21 x 12 | | 14 | 26 x 15 |
| | 15 | 23 x 13 | | 15 | 24 x 13 |
| | | | | 16 | 22 x 8 |
| | Average - | 22 x 11 | | Average - | 23 x 11 |

TABLE 1, Continued

| Healthy plants | | | Affected plants. | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches. | Plant No. | Leaf No. | Dimensions of leaf in inches. |
| 7 | 1 | 22 x 10 | 7 | 1 | 18 x 6 |
| | 2 | 24 x 12 | | 2 | 13 x 4 |
| | 3 | 30 x 16 | | 3 | 21 x 8 |
| | 4 | 28 x 16 | | 4 | 25 x 11 |
| | 5 | 29 x 15 | | 5 | 26 x 10 |
| | 6 | 30 x 14 | | 6 | 28 x 12 |
| | 7 | 29 x 15 | | 7 | 28 x 11 |
| | 8 | 30 x 16 | | 8 | 27 x 10 |
| | 9 | 31 x 16 | | 9 | 24 x 11 |
| | 10 | 30 x 17 | | 10 | 24 x 10 |
| | 11 | 30 x 15 | | 11 | 23 x 10 |
| | 12 | 27 x 14 | | 12 | 24 x 10 |
| | 13 | 26 x 12 | | 13 | 22 x 12 |
| | 14 | 25 x 11 | | 14 | 23 x 8 |
| | 15 | 24 x 10 | | 15 | 22 x 8 |
| | Average - | 28 x 13 | | Average - | 23 x 10 |
| 8 | 1 | 17 x 7 | 8 | 1 | 14 x 4 |
| | 2 | 22 x 11 | | 2 | 15 x 5 |
| | 3 | 26 x 12 | | 3 | 19 x 6 |
| | 4 | 26 x 12 | | 4 | 19 x 7 |
| | 5 | 28 x 15 | | 5 | 18 x 6 |
| | 6 | 30 x 18 | | 6 | 19 x 8 |
| | 7 | 30 x 17 | | 7 | 19 x 6 |
| | 8 | 31 x 16 | | 8 | 24 x 10 |
| | 9 | 31 x 18 | | 9 | 23 x 9 |
| | 10 | 32 x 18 | | 10 | 24 x 10 |
| | 11 | 28 x 17 | | 11 | 21 x 8 |
| | 12 | 29 x 11 | | 12 | 21 x 7 |
| | 13 | 26 x 12 | | 13 | 19 x 4 |
| | 14 | 24 x 10 | | 14 | 18 x 7 |
| | | | | 15 | 18 x 5 |
| | | | | 16 | 18 x 6 |
| | Average - | 27 x 14 | | Average | 19 x 7 |

TABLE 1, Continued

| Healthy plants | | | Affected plants. | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimensions of leaf in inches |
| 9 | 1 | 14 x 7 | 9 | 1 | 27 x 12 |
| | 2 | 18 x 8 | | 2 | 22 x 9 |
| | 3 | 20 x 9 | | 3 | 30 x 13 |
| | 4 | 24 x 10 | | 4 | 29 x 12 |
| | 5 | 23 x 12 | | 5 | 22 x 10 |
| | 6 | 26 x 13 | | 6 | 29 x 13 |
| | 7 | 24 x 14 | | 7 | 28 x 13 |
| | 8 | 28 x 15 | | 8 | 28 x 12 |
| | 9 | 26 x 14 | | 9 | 24 x 11 |
| | 10 | 28 x 17 | | 10 | 28 x 12 |
| | 11 | 23 x 10 | | 11 | 28 x 13 |
| | 12 | 24 x 11 | | 12 | 24 x 11 |
| | 13 | 23 x 11 | | 13 | 28 x 14 |
| | | | | 14 | 25 x 11 |
| | | | | 15 | 23 x 10 |
| | Average - | 23 x 11 | | Average - | 26 x 12 |
| 10 | 1 | 21 x 8 | 10 | 1 | 19 x 7 |
| | 2 | 24 x 12 | | 2 | 24 x 9 |
| | 3 | 23 x 11 | | 3 | 19 x 7 |
| | 4 | 27 x 13 | | 4 | 24 x 8 |
| | 5 | 27 x 13 | | 5 | 26 x 9 |
| | 6 | 24 x 13 | | 6 | 28 x 12 |
| | 7 | 28 x 12 | | 7 | 28 x 11 |
| | 8 | 27 x 13 | | 8 | 26 x 10 |
| | 9 | 25 x 12 | | 9 | 27 x 11 |
| | 10 | 26 x 14 | | 10 | 23 x 10 |
| | 11 | 25 x 12 | | 11 | 24 x 11 |
| | 12 | 24 x 11 | | 12 | 23 x 12 |
| | 13 | 25 x 11 | | 13 | 24 x 12 |
| | 14 | 24 x 10 | | 14 | 25 x 13 |
| | 15 | 20 x 8 | | 15 | 18 x 7 |
| | 16 | 22 x 9 | | | |
| | Average - | 25 x 12 | | Average - | 24 x 10 |

TABLE 1, Continued

| Healthy plants | | | Affected plants. | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimensions of leaf in inches. |
| 11 | 1 | 15 x 6 | 11 | 1 | 25 x 6 |
| | 2 | 16 x 7 | | 2 | 21 x 5 |
| | 3 | 22 x 7 | | 3 | 23 x 5 |
| | 4 | 23 x 8 | | 4 | 25 x 7 |
| | 5 | 24 x 8 | | 5 | 27 x 7 |
| | 6 | 32 x 16 | | 6 | 26 x 8 |
| | 7 | 35 x 18 | | 7 | 26 x 10 |
| | 8 | 32 x 15 | | 8 | 27 x 11 |
| | 9 | 32 x 15 | | 9 | 27 x 10 |
| | 10 | 32 x 18 | | 10 | 27 x 10 |
| | 11 | 31 x 16 | | 11 | 24 x 10 |
| | 12 | 30 x 18 | | 12 | 24 x 8 |
| | 13 | 29 x 16 | | 13 | 22 x 10 |
| | 14 | 28 x 17 | | | |
| | 15 | 26 x 12 | | | |
| | 16 | 23 x 12 | | | |
| | Average - | 27 x 13 | | Average - | 23 x 8 |
| 12 | 1 | 26 x 9 | 12 | 1 | 22 x 9 |
| | 2 | 27 x 11 | | 2 | 16 x 7 |
| | 3 | 26 x 13 | | 3 | 24 x 11 |
| | 4 | 28 x 15 | | 4 | 26 x 11 |
| | 5 | 27 x 12 | | 5 | 29 x 12 |
| | 6 | 27 x 12 | | 6 | 28 x 11 |
| | 7 | 28 x 13 | | 7 | 28 x 11 |
| | 8 | 26 x 12 | | 8 | 27 x 12 |
| | 9 | 27 x 13 | | 9 | 24 x 11 |
| | 10 | 24 x 11 | | 10 | 25 x 10 |
| | 11 | 26 x 12 | | 11 | 25 x 11 |
| | 12 | 27 x 11 | | 12 | 22 x 7 |
| | 13 | 26 x 12 | | 13 | 24 x 10 |
| | | | | 14 | 25 x 9 |
| | Average - | 27 x 12 | | Average | 24 x 10 |

TABLE 1, Continued

| Healthy plants | | | Affected plants | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches. | Plant No. | Leaf No. | Dimensions of leaf in inches |
| 13 | 1 | 19 x 5 | 13 | 1 | 29 x 12 |
| | 2 | 21 x 7 | | 2 | 25 x 9 |
| | 3 | 21 x 8 | | 3 | 29 x 11 |
| | 4 | 23 x 8 | | 4 | 27 x 10 |
| | 5 | 24 x 9 | | 5 | 29 x 11 |
| | 6 | 25 x 9 | | 6 | 25 x 14 |
| | 7 | 23 x 9 | | 7 | 23 x 10 |
| | 8 | 24 x 10 | | 8 | 22 x 10 |
| | 9 | 24 x 10 | | 9 | 23 x 10 |
| | 10 | 22 x 12 | | 10 | 18 x 10 |
| | 11 | 22 x 10 | | 11 | 22 x 11 |
| | 12 | 25 x 10 | | 12 | 24 x 12 |
| | 13 | 21 x 11 | | | |
| | Average - | 23 x 9 | | Average - | 25 x 11 |
| 14 | 1 | 23 x 9 | 14 | 1 | 15 x 5 |
| | 2 | 26 x 12 | | 2 | 16 x 8 |
| | 3 | 29 x 11 | | 3 | 18 x 8 |
| | 4 | 29 x 12 | | 4 | 21 x 9 |
| | 5 | 26 x 10 | | 5 | 21 x 9 |
| | 6 | 31 x 13 | | 6 | 21 x 10 |
| | 7 | 27 x 10 | | 7 | 20 x 9 |
| | 8 | 29 x 13 | | 8 | 23 x 12 |
| | 9 | 28 x 13 | | 9 | 23 x 11 |
| | 10 | 29 x 12 | | | |
| | 11 | 26 x 10 | | | |
| | 12 | 24 x 9 | | | |
| | 13 | 27 x 11 | | | |
| | 14 | 23 x 8 | | | |
| | Average - | 27 x 11 | | Average - | 20 x 8 |

TABLE 1, Continued

| Healthy plants | | | Affected plants. | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimensions of leaf in inches |
| 15 | 1 | 26 x 11 | 15 | 1 | 18 x 7 |
| | 2 | 25 x 11 | | 2 | 16 x 5 |
| | 3 | 28 x 13 | | 3 | 14 x 6 |
| | 4 | 29 x 13 | | 4 | 18 x 8 |
| | 5 | 28 x 14 | | 5 | 24 x 9 |
| | 6 | 29 x 14 | | 6 | 25 x 9 |
| | 7 | 28 x 13 | | 7 | 25 x 11 |
| | 8 | 28 x 13 | | 8 | 23 x 8 |
| | 9 | 24 x 14 | | 9 | 25 x 11 |
| | 10 | 25 x 13 | | 10 | 23 x 11 |
| | 11 | 26 x 14 | | 11 | 21 x 9 |
| | 12 | 31 x 14 | | 12 | 20 x 10 |
| | 13 | 24 x 11 | | | |
| | Average - | 27 x 13 | | Average - | 21 x 9 |

| | | | | | |
|---|---|---|---|---|---|
| 16 | 1 | 22 x 10 | 16 | 1 | 21 x 5 |
| | 2 | 25 x 12 | | 2 | 25 x 9 |
| | 3 | 27 x 11 | | 3 | 26 x 7 |
| | 4 | 28 x 11 | | 4 | 25 x 7 |
| | 5 | 30 x 12 | | 5 | 25 x 8 |
| | 6 | 30 x 16 | | 6 | 29 x 10 |
| | 7 | 33 x 15 | | 7 | 28 x 11 |
| | 8 | 32 x 16 | | 8 | 23 x 8 |
| | 9 | 31 x 14 | | 9 | 22 x 8 |
| | 10 | 26 x 13 | | | |
| | 11 | 24 x 13 | | | |
| | 12 | 27 x 16 | | | |
| | 13 | 24 x 11 | | | |
| | 14 | 29 x 13 | | | |
| | 15 | 26 x 16 | | | |
| | 16 | 27 x 12 | | | |
| | 17 | 28 x 12 | | | |
| | Average - | 27 x 14 | | Average - | 25 x 8 |

TABLE 1, Continued

| Healthy plants | | | Affected plants | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimensions of leaf in inches |
| 17 | 1 | 26 x 8 | 17 | 1 | 12 x 5 |
| | 2 | 28 x 12 | | 2 | 10 x 4 |
| | 3 | 27 x 12 | | 3 | 9 x 4 |
| | 4 | 29 x 13 | | 4 | 12 x 5 |
| | 5 | 31 x 13 | | 5 | 14 x 5 |
| | 6 | 30 x 12 | | 6 | 16 x 6 |
| | 7 | 29 x 13 | | 7 | 17 x 7 |
| | 8 | 26 x 14 | | 8 | 17 x 7 |
| | 9 | 28 x 12 | | 9 | 16 x 5 |
| | 10 | 27 x 12 | | 10 | 18 x 8 |
| | 11 | 26 x 11 | | 11 | 18 x 8 |
| | 12 | 26 x 12 | | 12 | 18 x 10 |
| | 13 | 25 x 11 | | 13 | 14 x 6 |
| | 14 | 26 x 10 | | | |
| | 15 | 24 x 9 | | | |
| | Average - | 23 x 12 | | Average - | 15 x 6 |
| 18 | 1 | 23 x 9 | 18 | 1 | 12 x 5 |
| | 2 | 25 x 11 | | 2 | 10 x 4 |
| | 3 | 26 x 12 | | 3 | 15 x 6 |
| | 4 | 28 x 13 | | 4 | 17 x 7 |
| | 5 | 30 x 14 | | 5 | 20 x 9 |
| | 6 | 27 x 12 | | 6 | 22 x 10 |
| | 7 | 29 x 12 | | 7 | 20 x 10 |
| | 8 | 31 x 13 | | 8 | 22 x 9 |
| | 9 | 30 x 14 | | 9 | 19 x 10 |
| | 10 | 28 x 12 | | | |
| | 11 | 26 x 11 | | | |
| | 12 | 25 x 9 | | | |
| | 13 | 25 x 10 | | | |
| | 14 | 24 x 9 | | | |
| | 15 | 24 x 11 | | | |
| | 16 | 23 x 10 | | | |
| | Average - | 26 x 11 | | Average - | 17 x 8 |

TABLE 1, Continued

| Healthy plants | | | Affected plants | | |
|---|---|---|---|---|---|
| Plant No. | Leaf No. | Dimensions of leaf in inches | Plant No. | Leaf No. | Dimension of leaf in inches |
| 19 | 1 | 24 x 12 | 19 | 1 | 13 x 5 |
| | 2 | 26 x 13 | | 2 | 15 x 5 |
| | 3 | 28 x 12 | | 3 | 17 x 6 |
| | 4 | 29 x 13 | | 4 | 20 x 8 |
| | 5 | 30 x 14 | | 5 | 12 x 4 |
| | 6 | 28 x 12 | | 6 | 20 x 7 |
| | 7 | 29 x 13 | | 7 | 20 x 8 |
| | 8 | 27 x 12 | | 8 | 16 x 7 |
| | 9 | 29 x 13 | | 9 | 22 x 6 |
| | 10 | 29 x 14 | | | |
| | 11 | 27 x 12 | | | |
| | 12 | 26 x 11 | | | |
| | 13 | 25 x 9 | | | |
| | 14 | 24 x 9 | | | |
| | Average - | 27 x 12 | | Average - | 17 x 6 |
| 20 | 1 | 21 x 9 | 20 | 1 | 13 x 4 |
| | 2 | 23 x 10 | | 2 | 14 x 5 |
| | 3 | 22 x 11 | | 3 | 16 x 6 |
| | 4 | 25 x 12 | | 4 | 19 x 8 |
| | 5 | 26 x 11 | | 5 | 21 x 8 |
| | 6 | 24 x 11 | | 6 | 23 x 9 |
| | 7 | 26 x 12 | | 7 | 22 x 9 |
| | 8 | 27 x 13 | | 8 | 23 x 10 |
| | 9 | 28 x 13 | | 9 | 23 x 8 |
| | 10 | 25 x 12 | | 10 | 20 x 7 |
| | 11 | 24 x 11 | | 11 | 19 x 6 |
| | 12 | 24 x 10 | | | |
| | 13 | 22 x 9 | | | |
| | 14 | 23 x 9 | | | |
| | 15 | 21 x 8 | | | |
| | Average - | 26 x 11 | | Average - | 20 x 7 |

TABLE 2 - SUMMARY OF RESULTS GIVEN IN TABLE 1.

| Healthy plants | | Diseased plants. | |
|---|---|---|---|
| Plant No. | Average leaf | Plant No. | Average leaf. |
| 1 | 27 x 13 | 1 | 19 x 8 |
| 2 | 27 x 14 | 2 | 20 x 9 |
| 3 | 23 x 9 | 3 | 17 x 9 |
| 4 | 27 x 11 | 4 | 25 x 12 |
| 5 | 27 x 13 | 5 | 28 x 11 |
| 6 | 27 x 12 | 6 | 23 x 11 |
| 7 | 25 x 12 | 7 | 23 x 10 |
| 8 | 22 x 11 | 8 | 19 x 7 |
| 9 | 28 x 13 | 9 | 26 x 12 |
| 10 | 24 x 10 | 10 | 24 x 10 |
| 11 | 21 x 12 | 11 | 23 x 8 |
| 12 | 26 x 11 | 12 | 24 x 10 |
| 13 | 28 x 13 | 13 | 25 x 11 |
| 14 | 27 x 14 | 14 | 20 x 8 |
| 15 | 23 x 11 | 15 | 21 x 9 |
| 16 | 25 x 12 | 16 | 25 x 8 |
| 17 | 23 x 12 | 17 | 15 x 6 |
| 18 | 26 x 11 | 18 | 17 x 8 |
| 19 | 24 x 10 | 19 | 17 x 6 |
| 20 | 27 x 12 | 20 | 20 x 7 |
| Average - | 25 x 12 | Average - | 21.5 x 9 |

A study of the data tabulated in Tables 1 and 2 shows that the leaves of the diseased plants are 15 per cent shorter and 25 per cent narrower than those of the healthy plants. From these data it is estimated that ringspot caused 20 per cent injury to the affected plants. In addition to the loss in size, the average number of leaves per plant was 2.25 per cent less for the affected ones. The healthy plants averaged 14.75 leaves per plant and the diseased ones averaged only 12.50 per plant. There was also considerable tissue injury to the leaves on the affected plants.

## THE PERCENTAGE OF PLANTS AFFECTED

Another phase of the ringspot problem, more important than the preceding one, is the determination of the percentage of affected plants. The field and plant bed inspections made during the growing season of 1927 throw considerable light on this point. The results of these observations are given in Table 3. One hundred and seventy-one fields in ten counties, and forty-nine plant beds in six counties, were inspected. An average of 2.5 per cent ringspot infection was found in the one hundred and seventy-one fields observed. In one field in Brunswick County ringspot occurred on 75 per cent of the plants. Alfalfa had grown on this soil for several years before the tobacco was planted and it is probable that infection was carried in the alfalfa plants that were left around the ditch banks and along the fence rows. There was a vegetable garden at one end of this field and some of the vegetable crops might have acted as a source of infection. Recent unpublished studies by Henderson and Wingard show that squash and cantaloupe act as natural hosts of the ringspot disease.

Table 4 shows the percentage of ringspot infection in Washington County during the season of 1928. Fifty representative tobacco fields were tabulated. In practically every field inspected Ringspot was found. An average of 3 per cent was recorded for that year.

Table 5 shows the percentage of field infection in Washington County in 1929. One hundred tobacco fields in different parts of the County were inspected and tabulated. Only two small fields were found that did not contain ringspot. Many fields were found with from 50 to 90 per cent infection, but the majority ran from 8 to 12 per cent. As reported elsewhere, the highest percentage of infection was found in the middle of the growing season when the plants were about a foot high. At topping time the ringspot symptoms had been masked on about 50 per cent of the plants in most fields. During the year many complaints were received from farmers concerning a serious disease of their tobacco when it was about half grown. In practically every case the injury was caused by ringspot. When the plants became affected early in the season, very severe injury resulted, which caused them to become stunted and deformed, and in many cases the grower removed them from the field as being worthless.

TABLE 3- Results of field and plant bed inspections made during the season of 1927.

| County | Number of fields inspected. | Number of plant beds inspected. | Average percentage of infected plants in field. | Average percentage of infected plants in plant bed. |
|---|---|---|---|---|
| Halifax | 22 | 10 | 3 | 0.1 |
| Appomattox | 18 | 8 | 2 | 0 |
| Amherst | 20 | 0 | 2 | 0 |
| Nelson | 15 | 6 | 1.5 | 0 |
| Pittsylvania | 23 | 8 | 2 | 0 |
| Mecklenburg | 12 | 0 | 3 | 0 |
| Charlotte | 16 | 7 | 3 | 0 |
| Brunswick | 25 | 10 | 5 | 0 |
| Dinwiddie | 10 | 0 | 2 | 0 |
| Hanover | 10 | 0 | 1.5 | 0 |

Average - - 17.1 fields 2.5 per cent.

TABLE 4 - Results of field inspections made in Washington County in 1928.

| Field number | Percentage of infected plants | Field number | Percentage of infected plants. |
|---|---|---|---|
| 1 | 8 | 26 | 3 |
| 2 | 2 | 27 | 4 |
| 3 | 3 | 28 | 3 |
| 4 | 2 | 29 | 3 |
| 5 | 3 | 30 | 2 |
| 6 | 2 | 31 | 6 |
| 7 | 5 | 32 | 3 |
| 8 | 2 | 33 | 0 |
| 9 | 3 | 34 | 2 |
| 10 | 4 | 35 | 4 |
| 11 | 1 | 36 | 2 |
| 12 | 0 | 37 | 4 |
| 13 | 2 | 38 | 3 |
| 14 | 3 | 39 | 2 |
| 15 | 2 | 40 | .5 |
| 16 | 4 | 41 | 4 |
| 17 | 7 | 42 | 4 |
| 18 | 3 | 43 | 3 |
| 19 | 5 | 44 | 3 |
| 20 | 4 | 45 | 5 |
| 21 | 2 | 46 | 2 |
| 22 | 1 | 47 | 6 |
| 23 | 5 | 48 | 0 |
| 24 | 0 | 49 | 2 |
| 25 | 1 | 50 | 2 |

Average - 3 per cent.

TABLE 5- Results of field inspections made in Washington County in 1929.

| Field number | Percentage of infected plants. | Field number | Percentage of infected plants. |
|---|---|---|---|
| 1 | 90 | 51 | .5 |
| 2 | 75 | 52 | 2 |
| 3 | 20 | 53 | 9 |
| 4 | 2 | 54 | 13 |
| 5 | 9 | 55 | 2 |
| 6 | 1.5 | 56 | 4 |
| 7 | 3 | 57 | 2 |
| 8 | 2 | 58 | 13 |
| 9 | 4 | 59 | 1 |
| 10 | 2 | 60 | 3 |
| 11 | 1 | 61 | 5 |
| 12 | 18 | 62 | 12 |
| 13 | 3 | 63 | 16 |
| 14 | 6 | 64 | 0 |
| 15 | 8 | 65 | 8 |
| 16 | 2 | 66 | 4 |
| 17 | 4 | 67 | 6 |
| 18 | 10 | 68 | 3 |
| 19 | 3 | 69 | 2 |
| 20 | 20 | 70 | 6 |
| 21 | 1 | 71 | 5 |
| 22 | 6 | 72 | 15 |
| 23 | 2 | 73 | 18 |
| 24 | 5 | 74 | 6 |
| 25 | 3 | 75 | 4 |
| 26 | 2 | 76 | 3 |
| 27 | 6 | 77 | 2 |
| 28 | 8 | 78 | 8 |
| 29 | 5 | 79 | 5 |
| 30 | 4 | 80 | 12 |
| 31 | 2 | 81 | 14 |
| 32 | 9 | 82 | 3 |
| 33 | 12 | 83 | 6 |
| 34 | 11 | 84 | 8 |
| 35 | 8 | 85 | 10 |
| 36 | 5 | 86 | 4 |
| 37 | 3 | 87 | 3 |
| 38 | 9 | 88 | 2 |
| 39 | 2 | 89 | 14 |
| 40 | 20 | 90 | 3 |
| 41 | 14 | 91 | 2 |
| 42 | 6 | 92 | 6 |
| 43 | 5 | 93 | 8 |
| 44 | 3 | 94 | 5 |
| 45 | 8 | 95 | 11 |
| 46 | 6 | 96 | 3 |
| 47 | 8 | 97 | 5 |
| 48 | 3 | 98 | 9 |
| 49 | 2 | 99 | 4 |
| 50 | 1 | 100 | 6 |

Average - 7.6 per cent.

## TOTAL LOSS FOR WASHINGTON COUNTY IN 1929.

The total loss for Washington County was found by multiplying the average extent of injury to the plant by the percentage of plants affected. According to the results given in Tables 1, 2, and 5, 7.6 per cent of all the plants in the County were affected with ringspot, and the value of the affected plants was reduced 20 per cent. The total loss for the County, therefore, was 20 per cent of 7.6 or 1.52 per cent of the entire crop.

It is estimated that in 1928 there was 2500 acres of tobacco grown in Washington County and that the average production was 1050 pounds per acre. The average price paid for burley tobacco on the Abingdon market was 32 cents per pound. Therefore, the total crop produced was 2,625,000 pounds and the loss due to ringspot 39,900 pounds. This makes a loss to farmers of Washington County of $12,768 from this disease in 1929.

## DISCUSSION OF RESULTS.

Ringspot undoubtedly is a disease of considerable economic importance to tobacco growers. My observations in eleven of the leading tobacco counties of Virginia with all types of tobacco grown - Burley, flue cured, fire cured, and sun cured lead me to believe that this disease is not any more virulent to one type or variety of tobacco than it is to another but that all are equally susceptible. Fromme, Wingard, and Priode ('27) produced the disease on five species of Nicotiana and a number of varieties of N.tobacum. Wingard ('28) developed ringspot on thirty-eight genera of plants representing seventeen families. This shows that the disease is capable of a wide range of infection. As more host plants become naturally infected, they in turn will, very probably, act as reservoirs for the dissemination of ringspot infection to tobacco. Wingard ('28) reported finding ringspot infection occurring naturally on sweet clover, and succeeded in producing infection on tobacco by inoculating with the sweet clover material.

For the past two years a large number of weeds have been sent to the Virginia Agricultural Experiment Station for inoculation tests on tobacco in an attempt to find natural hosts of ringspot. One particular field of tobacco which had 8 per cent ringspot infection in 1928 was again planted to tobacco in 1929, and 90 per cent infection developed. A great variety of weeds completely surrounded this field. Numerous specimens were tested, but the stick weed, sometimes called yellow crown beard (Verbesina alternifolia) was the only one found capable of transmitting the disease to tobacco. This may prove to be an important step toward the solution of the ringspot problem. If the natural hosts were known, it should be possible to control ringspot by their eradication as Doolittle and Walker ('25 and '26) and Gardner and Kendrick ('22 and '23) were able to control cucumber and tomato mosaic.

Allard ('17) found that under certain conditions tobacco mosaic could be transmitted to plants in the seed bed from refuse in the soil. He found that this could be prevented by steam sterilization of the seed bed. To determine the possibility of ringspot being transmitted in a similar manner, a seed bed sterilization experiment was conducted. The fields set from these beds showed a 30 per cent ringspot infection. Due to the presence of the disease in the fields set from sterilized plant beds it seems that ringspot comes from some source other than the plant bed soil. Webb ('27) produced mosaic on winter wheat by planting seed in infested soil, however, this mosaic occurs on the roots or crown of the plant. It has been shown by Wingard ('28) that the ringspot virus in expressed juice does not remain virulent for more than 24 hours at ordinary temperatures. It does not seem possible, therefore, for infection to be carried over in the plant bed soil or old tobacco or other plant refuse.

As reported above various plants and weeds were closely observed in an attempt to determine which were capable of harboring the ringspot virus. Twenty-five different species of plants were sent to the State Agricultural Experiment Station for inoculation testa with tobacco. Sweet clover and stick weed were the only plants found to be naturally affected with the ringspot virus. Doolittle and Walker ('25 and '26) found that when all mosaic host plants were removed that occurred within seventy-five yards of cucumber fields and all new shoots removed, that the infection was reduced from 39 per cent to 3 per cent. From my studies it appears that certain plants act as hosts for the ringspot virus. However, the method of dissemination of the disease from its hosts to tobacco has not been determined.

My experiments with the tobacco flea beetle (Epitrix parvula) seem to show that it does not transmit ringspot virus from diseased to healthy tobacco plants. In none of the cages did the insects definitely transmit the disease, however, due to the fact that the flea beetle is so common on tobacco, it seems that further study should be made of it as a carrier of the ringspot virus. The potato flea beetle (Epitrix cucumeris) did not survive when caged with tobacco plants alone, neither was this insect observed to feed on tobacco in the field. Since tobacco is such an unfavorable host, it does not appear that this flea beetle is concerned with the transmission of ringspot virus. Leaf hoppers (Empoasca fabea) and aphis (Marcosiphum solanifolia) were very similar in behavior to the potato flea beetle. They were not observed feeding on tobacco under natural conditions and they did not survive when placed on tobacco in insect proof cages. From the evidence given, it does not seem that these insects are of any importance in the transmission of the ringspot disease. The tobacco worm

(*Phelglthontius quinquemoculata*) and the common lightning bug (*Photinus scintillano*) were observed quite commonly on tobacco in the field. In the cage studies, the tobacco worms survived but did not transmit the disease. The lightning bugs dies after a few days when caged with the tobacco alone. These two insects do not appear to be responsible for the transmission of ringspot virus. It seems that some insect must be responsible for transmitting the ringspot infection from affected weed hosts to tobacco; however, results were not obtained to support this theory. Further study under varying conditions would perhaps throw more light on this subject.

Kunkel ('26) transmitted aster yellows, a virus disease similar to ringspot of tobacco, by the leaf hopper (*Cicadula sexonatata*) to more than 50 different species in 23 different families of plants. But neither adults or nymphs were capable of tramsmitting the virus to healthy plants until after a 10 day incubation period. He also found that many individuals retained the virus as long as they lived and that some seemed to lose it after a short time. He reported, though, that tobacco was an unfavorable host for this insect.

Gardner and Kendrick ('22) found the hosts of tomato mosaic and reported that aphis and flea beetles may be carriers of the virus from the host plants to tomatoes. Allard ('17) reports that some species of aphis become active carriers of infective mosaic material. The large plant louse (*Macrosiphum tobaci*) sometimes becomes an active carrier. Doolittle and Walker ('25 and '26) reported that mosaic is transmitted from the wild cucumber to the cultivated cucurbits by the stripped cucumber beetle, and to a lesser extent by the twelve-spotted cucumber beetle. The melon aphis acted as the chief agency in dissiminating the virus from other wild host plants. It seems that some insect must transmit the ringspot virus from the host to tobacco.

The rate of spread in the field is very confusing. Fields were observed in the middle of the growing season to be badly affected with ringspot. In one case as much as 90 per cent infection was found, later about topping time, the disease seemed to disappear, the markings were covered to such a degree that it became difficult to locate them and, finally, many disappeared altogether. The field that showed a 90 per cent infection on July 3rd, showed only a 35 per cent infection on August 3rd. This was true in practically every case. One very interesting fact noted was that the plants in the seven fields inoculated with ringspot virus received from Dr. S. A. Wingard, continued to show very distinct rings and markings. The plants appeared to be more stunted and of much poorer quality than plants naturally infected with ringspot. In another field where counts were made on July 3rd, 75 per cent infection was noted. On August 3rd, the infection had become masked to such an extent that only 30 per cent of the plants showed signs of the ringspot. The masking of the ringspot symptoms is very interesting. It occurs both in the field and under greenhouse conditions. It suggests the development of immunity which would be an interesting phenomenon if found to occur in connection with a plant disease.

The extent of injury to plants was rather difficult to determine due to the fact that even though the plant may have had the disease and have shown typical markings early in the season, at topping time and later when the studies were made, the disease in many cases had disappeared. It could not be definitely determined whether certain plants were dwarfed because of ringspot or because of some nutritional disturbance. However, measurements were obtained which give a fairly accurate idea of the injury caused by the disease. There may be considerable injury not noticeable in the weight and quality of the leaf. When this disease becomes systemic and the markings masked, the injury due to stunting may be greater than my studies actually indicate.

The percentage of plants affected is generally small, although in some fields at certain stages of growth infection is very high. Infection was found in 98 per cent of the fields inspected in 1929, with the percentage of infection running small as a rule. In 1927, inspections were made of one hundred and seventy-one tobacco fields and forty-nine plant beds, representing ten counties. An average of 2.5 per cent of the plants were affected with ringspot in the fields. In only one county was infection found in the plant bed, and these beds were inspected late in the season after all fields had been set. In Brunswick County one field was found in which 75 per cent of the plants were affected. In 1928 field inspections were made in Washington County. A number of plant beds were also inspected, but no infection was found in the beds. Fifty fields were studied and an average of 3 per cent infection was found. In some fields infection was as high as 8 per cent.

In 1929 the percentage of infected plants was considerably higher than in the two previous years. Several inspections were made in the middle of the season, during July, and a high percentage of infection was found. The field where 8 per cent infection was reported in 1928 was again planted in tobacco in 1929. This field had a hog pen at one end and a wood house and garden in the other. On July 3rd, when this field was inspected 90 per cent of the plants showed symptoms of ringspot. One month later, August 3rd, about 55 per cent of the plants had covered all signs of ringspot infection, only 35 per cent showed ringspot markings. In another field 75 percent infection was found on July 3rd. Several plants were removed from this field, the grower thinking them worthless. However, many of the plants recovered and made very good tobacco. One hundred tobacco fields were studied in 1929, and the average percentage of infection was found to be 7.6. This is a rather high average for any plant disease to run and further shows the importance of the ringspot disease in Washington County.

## PRECAUTIONARY MEASURES.

The burning or steaming of the plant bed should be done thoroughly in order to reduce the weeding to a minimum, and a new canvas should be stretched tightly over the plant bed to prevent insects from entering. Tobacco trash of any kind should not be used on the bed, either for burning or fertilizing, as stalks and pieces of leaves left about the bed may cause infection. Plant beds should be dusted with calcium arsenate to control insects. If only an occasional plant develops the disease in the field they should be removed as soon as found rather than left as centers of infection. A program of general destruction of all weeds may be advisable where they are abundant. While hoeing, worming, topping or suckering, diseased plants should not be touched with the hands as the infection may be spread to many of the healthy plants handled later. Either cut the diseased plants as soon as found or leave them undisturbed until they can be handled by themselves.

## SUMMARY.

1. Steam sterilization of the tobacco plant bed did not control ringspot.
2. The virus was not transmitted from diseased to healthy plants by the common tobacco flea beetle (*Epitrix parvula*) although they lived and multiplied on tobacco plants within the cages.

3. The cucumber flea beetle (*Epitrix cucumeris*), leaf hopper (*Empoasa fabea*), Aphis (*Macrosiphum solanifolii*) and the lightning bug (*Photinus scintillans*) did not survive when caged on tobacco, and no infection was obtained with them. The tobacco worm (*Phlegethontius quinquemoculata*) survived but did not transmit ringspot.

4. Stick weed, sometimes called yellow crown beard, (*Verbisina alternifolia*) and sweet clover (*Melilotus alba*) were found naturally affected with ringspot, infection was readily obtained on tobacco with the expressed juice from these plants.

5. Twenty-five other species of weeds were tested for ringspot with negative results.

6. The rate of spread of the disease was not definitely determined.

7. The percentage of ringspot infection in ten counties in Virginia in 1927 was 2.5. In Washington County in 1928 it was 3 per cent, and in 1929, 7.6 per cent.

8. There was an average injury of 20 per cent to the affected plants.

9. It is estimated that ringspot caused a total loss of $12,768.00 in Washington County in 1929.

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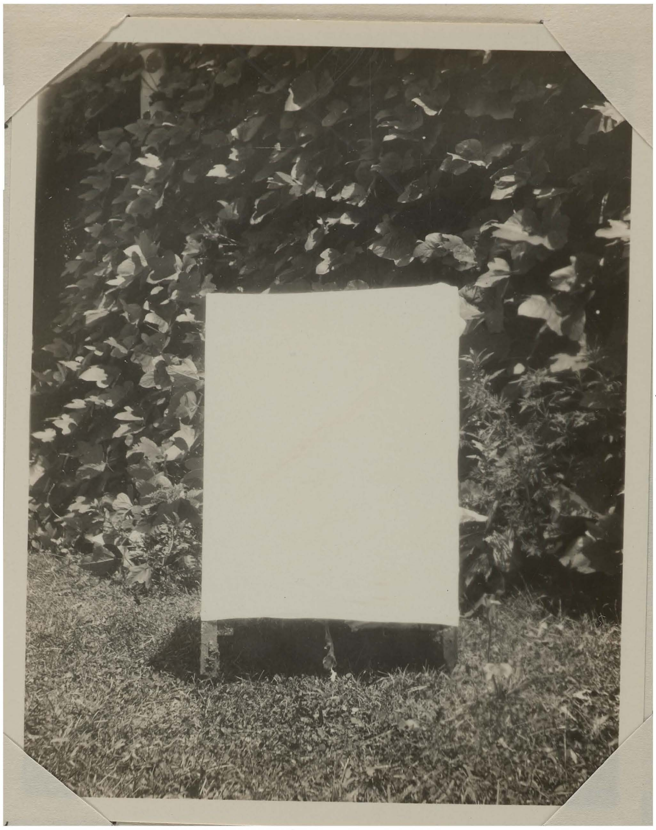

Fig. 1- Large insect cage used in ringspot studies.

Fig. 2- Small insect cage used in ringspot studies.

Fig. 3- Inverted pan used in sterilizing plant bed. The end of steamed bed can be seen in left hand corner of this picture.

Fig. 4- Steam sterilized plant bed with canvas removed at transplanting time. Note the freedom of bed of weeds.

Fig. 5- Washington County plant beds.

Fig. 6- Young Burley tobacco plant showing systemic ringspot infection. Note the stunting of the plant.

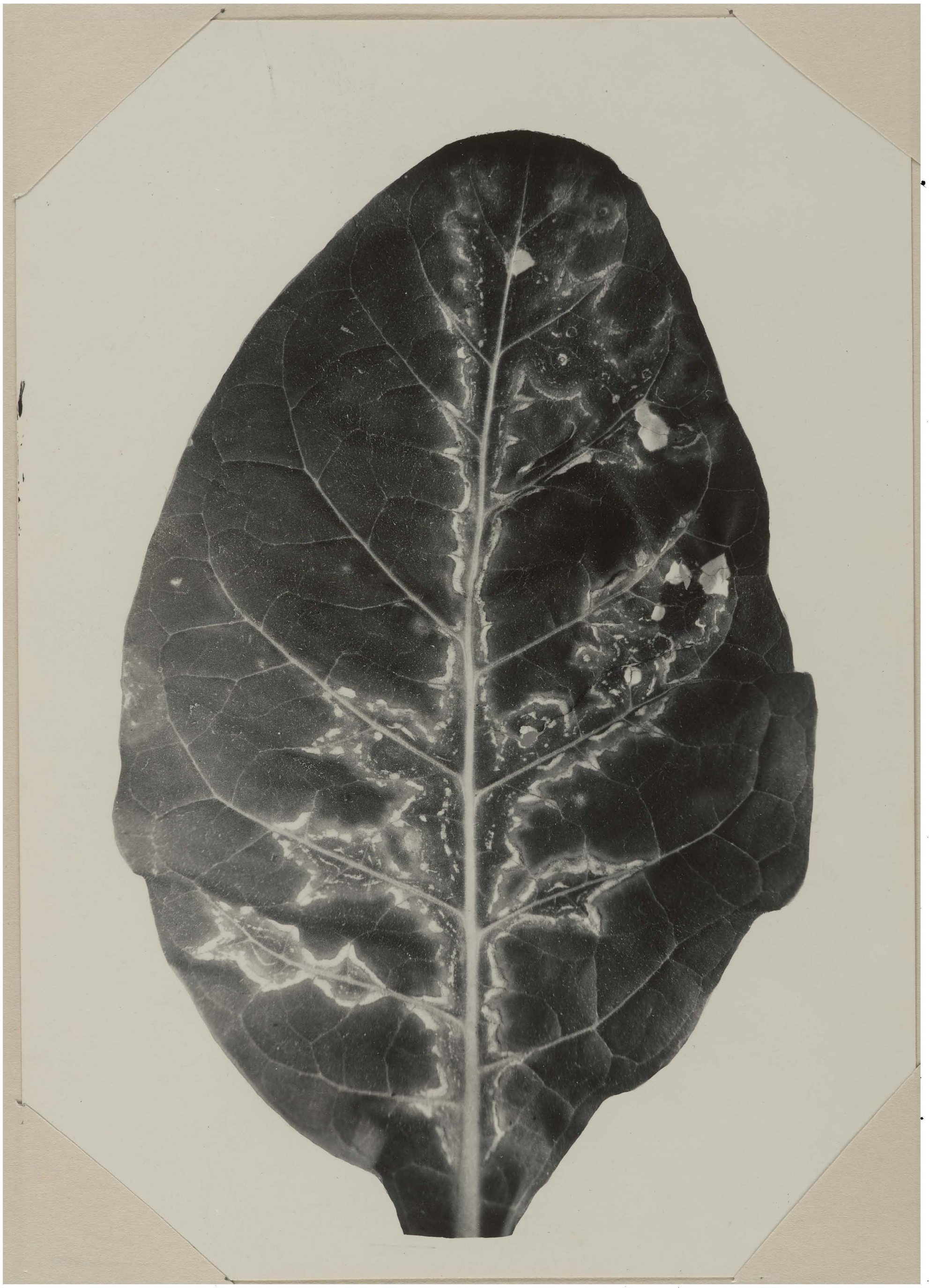

Fig. 7- Ringspot on Burley tobacco.
Ringspot infection on Burley tobacco leaf. Note how the necrotic lines follow the leaf veins.

Fig. 8- Ringspot on Burley tobacco. Severe ringspot infection on leaf of Burley tobacco. Such a leaf is practically worthless.