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CONTENTS

Page

Hosts and Symptoms of Ring Spot, a Virus Disease of Plants - - - - - - 127
S. A. WINGARD

Bacterial Pocket Disease of the Sugar Beet - - - - - - - - - - 155
NELLIE A. BROWN

Growth and Senescence in Red Danish Cows as Measured by the Rate of Milk Secretion - - - - - - - - - - - - - - - - - 169
W. L. GAINES and D. D. SHAW

Morphology and Taxonomy of the Pecan-Scab Fungus, Cladosporium effusum (Wint.) Comb. Nov. - - - - - - - - - - - - - - - - 181
J. B. DEMAREE

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. 37 WASHINGTON, D. C., AUGUST 1, 1928 No. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS [1]

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INTRODUCTION

The continual increase in prevalence of tobacco ring spot in Virginia after it was first observed in 1917, together with the severe damage that it often caused to the tobacco crop, showed it to be a disease of great economic potentiality. These facts led to a study of the disease, the results of which were published in a preliminary report by Fromme, Wingard, and Priode [3] in 1927. In this report ring-spot was shown to be an infectious disease whose causative agent is apparently a virus.

The further study of the disease, reported in this paper, relates to its occurrence and symptoms on a variety of hosts other than tobacco, and on a number of species and varieties of Nicotiana.

REVIEW OF LITERATURE

In 1922 Fromme and Wingard,[4] and in 1924 Wingard and Godkin [5] reported ring-spot infection on some of the agronomic varieties of *Nicotiana tabacum* L. in Virginia. Such reports have also been made by the Plant Disease Survey from Kentucky and Ohio. In 1927, Fromme, Wingard, and Priode [6] reported four additional species of Nicotiana as being susceptible to infection, namely, *glutinosa*, *langsdorffii*, *paniculata*, and *sylvestris;* and the following varieties of *N. tabacum: Atropurpurea*, *auriculata*, *brasiliensis*, *calyciflora*, *colossea*, *gigantea*, *lacerata*, *latissima*, *macrophylla*, and *microphylla*. The agronomic varieties, Burley, Green's Wildfire Resistant, Little Orinoco, Macedonian, and Maryland, were also shown to be susceptible.

Priode,[7] in 1928, reported ring-spot infection on beet (*Beta vulgaris* L.), pokeweed (*Phytolacca decandra* L.), petunia (*Petunia hybrida* Vilm.), and New Zealand spinach (*Tetragonia expansa* Murr.).

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[2] The writer wishes to acknowledge his indebtedness to Dr. F. D. Fromme for many valuable suggestions in this study and in the preparation of the manuscript. For the seed of the different species and varieties of Nicotiana used in the experiments the writer is especially indebted to Dr. P. J. Anderson, of the Connecticut Agricultural Experiment Station, Dr. R. E. Clausen, of the University of California; Dr. James Johnson, of the University of Wisconsin; and Dr. W. D. Valleau, of the University of Kentucky.

[3] FROMME, F. D., WINGARD, S. A., and PRIODE, C. N. RING SPOT OF TOBACCO; AN INFECTIOUS DISEASE OF UNKNOWN CAUSE. Phytopathology 17: 321–328, illus. 1927.

[4] FROMME, F. D., and WINGARD, S. A. BLACKFIRE OR ANGULAR-LEAFSPOT OF TOBACCO. Va. Agr. Expt. Sta. Tech. Bul. 25: 16–18, illus. 1922.

[5] WINGARD, S. A., and GODKIN, J. TOBACCO DISEASES IN VIRGINIA AND THEIR CONTROL. Va. Agr. Col. Ext. Bul. 90: 25–27, illus. 1924.

[6] FROMME, F. D., WINGARD, S. A., and PRIODE, C. N. Op. cit.

[7] PRIODE, C. N. FURTHER STUDIES IN THE RING-SPOT DISEASE OF TOBACCO. Amer. Jour. Bot. 15: 88–93, illus. 1928.

MATERIALS AND METHODS

The inoculum used throughout these studies was taken from a tobacco plant of the variety Kentucky Yellow obtained in Charlotte County, Va., August 20, 1926. The plant showed typical symptoms of ring spot on all of its leaves and as a result was considerably dwarfed. It was transplanted into the greenhouse bed at Blacksburg, where it continued to grow for over a year.

The ring-spot symptoms gradually failed to develop on the newly formed leaves, but the sap from the new growth continued to be infectious and would readily produce the disease on healthy plants. Infection was obtained on plants of *Nicotiana langsdorffii* Weinm. in December, 1926, and from then on transfers were made at regular intervals to healthy plants of *N. langsdorffii*, and *N. tabacum* in order to keep an abundant supply of disease on young vigorous plants.

Seed of many of the plants other than tobacco were obtained locally from growers and seedsmen, and many of the seeds were collected locally as seedlings and transplanted into the greenhouse beds. The plants in the majority of cases were grown in 4-inch pots which were sunk to about one-third of their depth in the soil of the greenhouse bed. In other cases, however, they were grown directly in the greenhouse bed soil. Plants, like tobacco, that make an erect growth were inoculated when they were 6 to 12 inches high.

The number of plants employed to represent the different hosts in the inoculation experiments varied from only 5 or 6 in a few cases to as many as 50 in others. In most cases the inoculations were repeated several times and at different stages in the development of the plant, new plants being used for each experiment, to make sure that the symptoms produced were typical. Material from 26 of the species of plants listed as hosts has been used as reinoculum on tobacco (*Nicotiana tabacum* L.), and typical ring-spot symptoms have developed in every instance. The species from which inoculum has been recovered and used to reinfect tobacco are indicated by an asterisk in the list of hosts given below. No attempt was made to obtain infection on tobacco from any of the other species, but there is no reason to believe that infection would not have been obtained on tobacco from these species had the inoculations been made.

The inoculum employed has consisted uniformly of the fresh sap and macerated tissues of heavily infected tobacco leaves, prepared by grinding the affected leaves in a mortar and adding enough tap or distilled water to dilute the sap to about 1 to 10. The inoculum was always applied immediately after being prepared in order to make sure of its virulency which disappears rapidly in expressed sap.

Infection has been transmitted readily by several methods of inoculation, but the method found to be most satisfactory and the one employed in this study is designated as the swabbing method. By this method the leaves of the trial plants are carefully swabbed with cotton plugs saturated with inoculum prepared as described above. Three or four leaves intermediate in size and age were inoculated on each plant. The inoculations were made late in the afternoon to avoid scorching of the leaves by the hot rays of the sun. No bell jars or covers of any kind were employed.

EXPERIMENTAL RESULTS

A wide selection of plants was inoculated in order to determine as far as possible the limits of the host range, and to study such variation in symptoms as might occur on different hosts. Especial attention was given to those plants which might be expected to carry the virus over winter in or near tobacco fields.

Infection was obtained on 38 genera of plants representing 17 families; namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae. The genera, species, and varieties of these families are listed below.

SOLANACEAE.—This family is well represented in the list of hosts, *Nicotiana acuminata* Grah., *N. clevelandii**[8] A. Gray, *N. glutinosa** L., *N. langsdorffii** Weinm., *N. longiflora* Cav., *N. paniculata* L., *N. plumbaginifolia** Viv., *N. quadrivalvis* Pursh, *N. quadrivalvis* var. *multivalvis* Gray, *N. repanda** W., *N. rustica* L. var. Iowa, var. English, and var. *jamaicensis*, *N. sanderae** (*Alta grandiflora* × *forgetiana*), *N. suaveolens* Lehm., *N. sylvestris** Speg., *N. tomentosa* Ruiz. and Pav., *N. trigonophylla* Dun., and *N. tabacum* L. developed infection. The following botanical varieties of *N. tabacum* also developed infection: *Atropurpurea*, *auriculata*, *brasiliensis*, *calycina*, *calyciflora*, *cavala*, *colossea*, *gigantea*, *lacerata*, *latissima*, *macrophylla*, *microphylla*, *purpurea*, *sanguinea*, and Turkish; and also the agronomic varieties, Adcock, Big Burley, Green's Wildfire Resistant, Kentucky Yellow, Little Orinoco, Lizard Tail, Macedonian, Maryland, Standup Burley, and Warne.

Other solanaceous plants found to be susceptible were *Datura stramonium** (Jimson weed), *Nicandra physalodes* (L.) Pers. (apple of Peru), *Petunia violacea** Lindl. (petunia), *Physalis angulata* L. (ground cherry), *Solanum carolinense** L. (horse nettle), *Solanum melongena* L. var. *esculentum* Nees (eggplant), *Solanum nigrum* L. (nightshade), and *Solanum pseudo-capsicum* L. (Jerusalem cherry).

CUCURBITACEAE.—Infection was readily obtained on the following representatives of this family: *Citrullus vulgaris* Schrad. (Monte Cristo watermelon), *Cucumis melo* L. var. *cantalupensis** Naud. (Honey Ball and Honey Dew cantaloupe), *Cucumis sativus** L. (Everbearing and Ideal White Spine cucumber), *Cucurbita pepo* L. var. *condensa** Bailey (Golden Summer Crookneck and Mammoth White Bush squash), *Cucurbita moschata** Duschene (Cushaw pumpkin), *Cucurbita pepo* L. (cornfield pumpkin), *Cucurbita pepo* L. var. *ovifera** Bailey (Nest Egg gourd), *Lagenaria leucantha* Rusby (Dipper gourd), and *Luffa cylindrica** Roem. (Dish Cloth gourd).

COMPOSITAE.—The list of ring-spot hosts in this family includes both weeds and cultivated plants, namely, *Ambrosia artemisiifolia* L. (small ragweed), *Ambrosia trifida* L. (giant ragweed), *Aster laevis* L. (Ostrich Plume aster), *Bidens discoidea* (T. and G.) Britton (Spanish needle), *Calendula officinalis** L. (pot marigold), *Callistephus chinensis* Nees (China aster), *Erigeron canadensis* L. (field erigeron), *Helianthus annuus* L. (Mammoth Russian sunflower), *Lactuca sativa* L. var. *capitata** L. (head lettuce), *Lactuca scariola* L. (prickly lettuce),

[8] Infection has been obtained on tobacco with an inoculum taken from the species that are marked with an asterisk.

Tagetes erecta L. (African marigold), and *Zinnia elegans** Jacq. (garden zinnia).

LEGUMINOSAE.—A number of species in this family have been shown to be susceptible to ring-spot infection, namely, *Dolichos lablab* L. (Broad Windsor bean), *Melilotus officinalis** Lam. (sweet clover), *Phaseolus lunatus* L. (small Lima or Sieva bean), *Phaseolus vulgaris** L. (kidney bean), and *Vigna sinensis* Endl. (black-eye cowpea).

The families listed below are represented by one or more host species as indicated.

CHENOPODIACEAE.—*Beta vulgaris** L. (garden beet and Silesian sugar beet), *Beta vulgaris* L. var. *cicla* L. (Swiss chard), *Chenopodium album* L. (lamb's-quarters).

VIOLACEAE.—*Viola papilionacea* Pursh (common violet), and *Viola tricolor* L. (giant pansy).

AIZOACEAE.—*Tetragonia expansa* Murr. (New Zealand spinach).

AMARANTHACEAE.—*Amaranthus paniculata* L. (pigweed).

CONVOLVULACEAE.—*Ipomoea purpurea* (L.) Roth. (morning-glory).

CRUCIFERAE.—*Barbarea Barbarea** (L.) MacM. (winter cress).

DIPSACEAE.—*Scabiosa atropurpurea* L. (sweet scabious).

EUPHORBIACEAE.—*Ricinus communis* L. (castor-oil plant).

LABIATAE.—*Salvia splendens* Ker. (scarlet sage).

MALVACEAE.—*Hibiscus esculentus* L. (okra).

PHYTOLACCACEAE.—*Phytolacca decandra** L. (pokeweed).

POLYGONACEAE.—*Polygonum hydropiper* L. (smartweed).

SCROPHULARIACEAE.—*Antirrhinum majus** L. (snapdragon).

In reading the list of host plants given above one is likely to conclude that the ring-spot virus is not very specific in its ability to infect. Such, however, does not seem to be the case, since many plants that failed to develop symptoms of any kind have been inoculated in these experiments. Of course, some of these may prove to be susceptible to infection when subjected to further experimentation, but in that event the number should be small.

No infection was obtained on the following plants: *Althaea rosea* Cav. (hollyhock), *Berberis vulgaris* L. (barberry), *Brassica alba* Rabenh. (mustard), *Brassica napobrassica* Mill. (rutabaga), *Brassica rapa* L. (turnip), *Brassica oleracea* L. var. *capitata* L. (cabbage), *Brassica oleracea* L. var. *acephala* DC. (collards), *Bryophyllum pinnatum* Kurz. (bryophyllum), *Capsicum frutescens* L. var. *grossum* Bailey (bell pepper), *Centranthus ruber* DC. (Jupiter's beard), *Chrysanthemum carinatum* L. (chrysanthemum), *Cirsium arvense* (L.) Scop. (Canada thistle), *Coleus blumei* Benth. var. *verschaffeltii* Lem. (coleus), *Cosmos diversifolius* Otto Ortg. (cosmos), *Dahlia merckii* Lem. (dahlia), *Dactylis glomerata* L. (orchard grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Dianthus barbatus* L. (sweet William), *Glycine hispida* Max. (soy bean), *Lycopersicon esculentum* Mill. (tomato), *Malconia maritima* R. Br. (stock), *Mentha piperita* L. (mint), *Oxalis repens* Thunb. (wood sorrel), *Pastinaca sativa* L. (parsnip), *Pisum sativum* L. (peas), *Phleum pratense* L. (timothy), *Pelargonium domesticum* Bailey (geranium), *Plantago major* L. (plantain), *Pyrus malus* L. (apple), *Rosa* spp. (rose), *Rumex patientia* L. (dock), *Rumex abyssinicus* Jacq. (rhubarb), *Raphanus sativus* L. (radish), *Solanum dulcamara* L. (bittersweet), *Solanum tuberosum* (potato), *Stellaria media* (L.) Cyrill. (chickweed), *Spinacea oleracea* L. (spinach), *Tragopogon porrifolius* L. (salsify), *Tradescantia flu-*

minensis Vill. (tradescantia), *Triticum vulgare* Vill. (wheat), *Tropaeolum majus* L. (nasturtium), and *Zebrina pendula* Schnizl. (Wandering Jew).

LEAF SYMPTOMS OF THE DISEASE

The symptoms of ring spot are restricted to the leaves of most of the host plants, but they may also appear on the stems and fruits in some instances, as will be described later. The symptoms vary considerably on different hosts, but still show a remarkable similarity in

FIG. 1.—Ring-spot infection on leaf of Turkish tobacco four days after inoculation. Natural size

practically all cases. Infection on the leaf usually results in the development of rings and lines that spread out in a zigzag manner, and these are the most characteristic symptoms of the disease.

Since the symptoms vary somewhat in even the most closely related plants, it might appear advisable to describe them in detail for each host. This procedure, however, is readily seen to be impractical when the large number of susceptible plants is considered. Therefore, instead of treating each host separately, those which show strikingly similar symptoms will be grouped together with a description of the symptoms that are characteristic of the group.

GROUP 1

The symptoms of the ring-spot disease are quite similar for the following varieties of *Nicotiana tabacum: Atropurpurea, auriculata, brasiliensis, calycina, calyciflora, cavala, colossea, lacerata, latissima, macrophylla, microphylla, purpurea, sanguinea,* and Turkish; and also for the agronomic varieties, Adcock, Little Orinoco, Lizard Tail, Green's Wildfire Resistant, Kentucky Yellow, Macedonian, Maryland, and Warne. The symptoms occur only on the leaves of this group of plants, and are first evident within 48 to 60 hours after inoculation.

FIG. 2.—Ring-spot infection on Turkish tobacco leaf eight days after inoculation. Note the difference in type of spot produced on the intervein tissue and that along the veins. Natural size

The initial spot consists of a small ring of necrotic tissue surrounding an island of apparently normal tissue. (Fig. 1.) The primary rings are usually not more than 1 mm. in diameter, and in some cases they are so small that they appear as mere dots. The necrotic rings or margins of the young spots appear as translucent lines in transmitted light. They become blanched or brown in color in a few days after they appear. A secondary ring or margin, 3 to 4 mm. in diameter, appears in four days (fig. 2); and a third one, 6 to 7 mm. in diameter, in about five days. This process continues when the spots are thinly distributed over the leaf until finally there are several of these necrotic rings with alternating zones of normal-appearing tissue, as in Figure 3. The center of the spot consists of the primary ring and its island of encircled tissue. The tissue in the center of the spot often dies in five or six days after the spot appears, especially if the primary ring is very small in diameter. In such cases the center of the spot has the appearance of a small dot.

The outlines of the spot vary according to the location. They are circular, as described above, when centered on intervein tissue, but when centered on the larger veins and midrib they are very irregular in outline. (Figs. 2, 4, and 5.) The infection follows the vein and

its branches, and the outline of the spot often suggests that of a deeply lobed leaf. (Figs. 4 and 5.)

Infection becomes systemic in 8 to 14 days after inoculation. If some of the leaves of a medium-sized plant are inoculated, local symptoms will appear in 48 to 60 hours, and in about 6 days symptoms will begin to appear on the uninoculated leaves, especially on the upper ones. The infection will continue up the plant, and in 10 to 14 days all the tiny bud leaves will show typical ring-spot symptoms. The symptoms of systemic infection show a marked tendency to fol-

FIG. 3.—Leaf of Green's Wildfire Resistant tobacco showing ring-spot infection three weeks after inoculation. Natural size

low the midrib and larger veins of the leaves, yet the characteristic circular spots appear on the intervein tissue. (Fig. 5.) As soon as the infection becomes systemic and symptoms appear on all the leaves of the plant—in about 10 to 14 days after inoculation—new leaves will appear on which symptoms may either fail to develop altogether or will develop only slightly, appearing only on the tip half of the leaf. The symptoms gradually fail to develop on the new growth, and those that do develop are located nearer and nearer the tips of the leaves until finally only faint symptoms can be detected at the very tip of the leaf. (Fig. 6.) New leaves produced after this stage

has been reached may appear normal, or they may show a faint grayish mottling. In some cases these apparently normal leaves on affected plants develop numerous brown necrotic specks. The margins are necrotic in the first spots that appear on these plants, but as new leaves appear and as the symptoms begin to become less conspicuous the margins of the spots are no longer necrotic but are chlorotic or only of a lighter green color than the normal tissue.

The leaves of plants on which the ring-spot symptoms have become masked appear to be a little thicker and to have more of a leathery

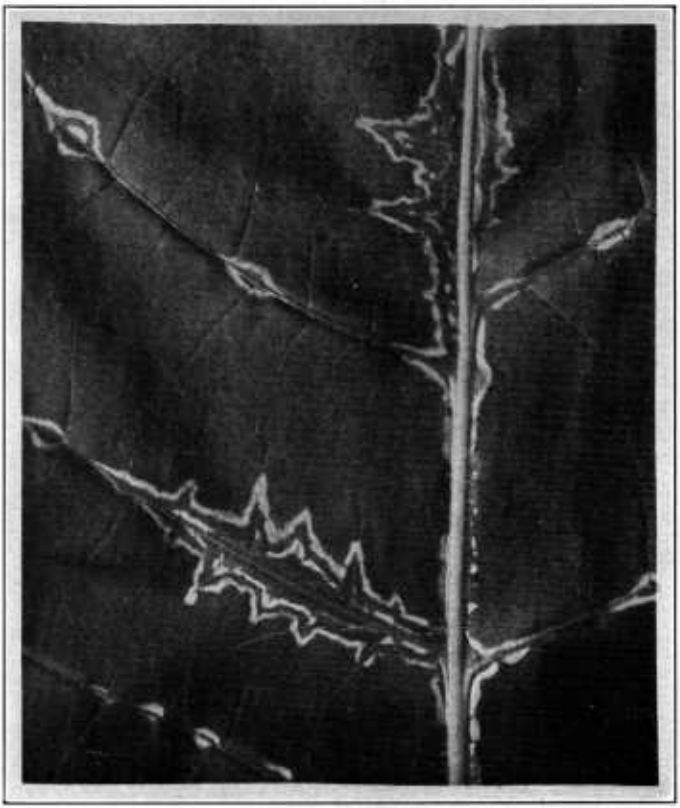

FIG. 4.—Symptoms of ring spot on a leaf of *Nicotiana tabacum* var. *purpurea* as a result of systemic infection. Natural size

texture than those of normal plants. (Fig. 7.) The leaves of suckers that develop from these old plants usually appear normal or show only a grayish mottling. However, typical ring-spot symptoms have appeared on the leaves of such suckers in a few instances. Several attempts have been made to produce ring-spot symptoms on the young leaves of both suckers and plants that have reached the stage of masked symptoms by reinoculating them with virus from severely affected leaves, but the results have been negative in every instance. On the other hand, the juice from these apparently healthy leaves readily produces typical symptoms when used to inoculate healthy plants. This masking of symptoms, or development of immunity, or

whatever it is, seems to hold under greenhouse conditions for practically all the plants tested.

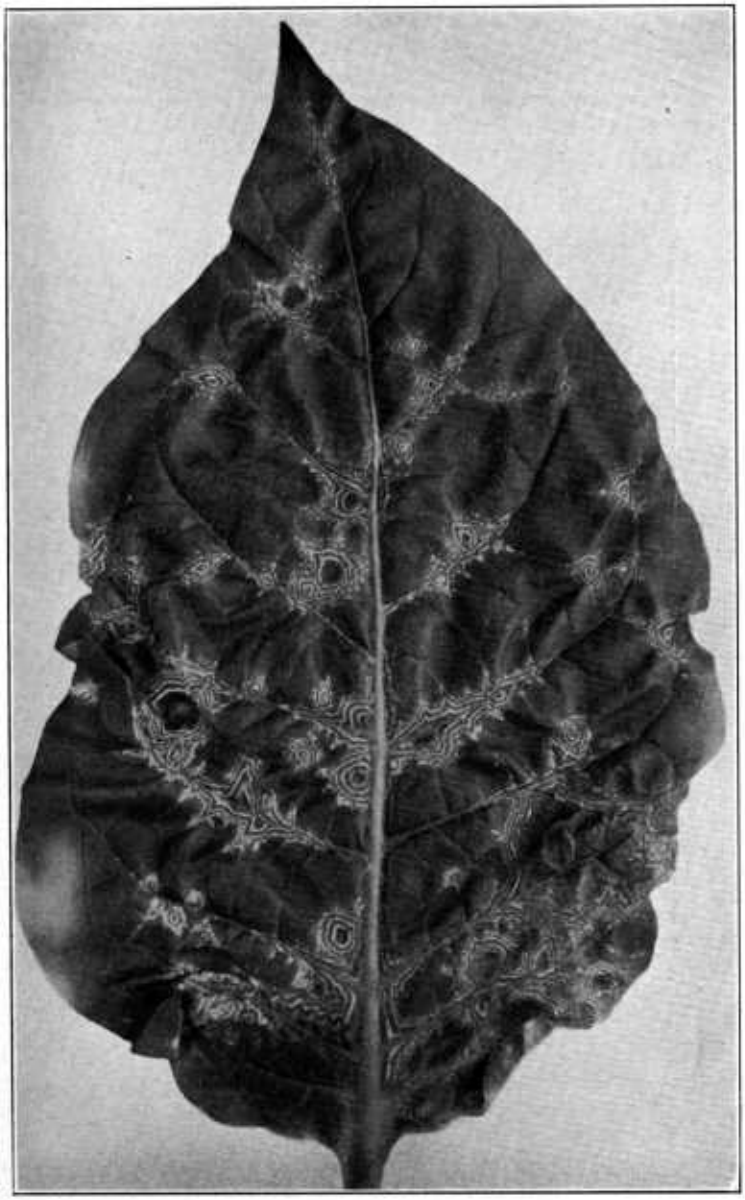

FIG. 5.—Systemic ring-spot infection on a leaf of Turkish tobacco. The lower leaves on the plant from which this leaf came were inoculated five weeks before this leaf was photographed. Natural size

GROUP 2

This group is represented by *Nicotiana rustica* var. English, *N. tabacum* var. *gigantea*, and the Burley strains of *N. tabacum*, namely,

Big Burley and Stand-up Burley. The ring-spot symptoms begin to appear on the leaves of these plants in about three days after inoculation. The first sign of infection is the development of a small necrotic spot which is surrounded by a definite light yellow halo. (Fig. 8.) The spots at this stage look almost exactly like wildfire spots, caused by *Bacterium tabacum* Wolf and Foster. The center of the spot may begin as a small brown speck or it may at first be composed of a small ring of necrotic tissue surrounding an island of apparently normal tissue. The tissue within the ring becomes chlorotic and then necrotic, giving the spot its characteristic center. The necrotic centers, 1 to 5 mm. in diameter, are surrounded by a definite light

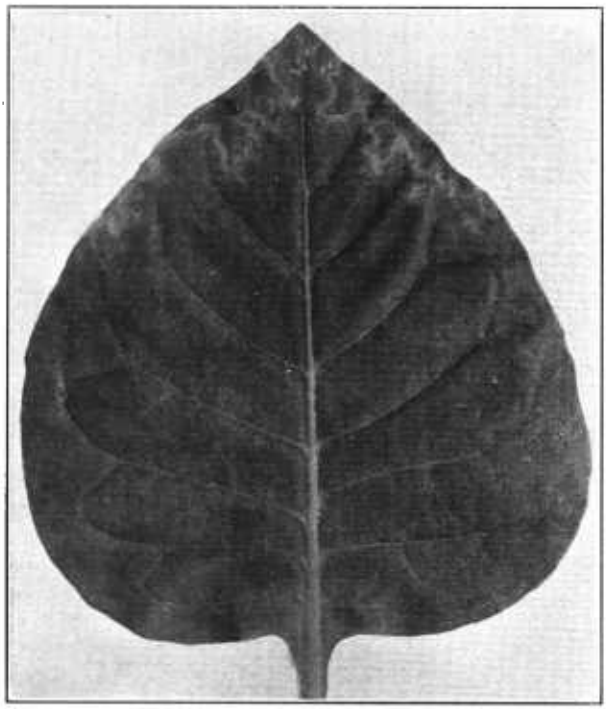

FIG. 6.—One of the top leaves of a Turkish tobacco plant showing the last trace of ring-spot symptoms on its tip. Lower leaves from the same plant were severely affected. Slightly reduced

green halo, at the margin of which a broken or a continuous line of necrotic tissue develops. This ring soon becomes surrounded by a light green halo, which in turn develops a necrotic line at its margin. This process continues until several such rings are developed and the spot measures 10 to 20 mm. in diameter. Numerous fine specks often develop in the alternating zones of living tissue.

Infection becomes systemic in 10 to 14 days and the symptoms begin to appear on the bud leaves. The leaves of intermediate size develop circular necrotic spots, or lines of necrotic tissue surrounding circular islands of living tissue, and zigzag lines of necrotic tissue running at random in the intervein tissues. (Fig. 9.) The young bud leaves are often almost completely destroyed. Big necrotic spots

appear and the leaf becomes twisted and distorted and never makes much growth. Another characteristic symptom on the leaves of Burley tobacco is the development of irregular lines of necrotic tissue

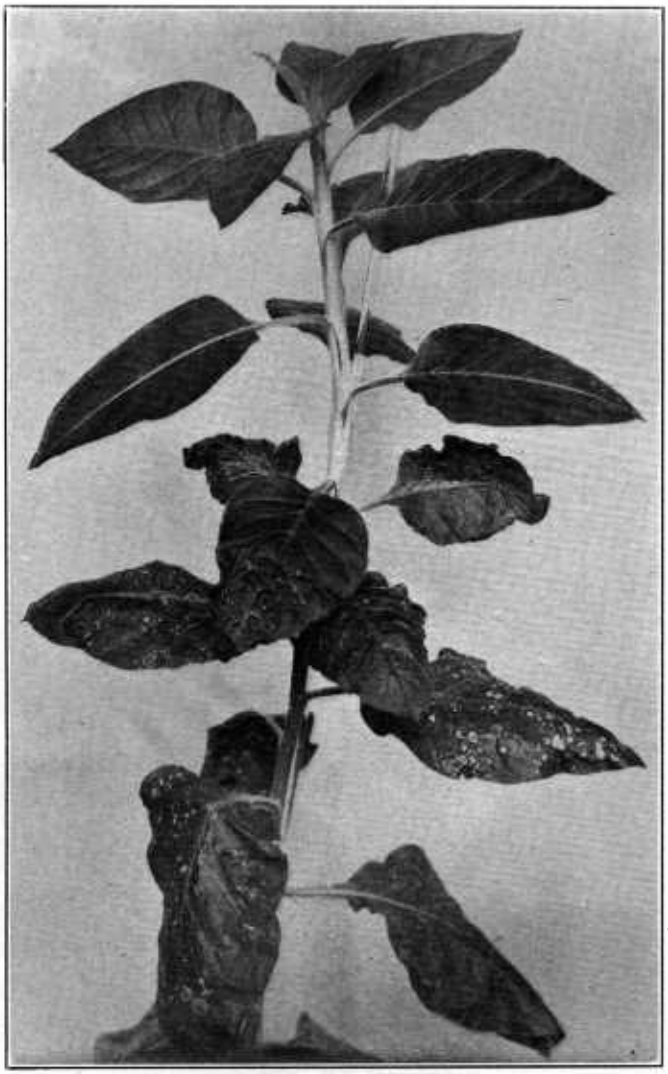

FIG. 7.—Turkish tobacco plant 23 days after inoculation with ring-spot. Note the gradual decline in the development of ring-spot symptoms on the upper leaves until finally the top leaves appear perfectly normal. Much reduced

along the midrib and larger veins as shown in Figure 10. There is a marked dwarfing of the entire plant as a result of infection.

The plants in this group also, sooner or later, develop an apparent immunity or tolerance to the disease. New leaves develop on which no symptoms appear, and yet the sap from such leaves is quite virulent when used as inoculum on healthy plants.

GROUP 3

This group includes pokeweed, petunia, *Nicotiana langsdorffii*, and *N. rustica* var. Iowa and var. *jamaicensis*. The symptoms of infection on these plants are similar in many respects to those of the plants in Group 1. The spots appear in about three days after inoculation

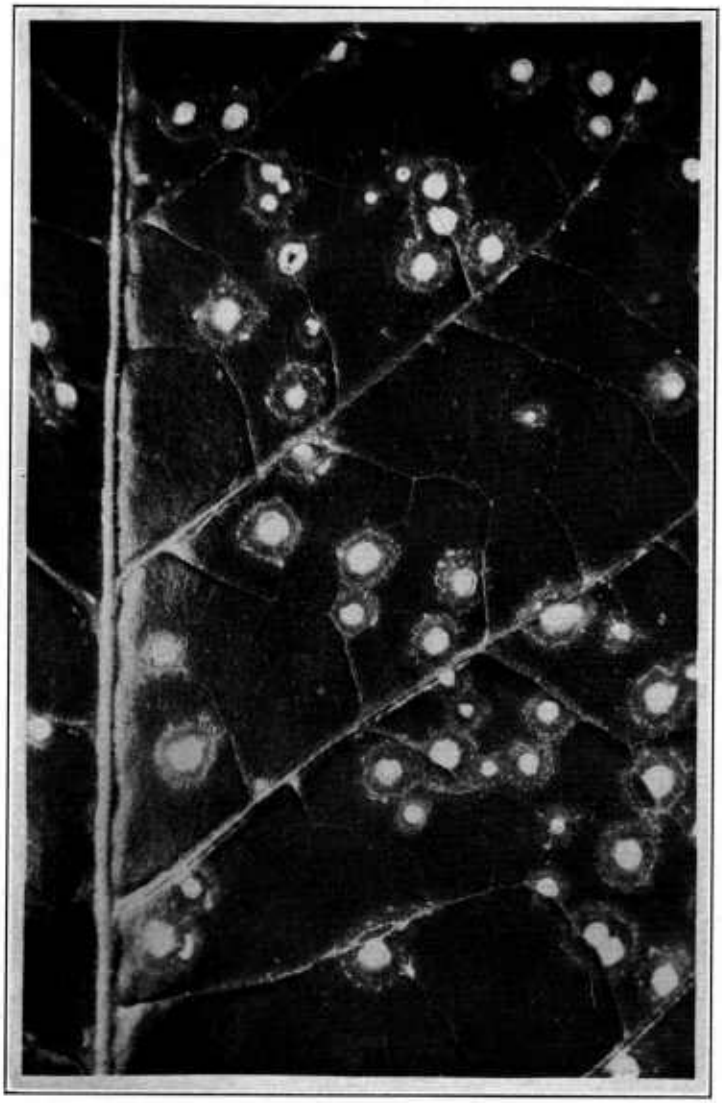

FIG. 8.—Ring-spot infection on leaf of Big Burley tobacco 10 days after inoculation. About natural size

and are composed of an island of normal green tissue surrounded by a continuous line of necrotic tissue which at first has a water-soaked appearance, but after a few days dries out and becomes perfectly white, as shown in Figure 11. The spots on pokeweed usually agree

with the above description, but in some cases they are more compact and the marginal lines instead of being composed of continuous lines of necrotic tissue are made up of a series of necrotic specks. (Fig. 12.)

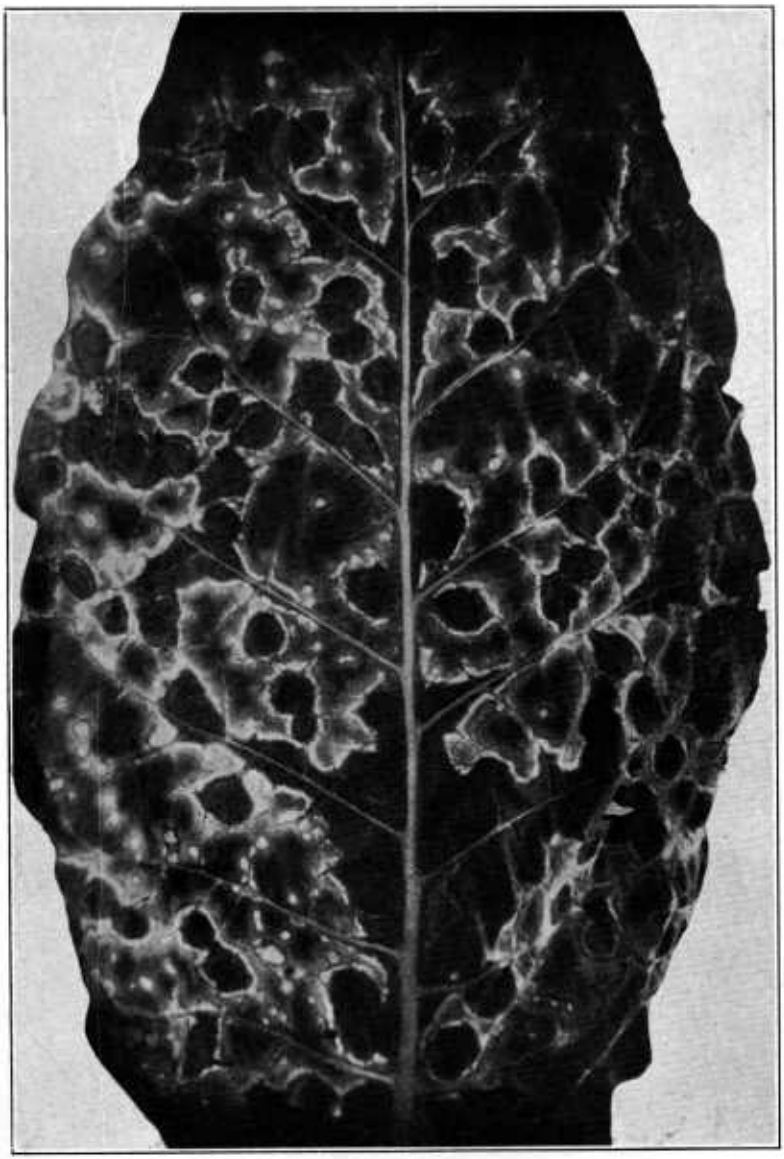

FIG. 9.—Systemic ring-spot infection on one of the top leaves of a Big Burley tobacco plant six weeks after some of the lower leaves had been inoculated. Slightly reduced

The primary rings are followed in two or three days by secondary rings of the same type and the secondary rings are in turn followed by tertiary ones. The tissue intervening between the necrotic mar-

gins retains its normal green color. Infection becomes systemic and for a time the new leaves will develop the typical spots, but after two to four weeks new leaves form on which the symptoms develop only faintly with the marginal lines no longer becoming necrotic. This continues until finally no symptoms appear on the new growth. The old spots on pokeweed leaves become masked by a thin velvety growth which is of a Tyrian rose color.

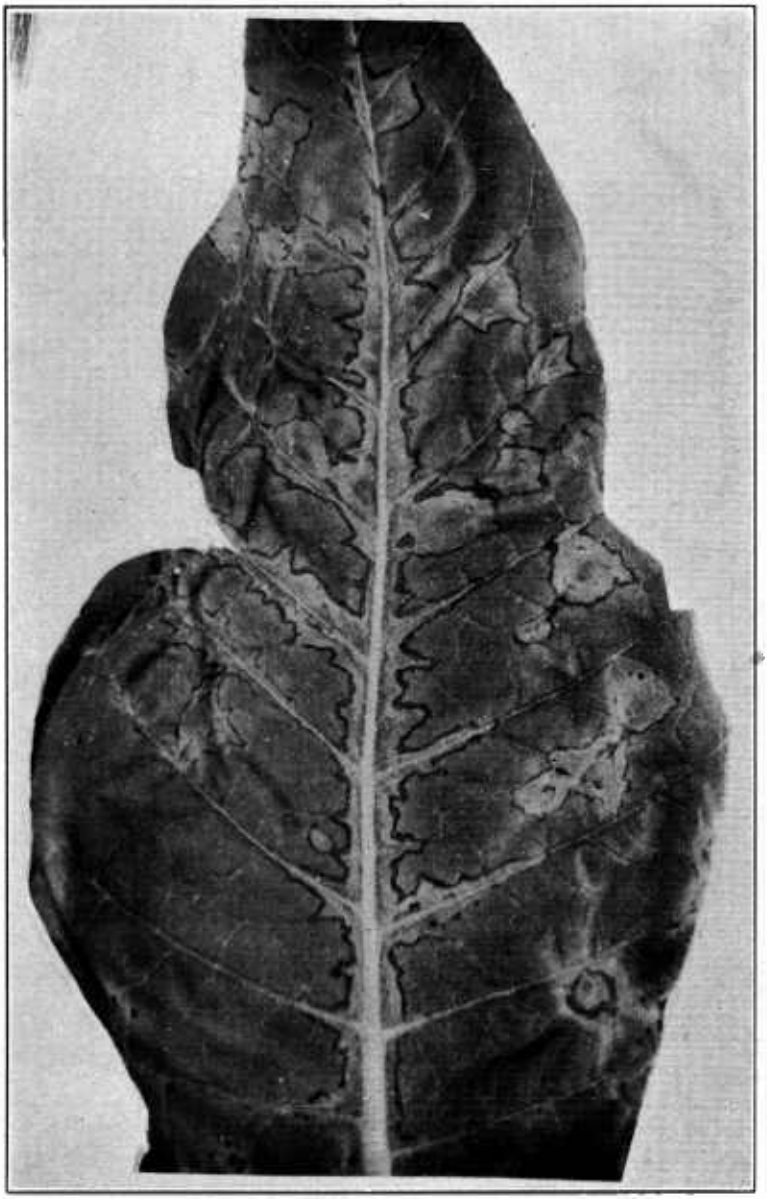

FIG. 10.—Systemic ring-spot infection on leaf of Big Burley tobacco. Note how the necrotic lines follow the midrib and veins of the leaf. Slightly reduced

GROUP 4

This group is represented by a wide variety of plants, all of which seem to be hypersensitive to the ring-spot virus. It includes the following species of Nicotiana: *Clevelandii, glutinosa, longiflora, multivalis, plumbaginifolia, quadrivalis, repanda, sanderae, suaveolens, sylvestris,* and *trigonophylla;* also garden aster, China aster, kidney bean, Lima bean, Broad Windsor bean, castor-oil bean, eggplant, water-

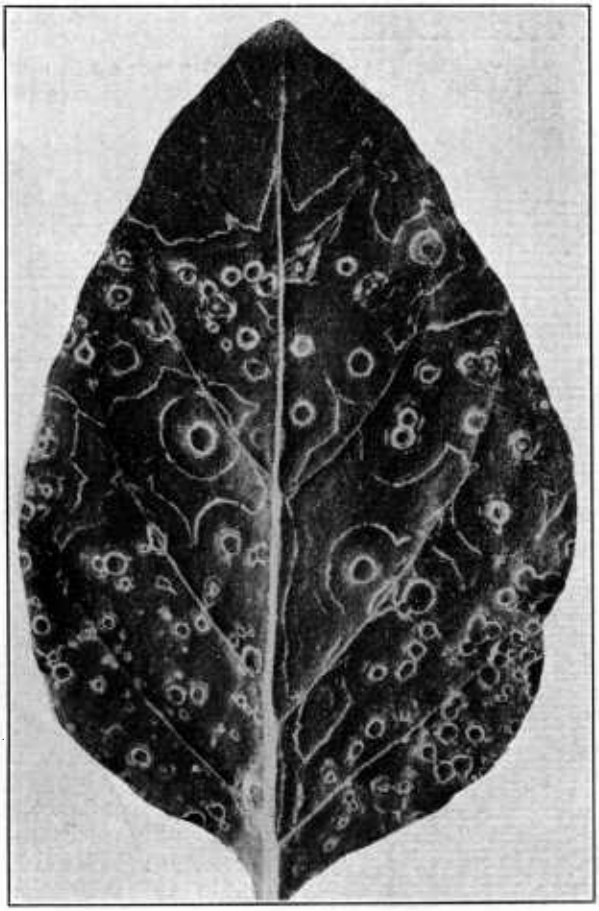

FIG. 11.—Ring-spot infection on leaf of *Nicotiana langsdorffii* three weeks after inoculation. About two-thirds natural size

melon, Jimson weed, prickly lettuce, horse nettle, New Zealand spinach, ground cherry, and snapdragon.

The most characteristic symptom of infection for this group is the development of spots that are necrotic throughout. The primary infection on certain of these plants, namely, *Nicotiana sylvestris, N. repanda, N. glutinosa,* Lima bean, garden bean, castor-oil bean, cowpea, watermelon, and horse nettle, appears as definite necrotic spots in three days after inoculation. The spot has a light center about 1 mm. in diameter (figs. 13 and 14) which is surrounded by a band

of dark brown tissue. The margin of the spot consists of newly killed tissue which has a black, water-soaked appearance. This marginal tissue turns brown on drying and a new necrotic band is formed. Thus the spots enlarge until they finally coalesce and either kill the entire leaf or large sections of it. (Fig. 15.) The initial symptoms on certain other plants of this group, of which *N. sanderae*, *N. multivalvis*, snapdragon, and New Zealand spinach are typical examples, are typical rings as described for plants in Group 1. (Fig. 16.) The spots remain as alternating zones of living and necrotic tissue for about a week and then the entire spot becomes necrotic and the necrosis spreads until the spots coalesce and kill the entire leaf or large areas thereof. (Fig. 15.) The original necrotic lines can still be seen in the necrotic spots on this leaf.

FIG. 12.—Systemic ring-spot infection on pokeweed leaf. Natural size

The foregoing relates to symptoms that develop on the parts of the leaf to which the inoculum was applied. After infection has become systemic in these plants, spots develop on the new leaves that are composed of rings of necrotic tissue intervening between zones of apparently normal tissue. (Fig. 17.) Finally, the symptoms fail to appear on the new growth.

GROUP 5

This group is represented by cantaloupe (Honey Ball and Honey Dew), cucumber (Everbearing and Ideal White Spine), gourd (Nest Egg, Dipper, and Dish Cloth), pumpkin (Cornfield and Cutshaw), and squash (Golden Summer Crookneck and Mammoth White Bush). These plants are very susceptible to ring-spot infection, as shown by the abundant development of symptoms within three days after inoculation. The young spots on the leaves consist of a small yellow to brown pin-pointlike center surrounded by a light-yellow margin or halo. (Fig. 18.) The pin-point center in some cases becomes necrotic, but as a rule the necrosis does not spread throughout the spot. Although the type of spot described above is the most typical for this group of plants, there is still a tendency for definite rings to appear, especially on the leaves to which the inoculum is

applied. In some cases the tiny brown centers are surrounded by two or three necrotic rings.

Infection becomes systemic in about 10 days and the halo type of spot appears in great numbers on all the new leaves. (Fig. 18.) In the case of the dipper gourd the leaf is literally covered with light-yellow spots no more than 1 mm. in diameter. These tiny spots have definite pin-pointlike centers that are water soaked in appearance. On squash and Nest Egg gourd the spots are much larger (figs. 18 and 19) but are of the same type. On pumpkin the spots are

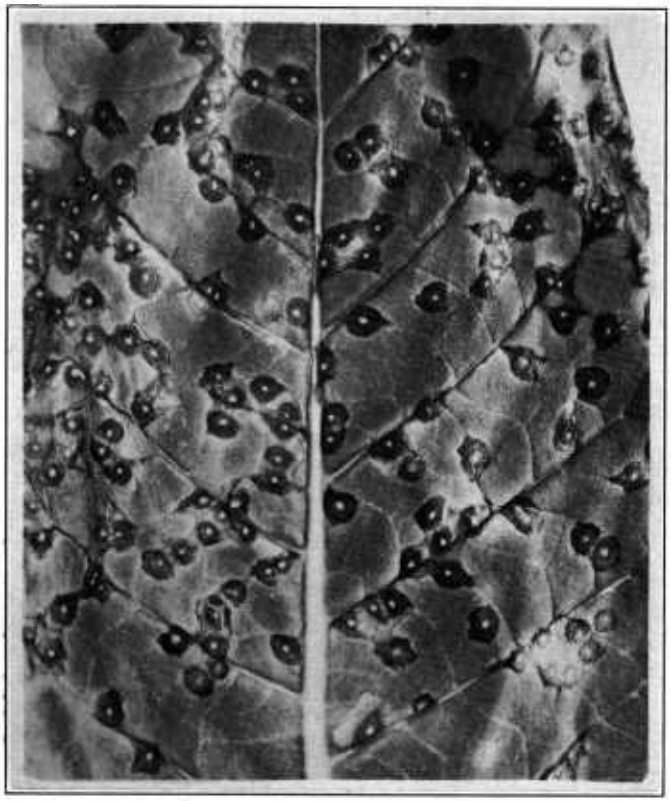

FIG. 13.—Ring-spot infection on leaf of *Nicotiana sylvestris* 10 days after inoculation. Note the light centers and brown margins of the spots. Natural size

restricted primarily to the leaf veins. (Fig. 20.) The halo type of spot also appears on cantaloupe, cucumber, and Dish Cloth gourd; but in addition to this definite rings and zigzags often develop. The lines and zigzags are much lighter in color than the normal green leaf tissue but as a rule there is no necrosis.

GROUP 6

This is a miscellaneous group in which all the remaining host plants have been placed, even though their ring-spot symptoms may differ slightly from each other. In general, infection on these hosts

produces either the ringlike spot or the fine zigzag lines that run at random across the leaf. There is not sufficient difference in the symptoms on these plants to warrant separating them into main groups, but they will be placed in subgroups according to their ring-spot symptoms.

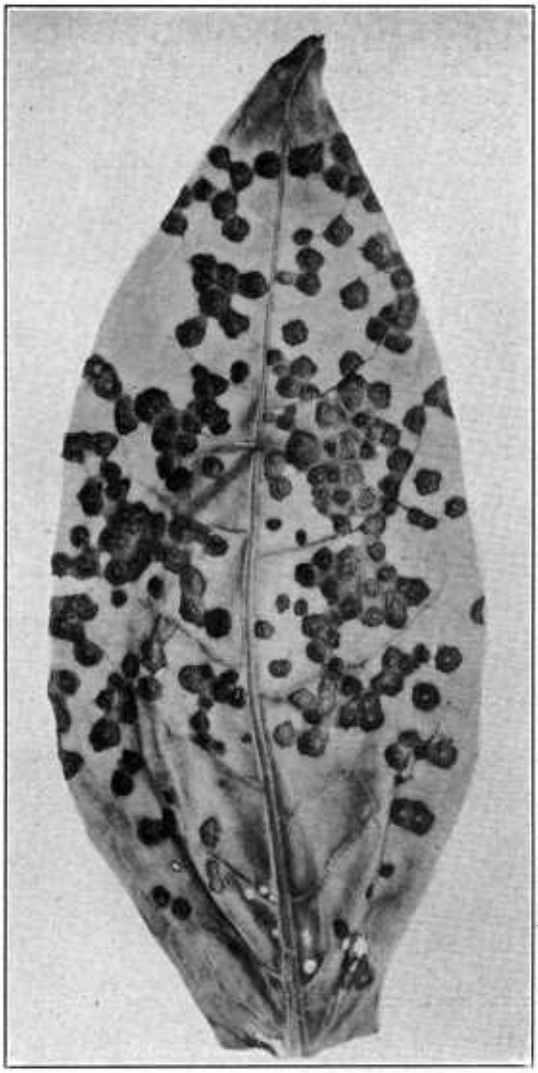

FIG. 14.—Ring-spot infection on leaf of *Nicotiana repanda* two weeks after inoculation. Note the brown necrotic spots. Natural size

Garden beet, sugar beet, Swiss chard, pot marigold, and *Nicotiana tomentosa* develop very faint zigzag lines on their leaves as a result of ring-spot infection. These lines are of a lighter color than the normal tissue but are never necrotic.

Infection on *Nicotiana acuminata*, pigweed, lamb's-quarters, field crigeron, morning-glory, winter cress, small ragweed, giant ragweed, violet, giant pansy, sweet scabious, night shade, apple of Peru, and sweet clover produces definite necrotic rings with the tissue within the ring usually remaining green.

On Spanish needle, African marigold, and smartweed the symptoms develop as very fine zigzag lines which may or may not form inclosures with irregular borders. This type of infection is shown in Figure 21. The tissue in the marginal lines becomes necrotic and turns white.

Infection on Jerusalem cherry results in the formation of both rings and zigzags on the leaves. (Fig. 22, A.) The rings and zigzags are made up of chlorotic tissue which never becomes necrotic. On scarlet sage definite light-green lines develop along the midrib and larger veins of the leaf. This is especially true of the symptoms that develop on this plant as a result of systemic infection. Infection on sunflower leaves produces a large circular spot, composed of a

center of normal green tissue surrounded by a broad band of light green. This light green border becomes necrotic with age and takes

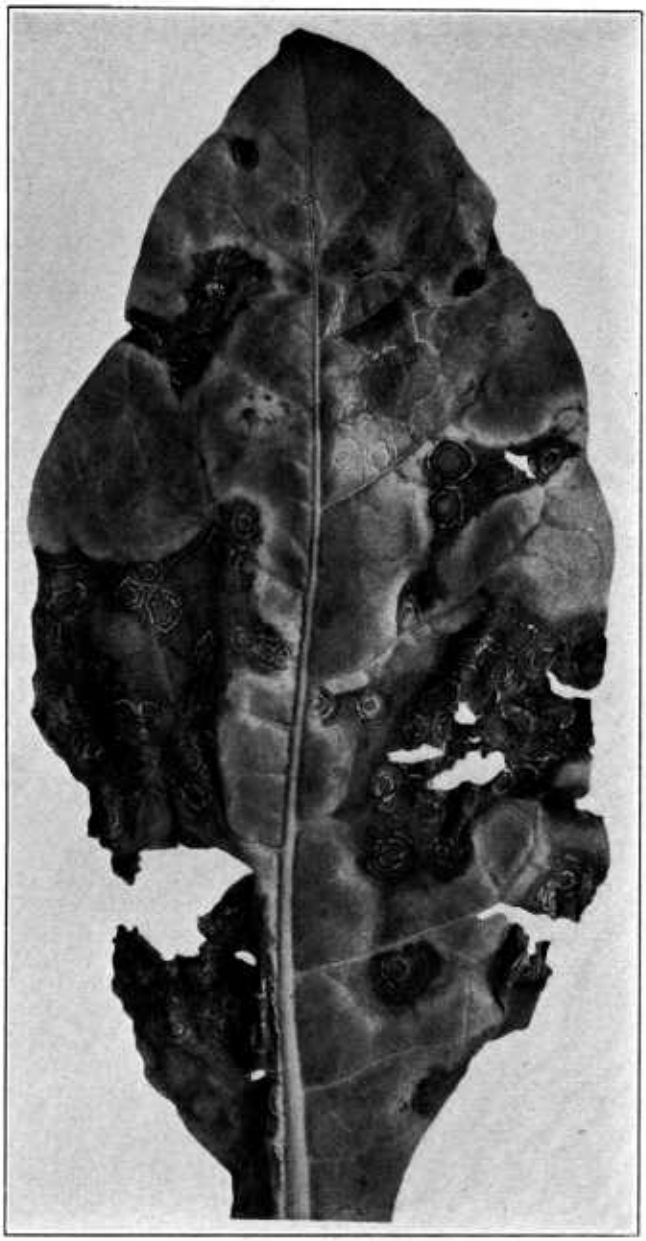

FIG. 15.—Ring-spot infection on leaf of *Nicotiana sanderae*. Note the definite concentric rings which are later followed by a complete necrosis of all the surrounding tissues. Four-fifths natural size

on a brown color. Some of the spots are a centimeter or more in diameter. Infection becomes systemic and produces brown necrotic

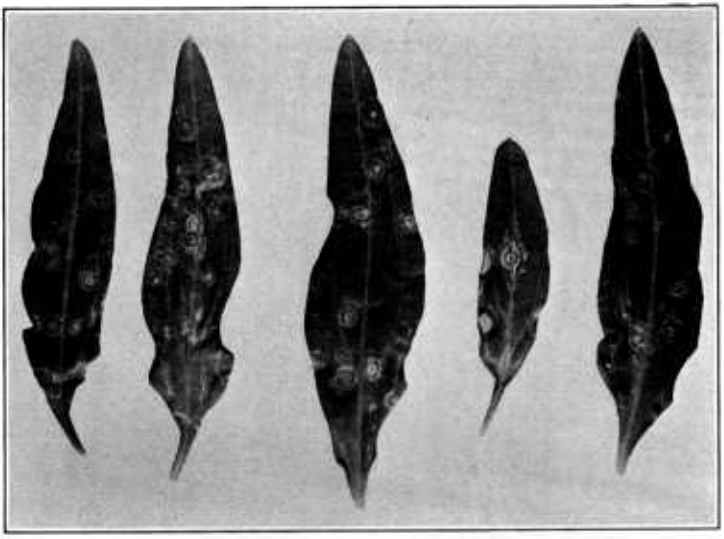

FIG. 16.—Ring-spot infection on snapdragon leaves a week after inoculation. Natural size

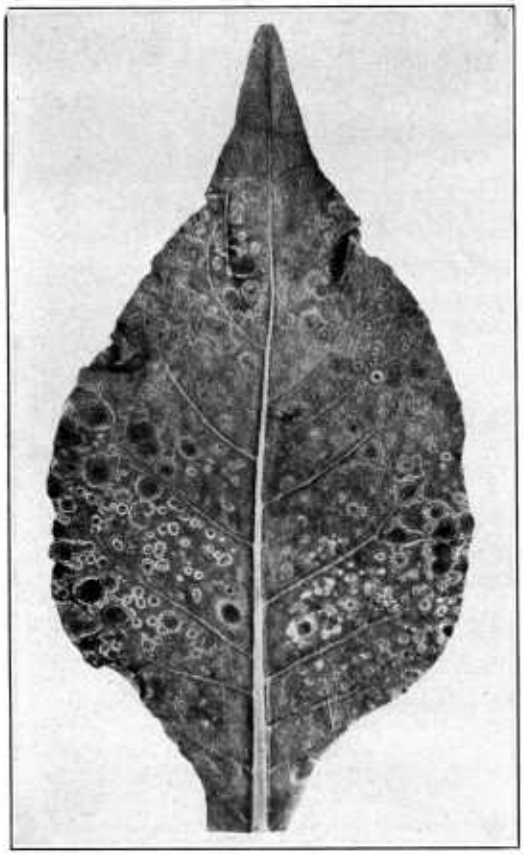

FIG. 17.—Systemic ring-spot infection on leaf of *Nicotiana plumbaginifolia*. Natural size

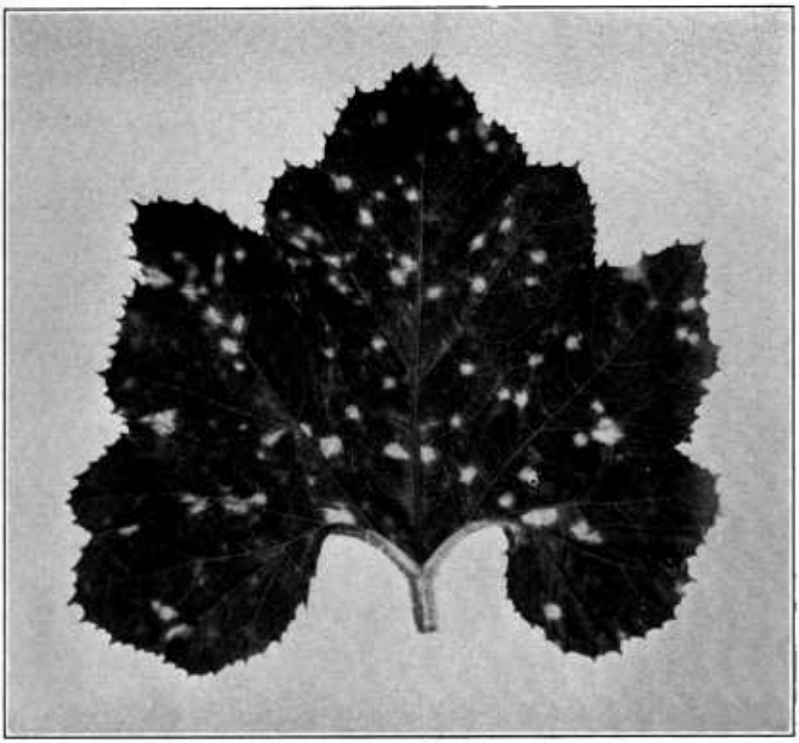

FIG. 18.—Systemic ring-spot infection on leaf of Golden Summer Crookneck squash. Natural size

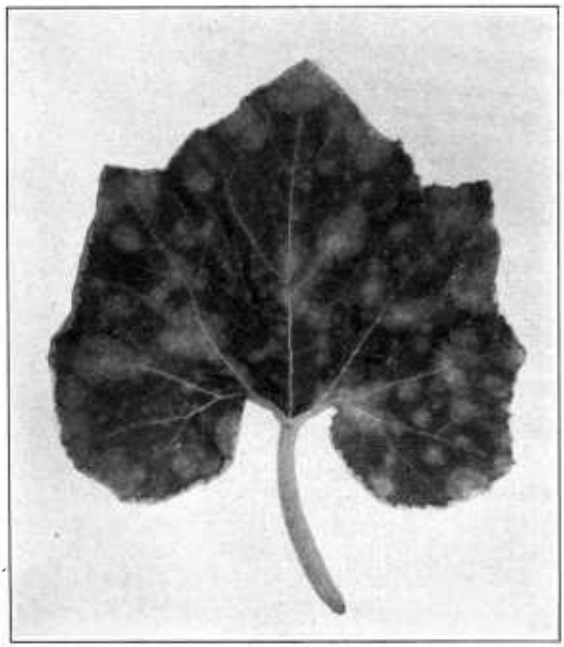

FIG. 19.—Systemic ring-spot infection on leaf of Nest Egg gourd. Natural size

rings on the involucral bracts of the flower. The symptoms on okra leaves are similar to those on sunflower except that the margins of the spots never become necrotic. No definite ring symptoms develop on the leaves of zinnia and head lettuce, and yet infection becomes systemic in these plants. The new leaves that develop after these plants have been inoculated show a marked stunting and in some cases are slightly crinkled but no other symptoms have been observed. The juice from such leaves, however, produces typical ring-spot symptoms when used as inoculum on Turkish tobacco.

STEM SYMPTOMS OF THE DISEASE

As a rule, symptoms do not occur on the stem of the host plant as a result of ring-spot infection; but in exceptional cases the stem

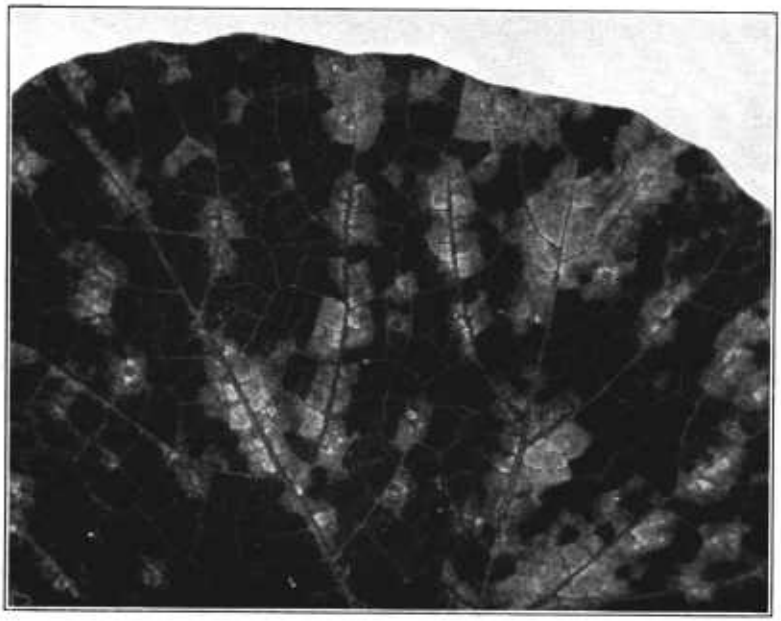

FIG. 20.—Systemic ring-spot infection on leaf of cornfield pumpkin. Natural size

shows marked symptoms and the entire plant may be killed. Stem lesions are most likely to occur on plants that are hypersensitive to the ring-spot virus, such as those listed in Group 4.

Representatives of the following plants have been killed outright in one or more of the inoculation experiments: *Nicotiana clevelandii*, *N. glutinosa*, *N. longiflora*, *N. multivalvis*, *N. plumbaginifolia*, *N. quadrivalvis*, *N. sanderae*, and *N. sylvestris*. The symptoms first appear on the leaves as dark circular necrotic spots. Then dark sunken lesions develop on the leaf petioles, and in about 10 days similar lesions appear on the main stem of the plant, in some cases extending its full length. The plant becomes twisted and dies in two or three weeks. The vascular tissues of the stem and roots become black before the plant dies. One of the *N. glutinosa* plants was

killed only to the ground line and later sent up suckers. This is very unusual, however, because the roots are usually destroyed in cases of such severe infection.

As noted, the above-named plants may or may not be killed outright by the ring-spot infection; but the following species are apparently always killed outright: Snapdragon, kidney bean, Lima bean,

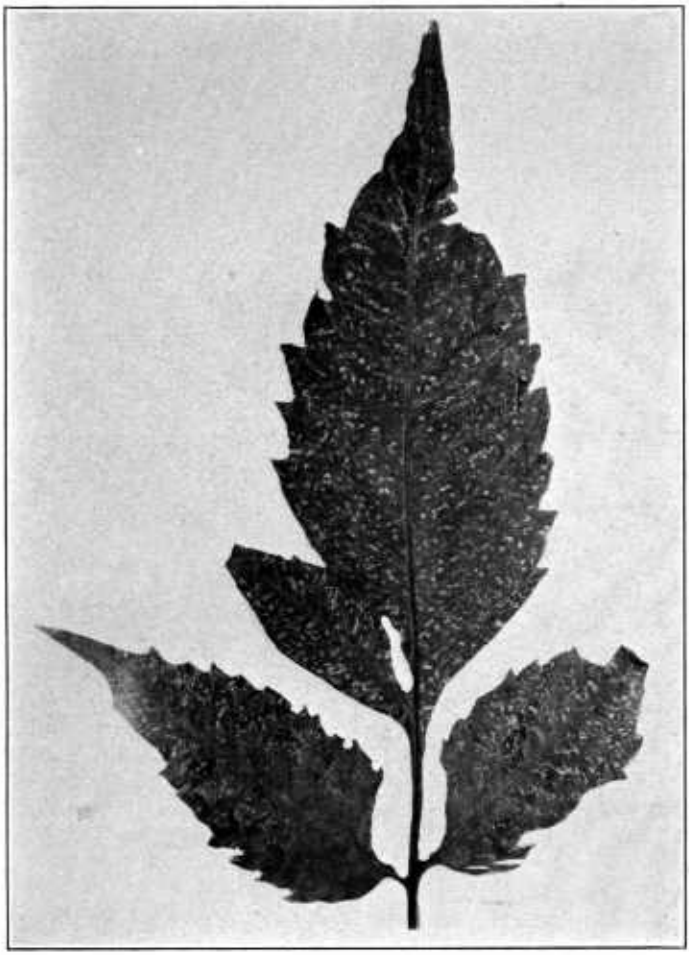

FIG. 21.—Ring-spot infection on leaf of Spanish needle. Note the very fine lines on this leaf. About natural size

Broad Windsor bean, cowpea, and New Zealand spinach. The necrotic spots that appear on the leaves of these plants are followed in two or three days by an infection of the leaf veins and petioles. The veins turn black and shrivel, and the lesions on the petioles become dark and sunken; and in the case of kidney bean, the infection looks very much like that of anthracnose. Dark sunken lesions also appear on the main stem, and the plants die in 10 to 14 days.

FRUIT SYMPTOMS OF THE DISEASE

Symptoms of ring-spot infection have recently been obtained on the fruits of some of the hosts; and, so far as the writer knows, this

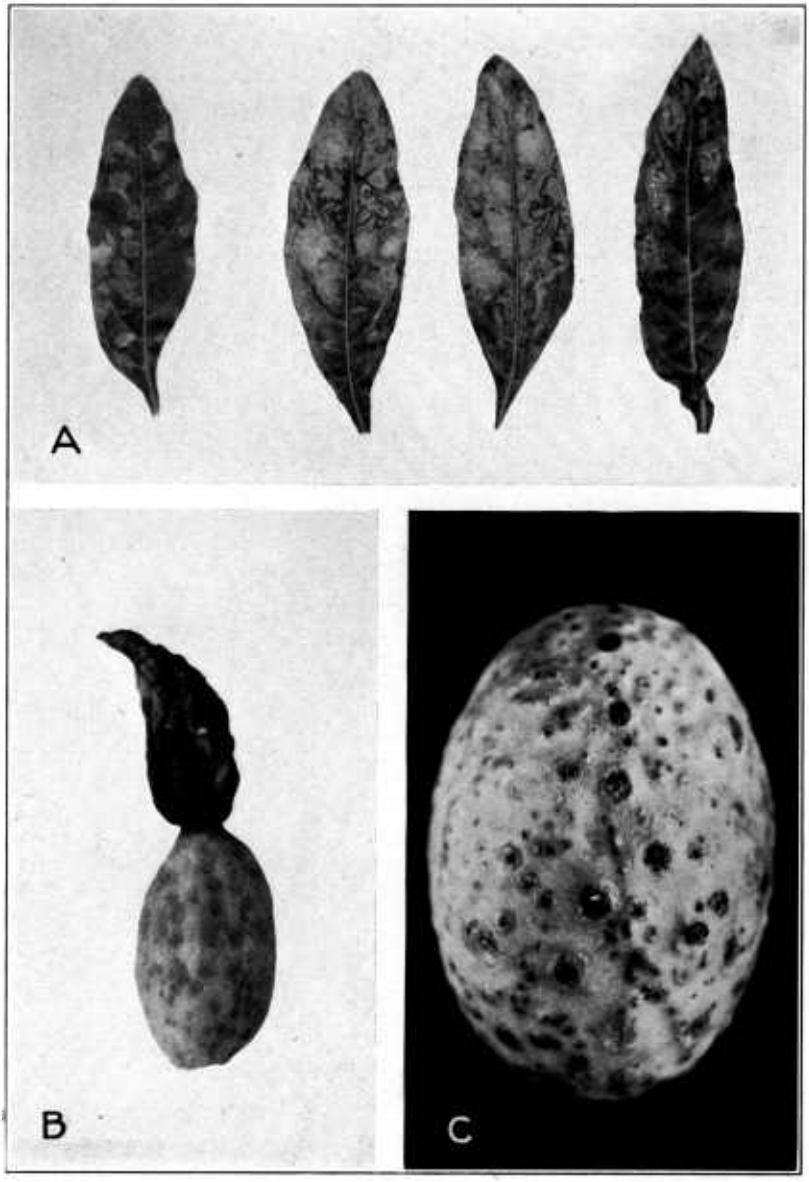

FIG. 22.—A, systemic ring-spot infection on the leaves of Jerusalem cherry. B, ring-spot infection on a young Nest Egg gourd; infection came from the vine. C, mature fruit of Nest Egg gourd showing ring-spot symptoms as a result of systemic infection in the plant on which it was produced; note the concentric rings of the spots. All natural size

is the first time the symptoms have ever been observed on fruits. These observations were made on the fruits of Nest Egg gourd and Golden Summer Crookneck squash, which were grown in the green-

house. The leaves of these plants were inoculated with the ring-spot virus when the plants were about 2 weeks old. Infection became systemic, and all the new leaves developed typical symptoms; and when the young fruits appeared they also showed the characteristic ring-spot symptoms.

The fruits show the ring-spot symptoms very early and often drop when they are only 1 or 2 inches in length. The symptoms first appear as small, circular, water-soaked spots not more than 1 mm. in diameter. (Fig. 22, B.) They become depressed in a few days and give the surface of the fruit a pitted appearance. (Fig. 23.) These pits become encircled by a very narrow line of water-soaked tissue in four or five days. These circular margins or rings are more easily seen when thin horizontal sections are cut from the surface of the fruit. The spots penetrate to a depth of 2 to 3 mm. as a rule, and in some cases can be traced all the way to the seed cavity. The spots develop a deep green pigment in contrast to the white or yellow pigment of the normal tissue. (Fig. 22, C.) Three of the infected Nest Egg gourd fruits grew to maturity, and the spots which had at first been depressed gradually became elevated and finally appeared as pimples on the surface of the mature fruit. (Fig. 22, C.) The spot in its final stage is composed of a small elevated center surrounded by one or more definite rings.

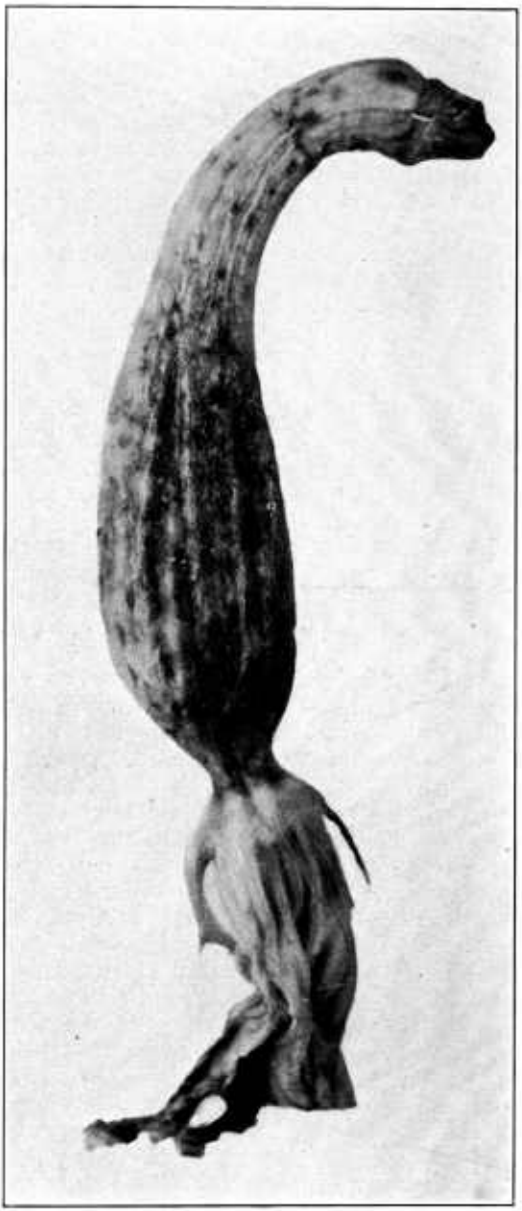

FIG. 23.—Ring-spot infection on a young Golden Summer Crookneck squash. Infection was systemic in the plant on which this fruit was produced. Natural size

DISCUSSION OF RESULTS

The results of the inoculation experiments show that the ring-spot virus has a very extensive host range. They also show that the virus is very specific in its infective properties, since so many of the plants that were tested failed to develop infection, and especially the solanaceous plants, pepper, potato, and tomato. One should naturally expect plants that are so closely related to tobacco to be susceptible. Repeated attempts were made to infect these plants but the results were always negative.

The list of susceptible plants includes horse nettle, pokeweed, and sweet clover, all of which may play an important part in the overwintering of the ring-spot virus. Horse nettle is a common weed in tobacco fields throughout Virginia, and it may serve as a constant source of infection.

Pokeweed is common in old plant-bed sites and around the borders of tobacco fields. This plant is very susceptible to ring-spot infection, and the sap from its infected leaves is very infectious on tobacco. Infection becomes systemic in this plant, but the symptoms fail to appear on the new growth a short time after the plant becomes infected. It appears possible, therefore, that apparently normal pokeweed plants may carry the virus and act as a reservoir of inoculum for tobacco.

These results also help to explain why ring-spot is usually present on tobacco that is grown in old garden and building sites. Pokeweed, Jimson weed, and horse nettle are always in evidence around such places and, if infected, may serve as a source of infection for tobacco.

Ring-spot infection is often very severe on tobacco crops that follow clovers and alfalfa in rotation. Such fields have been observed in which 50 per cent of the plants were heavily affected with ring-spot. These observations suggested that the leguminous crops were in some way responsible for the infection on tobacco, especially when sweet clover was found to be very susceptible to infection by the ring-spot virus. Natural infection has recently been found rather commonly on sweet clover that is growing on the experiment station plots, and in rotation on the college farm. Inoculum from both the artificially inoculated and the naturally infected sweet-clover plants readily produced the typical ring-spot symptoms on tobacco. Other clovers are no doubt susceptible to infection. These results help to account for infection on tobacco following clover in rotation.

The virus loses its potency at ordinary temperatures as soon as the infected plant tissue decays or becomes thoroughly dry, and in the expressed sap in about 24 hours. It seems, therefore, that the virus must overwinter in biennial or perennial plants, or in the embryo of infected seed. Since the virus appears to retain its potency indefinitely regardless of temperature as long as it is contained in living tissue, it seems that the embryo of the seed should carry it through the winter months. The systemic nature of infection should provide ample opportunity for the seed to become infected. The lesions on the young squash and gourd fruits penetrate the wall of the ovary and may extend to the placental tissue. Embryonic seeds might possibly become infected by their proximity to infected tissue.

Plants seem to develop an immunity to the ring-spot disease. Symptoms appear in abundance when the plants are inoculated and infection soon becomes systemic, as shown by the development of symptoms on the leaves that were uninoculated. New growth takes place and the ring-spot symptoms will appear on it. This process continues for 10 days to 2 weeks and then symptoms appear only faintly on the new leaves and suckers. Finally, the symptoms fail to appear on the new growth and the leaves appear normal. Attempts have been made to produce symptoms on these leaves by reinoculation with virus from heavily infected leaves from other plants, but without success. The sap from these apparently normal leaves is just as virulent as that from leaves that show the disease symptoms, indicating that the virus is present in such leaves even though the disease symptoms fail to appear.

SUMMARY

The ring-spot virus is capable of producing disease symptoms, more or less typical, on a wide variety of plants.

Plants of 72 genera have been inoculated, 38 of which developed infection, representing 17 families, namely, Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Dipsaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Phytolaccaceae, Polygonaceae, Scrophulariaceae, Solanaceae, and Violaceae.

The first symptoms appear in about 3 days after inoculation and infection becomes systemic about 10 days later.

The symptoms of the disease are restricted to the leaves of the majority of the plants, but they may also appear on the stems and fruits of some of the hosts.

Symptoms appear on the fruits of Nest Egg gourd and Golden Summer Crookneck squash as a result of systemic infection of the plant.

Certain plants are often killed outright as a result of ring-spot infection, namely, *Nicotiana clevelandii, N. glutinosa, N. longiflora, N. multivalvis; N. plumbaginifolia, N. quadrivalvis, N. sanderae, N. sylvestris*, Broad Windsor bean, Black-eye cowpea, kidney bean, Lima bean, snapdragon, and New Zealand spinach.

Infection has been found occurring naturally on sweet clover and the commercial varieties of tobacco.

Tobacco (*Nicotiana tabacum*) has been infected with virus recovered from the following plants: *N. clevelandii, N. glutinosa, N. langsdorffii, N. plumbaginifolia, N. repanda, N. sanderae, N. sylvestris*, Jimson weed, garden petunia, horse nettle, cantaloupe, cucumber, squash, pumpkin, gourd, calendula, head lettuce, garden zinnia, kidney bean, garden beet, winter cress, pokeweed, snapdragon, and sweet clover.