VIRUS DISEASES IN WILD PLANTS

A SURVEY OF VIRUS DISEASES IN WILD PLANTS

by

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SCOPE AND CONTENT: This thesis consists of a part of a long-term statistical survey of virus diseases in wild plants being conducted by Dr. W. D. MacClement. The Royal Botanical Gardens, Hamilton, Ontario, is the field of investigation. The distribution, frequency, season variation and rate of spread of the virus diseases were studied.

The research was conducted in six parts:

(a) A survey of virus in wild plants;

(b) A detailed study of virus in one genus in one area;

(c) The study of virus in three species in each of three line transects;

(d) Spread of virus in lawn weeds;

(e) Comparison of natural virus infection in crop and wild plants.

(f) Carry-over of virus in over-wintering plant structures.

ACKNOWLEDGEMENTS

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INTRODUCTION

Viruses are difficult entities to define. Two definitions which have some operational value are: "Viruses are sub-microscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only" (1) or, according to Stanley (2) "Viruses are auto-catalytic proteins which may be assumed to require the presence of living cells for multiplication." There is no single criterion by means of which viruses can be differentiated from bacteria, yet the virus group has been segregated by means of certain general characteristics. Among the most important of these are small size, the ability of reproduce or multiply when within the living cells of a given host, the ability to change or mutate during multiplication, and the inability to reproduce or grow on artificial media. The sole means of recognizing the existence of a virus is provided by the manifestation of disease which results from the growth of the virus.

Although virologists have not been able to satisfactorily define this entity, great strides have been made in recent years in the field of virus physiology and biochemistry. Through the use of electron microscopes and crystalline material, many common viruses can be defined in size and shape. Tobacco mossaic virus, for instance, is 15 mμ in diameter and infective if 280 mμ or longer in length (3). With respect to size, then,

the smallest viruses, such as alfalfa mosaic virus, are smaller than certain accepted protein molecules, such as the Busycon hemocyanin molecules. On the other hand, certain large viruses, such as vaccine virus, are larger than certain accepted organisms, such as the minimal reproductive units of the microorganisms of the pleuropneumonia group. Viruses, then, overlap with molecules at one extreme and with organisms at the other extreme. The data now available on viruses indicate that, as one goes from the smallest to the largest viruses, there is, with increase in mass, an increase in complexity of composition, structure and function. The viruses appear to provide a bridge between proteins and organisms.

The plant pathologist is, of course, interested in the manifestation of disease as recognized by the host plant reactions. These reactions of plants to infection with viruses range from no perceptible change, through diseases of different degrees of severity, to rapidly fatal conditions. Viruses are obligate parasites, and if they kill their hosts they also eliminate themselves. Hence, in the field, viruses occur most commonly in plants that to some extent tolerate their presence. The acme of tolerance is the ability to be infected and suffer not at all; to act as a symptomless carrier, a condition found to be far from uncommon, for many plants can carry one or more viruses. The more common symptoms of plant virus disease are as follows:

Vein-clearing and vein-banding. These occur prior to the mottle or mosaic in systemic infection. According to Sheffield (4), no anatomical or cytological abnormalities occur during the vein-clearing process. The yellow is due to retardation of chlorophyll formation.

Mosaic Mottling. Mosaic has been used to describe the different shades of green and yellow which develop on the tobacco plant when infected with the classical tobacco mosaic virus, the first virus to be described. Many extremes of discoloration are found in this group.

Rings (5). These are characteristic of a number of virus diseases. They may be single or concentric; they frequently have a central spot. Single rings are produced with necrotic or chlorotic walls.

Lesions (5). Necrotic-type lesions involving the death of the cells usually develop within a few days at the site of inoculation. They may be in the form of rings or a solid spot of dead cells.

Outgrowths (5). Abnormal growths associated with plant viruses are: internal galls, external tumors, enations and swellings.

Distortion. The leaves of virus-infected plants are distorted in various ways, such as crumpling, crinkling, rolling malformation, suppression of the laminae and twisting of the veins.

Necrosis (5). Death of cells is a common symptom. The necrosis may be in the form of the local spots or lesions already mentioned, or it may be systemic, leading to the death of the whole plant.

Chlorosis (5). One form of chlorosis is leaf-mottling of the mosaic disease. A second form is its "yellows" type, where there is no mottling but a uniform yellowing of the leaves.

Flower-colour changes (5) - The most usual effect of virus infection on the flower is to cause a characteristic change in the colour or "break" as it is called, consisting, for the most part, of a delicate pencilling or feathering of the colours.

Hence the gradation of tolerance referred to earlier can be seen to extend from a symptomless carrier, through systemic reactions to complete intolerance where the plant cell reaction is so complete as to cause death to the cell and to the virus.

After having thus learned how to identify virus diseases, the virologist's further concern was that of transmission of the virus. The known methods of transmission are by grafting; by inoculation; by soil transmission by contact; by seed; by pollen; by vegetative reproduction; by air and water, and by contamination of implements. by insects

All viruses which are systemic in their hosts can be transmitted by grafting. Cleft grafts, inarch grafts, patch grafts, and core grafts and budding have been used for studying virus diseases. In some cases, systemic infection can be induced by grafting when sap-inoculation produces local lesions only. The parasitic plant dodder (Cuscuta app.) acting as a living graft also transmits virus.

Inoculation as employed by a virologist consists essentially of inflicting a minute wound, or breaking of a trichome, to permit the entry of virus. A favourite method at one time was to scratch with a needle through a drop of inoculum placed on the leaf surface. Another method is to use a piece of cheesecloth dipped in the virus solution and rub it lightly over the leaf (6). Similarly, inoculations can be made by rubbing a glass spatula with a ground glass face, or even the finger,

over a leaf which has previously been dusted with carborundum to act as an abrasive (7). The entry of virus into a plant cell and consequent infection are presumably governed by a number of factors such as type of wound, whether in epidermal cells or trichome, toughness of epidermis, concentration of virus in inoculum, and so forth.

Soil Transmission of virus is rare but does occasionally occur in the case of tobacco mosaic and tobacco necrosis viruses. The latter is normally confined to the roots and natural transmission is through the soil and through root wounds.

Transmission by Contact, i.e. by contact between plants and parts of plants. It is largely those viruses which occur in high concentration in their host plants which are spread by contact between diseased and healthy plants.

Transmission by Seeds is of comparatively rare occurrence. There are a few cases known of transfer of bean mosaic by seeds. The percentage of infection of the bean seed is, however, very irregular, varying from 13 to 50%, and not all the seeds in one pod may be affected (Ray Nelson (1932)) (8). A few other isolated cases of seed transmission are listed by K. M. Smith.

Transmission by Pollen occurs in rare cases (9).

Transmission by Vegetative Reproduction (9). It is a general rule that all cuttings, tubers, runners, rhizomes, bulbs etc., if taken from a virus-diseased plant, will give rise in turn to plants also virus-diseased.

It is for this reason that these diseases are of paramount importance in such crops as potatoes, raspberries, strawberries and flower

bulbs, all of which are vegetatively propagated.

By Air and Water. These are modes of transmission of tobacco necrosis viruses which are akin to that of fungal spores and seem to be unique among plant viruses in this respect.

Transmission by the Contamination of Implements. T. M. V. is known to be readily transmitted on the hands and knives of workers during routine tending of the plants. One or two diseased plants are sufficient to infect several thousand by this means.

Transmission by Insects. Most viruses are dependent for transmission on the activity of insects. Two types of transfer occur with the aid of insects. The first is a simple mechanical carriage of infective sap on the exterior of the insect. The second and usual form depends on the insect's first feeding on an infected plant, where they acquire virus, and then moving to and feeding on healthy plants. This is sometimes one and the same as the first method, but usually the insects act as vectors. This is especially true of insects with piercing and sucking mouthparts.

Individual viruses are usually transmitted by one species of insect, or by a few closely related species. The degree of specificity varies with type of virus and strain of virus. Aster yellows was one of the first known cases of a virus infecting both a plant and an animal, i.e. it actually multiplied within the insect vector. Bawden (10) lists the following insect vectors: Grasshoppers; Earwigs, Thrips; Froghoppers (4 species); Leafhoppers (25 species); white flies (3 species); aphids (28 species); Mealy-bugs (3 species); Beetles (9 species). These insects transmit nearly one hundred known plant viruses.

and new names are being added to the list.

Plant virus investigation has followed the line of host-pathogen relations of certain specific viruses of commercial importance. Investigators have sought to find out all they can about the virus in question, such as its chemical make-up, pH inactivation point, isoelectric point, thermal inactivation points etc. They have also concerned themselves with the means of spread of a specific virus and control of vectors. Another line of investigation is the development of resistant varieties to specific virus.

Hence, the literature on virus research during the last thirty years consists of isolated pieces of information gleaned for the benefit of agriculturists generally. Prior to this research we had no knowledge about the quantity of naturally-occurring diseases. We knew little about the general distribution and frequency pattern of viruses in general. No attempt has hitherto been made to estimate the general seasonal variations of quantity and spread of virus, or the rate of spread.

The total field of references for virus in wild plants is extremely limited (18-25, 28-41). Experimental work has been limited to testing whether a given virus will infect a particular wild plant, rather than to investigating natural infection. It has long been suspected, however, that wild plants act as intermediate hosts or reservoirs of infection.

Hence, this project was proposed by Dr. W. D. MacClement in order to study some of these very important fundamental problems and answer a few of the basic questions about Virus Diseases in Wild Plant Life.

The project has been divided into six parts:

(a) A Survey of Virus in Wild Plants;

(b) A Detailed Study of Virus in One Genus in One Area;

(c) The Study of Virus in Three Species in Each of Three Line Transects;

(d) Spread of Virus in Lawn Weeds;

(e) Comparison of Natural Virus Infection in Crop and Wild Plants;

(f) Carry-over of Virus in Over-wintering Plant Structure.

A SURVEY OF VIRUS IN WILD PLANTS

During the summer of 1951, Miss Bonkoff, under the direction of Dr. W. D. MacClement, began a survey of virus diseases found in food and shelter plants in the Royal Botanical Gardens, Hamilton, Ontario.

The first half of the summer season was spent in sampling all types of plants found on one transect along the north shore of Cootes Paradise, on another along the south shore, and on a third through the Rock Gardens.

This survey suggested that the frequency of virus disease in wild plants is high.

During the latter half of the summer, quadrats 3 x 3 meters square were established on 8 surface cover areas. Each type of area was established in three widely-spaced repetitions. At intervals throughout the rest of the summer, five or more plants from each area were sampled and tested for virus.

In a report made to the Research Council of Ontario, it was stated that of the 634 samples taken during the summer, 131 showed some reaction in the test-plants.

The present investigator took over the project in the summer of 1952. After reviewing the results of the 1951 project, it was decided that the number of sampling areas should be decreased to 18, i.e. 6 surface types repeated in 3 areas, and the plants sampled each two weeks were to be the same species. Hence, fewer species

Opposite Page 10

Plate I

Ravine Bottom

Plate II

Ravine Slope

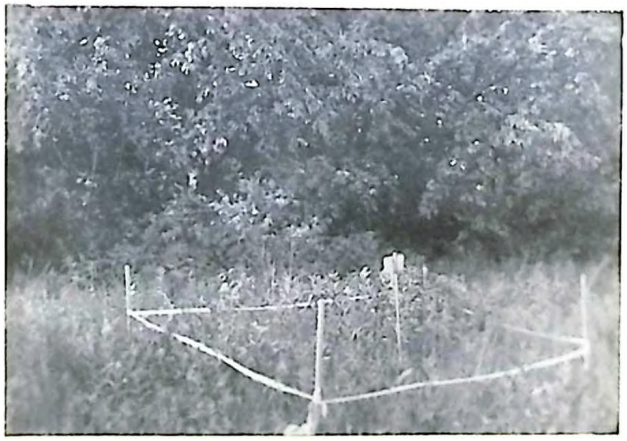


Plate III

Uncultivated Pasture

were inspected, but these were sampled more often to study virus frequency and distribution patterns. This decrease in number of species was due to shortage of space for growing and maintaining test plants.

Surface Types

Ravine Bottom:	an accumulation of muck and silt at the bottom of a steep-sided ravine.
Ravine Slope:	a shaded slope above the ravine bottom type area.
Uncultivated Pasture:	a pasture which is not cut or grazed.
Cultivated Pasture:	pasture which is cut once or twice a year.
Open Marsh:	floating root mass islands surrounded by water in the marsh.
Open Water:	2' to 6' deep in Cootes Paradise.

Each of the above classified areas was established in triplicate to minimize purely local conditions. This provided repetitious data concerning distribution of virus in various ecological zones.

Location - Refer to Map No.1

The Ravine Bottom quadrats were located as follows: (2a) a marshy area in the Royal Botanical Gardens west of Highway 102, directly north of Thorndale Crescent; (2b) an area at the end of the ravine inlet east of the President's house on the campus; (2c) an area just north of the culvert under the road through the ravine on the south shore.

These quadrates all contained Symplocarpus, Viola, Impatiens, Rubus and Corylus.

The three Ravine Slope areas were: (6a) a plot west of Highway 102 north of Thorndale on the slope above the before-mentioned Ravine Bottom

Opposite Page 11

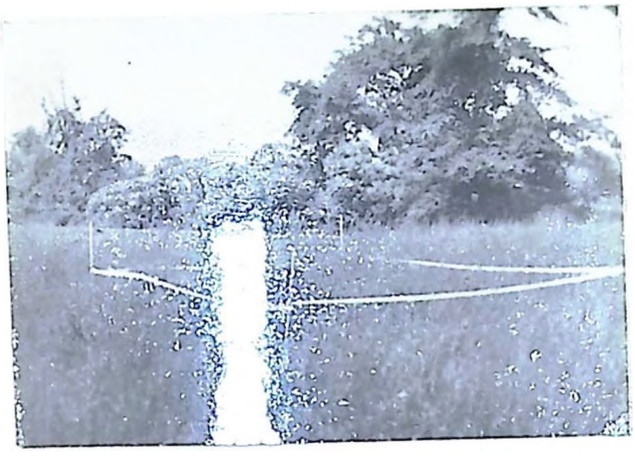


Plate IV.

Cultivated Pasture

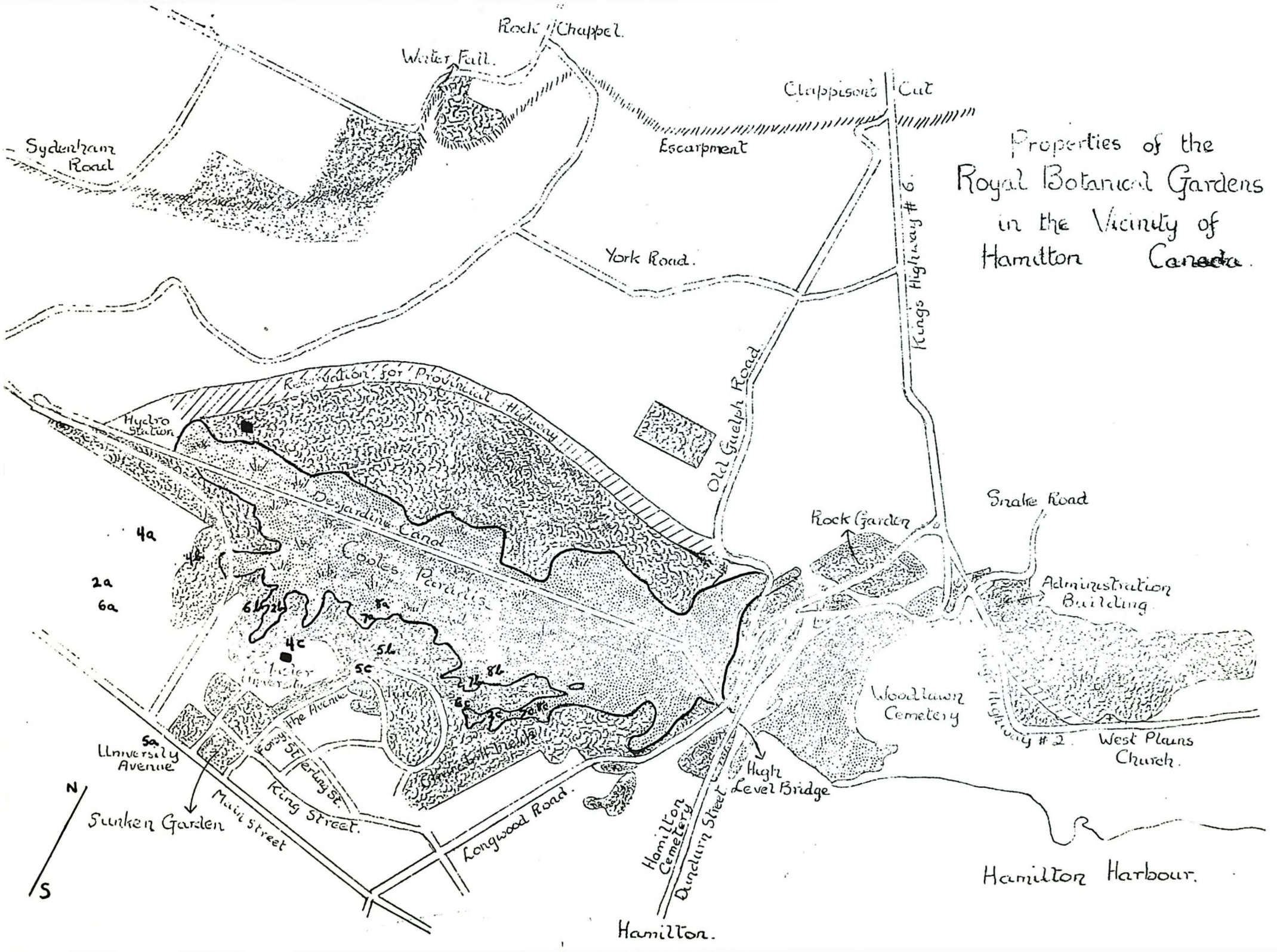
Plate V.

Open Marsh

Plate VI.

Open Water

(prints from coloured transparencies)


Properties of the
Royal Botanical Gardens
in the Vicinity of
Hamilton Canada.
Rock Chappel.
Water Fall.
Sydenham Road
Escarpment
Chappison's Cut
York Road.
Kings Highway # 6.
Old Guelph Road
Reservation for Provincial Highway
Hydro Station
Desjardins Canal
Cootes Paradise
Rock Garden
Snake Road
Administration Building
Woodlawn Cemetery
Highway # 2.
West Plains Church.
Hugh Level Bridge
Hamilton Cemetery
Dundurn Street
Hamilton Harbour.
4a
2a
6a
4b
6b
2b
7a
4c
5b
5c
8b
7b
6c
3c
2c
8c
The Avenue
Forsyth Fields
5a
University Avenue
Forsyth St.
Sterling St.
King Street.
Main Street
Sunken Garden
Longwood Road.
N
S
Hamilton.

area; (6b) an area sloping east beside the President's house; (6c) an area on slope of south side of cultivated pasture at extreme east end of campus.

The indicator plants of these areas were Trillium, Podophyllum, Arisaema, Rubus and Smilacina.

The three Uncultivated Pasture areas were (4a) a pasture plot on top of hill, west of Highway 102 northwest of Thorndale Crescent; (4b) the pasture land immediately west of Highway 102 opposite the President's house; (4c) the uncut pasture back of McMaster faculty gardens.

The indicator plants for these areas were Potentilla, Solidago, Gramineae, Phleum and Rubus.

The three Cultivated Pasture areas were (5a) a quadrat in the field south of the King Street extension opposite College Drive; (5b) a pasture field at the northeast end of the campus, just above the road into the wood; (5c) in 1952, under the ancient oak at the west end of the children's gardens at the Royal Botanical Gardens; in 1953, due to a change in cover plants, on Royal Botanical Gardens property north of Forsythe.

The sampled plants in these areas were Trifolium, Arctium, Gramineae, Phleum and Potentilla.

The three Open Marsh areas were (7a) the first deep inlet east of Grassy Point along the south shore; (7b) the east side of the deep double inlet midway between (7a) and Cockpit Island; (7c) a marsh area down the long inlet by Cockpit Island.

Indicator plants of Open Marsh quadrats were Polypodiaceae, Salix Sparganium, Panicum and Impatiens.

The open water areas were established in freely flowing water near each of the three open marsh areas.

Sampled plants here were Lemna minor, Potamogeton pectinatus, Potamogeton crispus, Ceratophyllum and Nymphaea.

Soil Analysis:

Chemical Soil Analyses were made of each of the land area quadrats. These tests were made with water extracts which rarely show the total quantity of soluble soil substance available but rather whether a soil is unusually low in one or more fertility constituents, or if a toxic condition is present.

The chemical analyses were made using the Simplex Soil Test Outfit and following the procedure therein. Only four results were used - blank, low, medium, high. This classification is used comparatively. A blank test result means that the proper colour or precipitation is not obtained in the testing operation and indicates, therefore, the amount of substance for which the test is made is so low as to be beyond the sensitivity of the test reaction used. A blank test does not necessarily mean that the substance under consideration is entirely absent from the soil extract. The words, "low," "medium," and "high" have their usual significance, i.e. as compared to usual agricultural soil standards.

TABLE I.

Chemical Soil Analysis of Ravine Bottom Areas
1953

	2a. North of Thorndale	2b. East of President's House	2c. In Mid Ravine
Type of Soil	Boggy soil frequently under water		
Organic Matter	Med. 18%	Med. 14%	High 32%
Soil Reaction	pH 7.5	pH 5	pH 6.5
Carbonates	High	Blank	High
Nitrates	Blank	Blank	Blank
Phosphorus	Blank	Low	Blank
Potassium	Medium	Low	Low
Calcium	High	High	High
Magnesium	High+	High	High+
Aluminum	Blank	Blank	Blank
Iron	Blank	Low	Low+
Manganese	Blank	Low+	Low
Sulphates	Blank	Blank	Blank
Ammonia	Low	Low	Low
Nitrites	Blank	Low	Blank

TABLE Ia.

Chemical Soil Analysis of Ravine Slope Areas
1953

	6a. North of Thorndale	6b. East of President's House	6c. South East end of campus
Type of Soil	Clay slope shaded by high oaks		
Organic Matter	Low 4%	Low 2%	Low 2%
Soil Reaction	pH 8	pH 5	pH 4
Carbonates	High	Low	Blank
Nitrates	Blank	Blank	Blank
Phosphorus	Blank	Low	Low (very)
Potassium	Low	Medium	Medium
Calcium	Blank	High	Low
Magnesium	High +	High	Medium
Aluminum	Blank	Blank	Blank
Iron	Blank	Blank	Blank
Manganese	Blank	Blank	Blank
Sulphates	Low	Blank	Blank
Ammonia	Low	Low	Low
Nitrites	Blank	Low	Blank

TABLE II.

Chemical Soil Analysis of Uncultivated Pasture Areas
1953

	4a. Hilltop North of Thorndale	4b. West of Highway 102	4c. Back of Faculty Gardens
Type of Soil	Clay	Clay	Clay
Organic Matter	Low 1.5%	Low 2.8%	Low 1.6%
Soil Reaction	pH 4.5	pH 6.5	pH 4.5
Carbonates	Blank	Medium	Blank
Nitrates	Blank	Blank	Blank
Phosphorus	Low (very)	Blank	Low (very)
Potassium	Low	Low	Low
Calcium	High	Low	Low
Magnesium	Medium	High +	High
Aluminum	Blank	Blank	Blank
Iron	Blank	Blank	Blank
Manganese	Blank	Blank	Blank
Sulphates	Blank	Blank	Blank
Ammonia	Low	Low	Low
Nitrites	Blank	Blank	Blank

TABLE IIa.

Chemical Soil Analysis of Cultivated Pasture Areas
1953

	5a. 20 yds. South of King Street W.	5b. North East of Campus	5c. North of Forsythe
Type of Soil	Heavy clay		
Soil Reaction	pH 5	pH 4.5	pH 4
Carbonates	Blank	Blank	Blank
Nitrates	Blank	Blank	Blank
Phosphorus	Low	Low (very)	Low (very)
Potassium	Medium +	Medium	Medium
Calcium	Low	Medium	Blank
Magnesium	High +	High	High
Aluminum	Blank	Blank	Blank
Iron	Blank	Low	Blank
Manganese	Blank	Blank	Blank
Sulphates	Blank	Low	Blank
Ammonia	Low	Low	Low
Nitrites	Blank	Blank	Blank

Analysis of Microflora of Soil Samples:

A spade full of earth was removed the width and depth of the spade face. The clod was broken open by hand, and from the near centre of the clod at a depth of 6-7 inches a few spoonfuls of earth were removed, using a sterile spoon and sterile containers. These precautions were taken so that the count of microflora were nearly correct. Had the sample been taken from the spade face, the microflora might have been carried down from the surface to the depth of sampling.

Dilutions were made to 1/10,000 and 1/1 million. Each dilution was plated out in three petri dishes of potato dextros agar. This medium was chosen as it supports growth of bacteria which attack plants. Sterile micro technique was observed throughout and check plates of water and P.D.A. were made each time before adding soil.

No inhibitor was used in any medium as comparative figures rather than exact counts were to be emphasized.

These studies enable one to study the similarities or variations between the members of ecological zones.

Readings from the three plates of both dilutions were added and averaged to give an expression of average of microflora per gram of soil.

TABLE III.

Microflora

Readings = average of 6 plates expressed as number of organisms per gm. soil

1953

Ravine Bottom	2a	2b	2c
Fungi	104,300	310,000	300,000
Bacteria	63,600	560,000	520,000
Actinomycetes	106,000	5,000	1,500
Ravine Slope	**6a**	**6b**	**6c**
Fungi	10,060	20,030	5,760
Bacteria	5,930	451,960	20,000
Actinomycetes	133	20,000	19,130
Uncultivated Pasture	**4a**	**4b**	**4c**
Fungi	240,000	31,500	5,000
Bacteria	821,200	3,245,000	423,500
Actinomycetes	20,000	25,000	20,000
Cultivated Pasture	**5a**	**5b**	**5c**
Fungi	39,267	200,500	25,000
Bacteria	39,000	9,003,900	316,500
Actinomycetes	30,000	102,500	70,000

TABLE IV.

Microflora per Type Area
per Gram of Soil

	Ravine Bottom	Ravine Slope	Uncultivated P.	Cultivated P.
Fungi	238,266	11,950	186,600	88,256
Bacteria	381,200	159,296	1,496,566	3,119,800
Actinomycetes	37,500	13,088	21,660	67,500

Sampling Procedures:

On each sampling occasion the five specified species of plants of each area were taken by slipping a new waxed paper bag over a leaf or stem, pinching off the rest of the plant, then folding the sample into the bag without touching it.

These samples were brought back to the greenhouse and mechanically inoculated to leaves of at least three kinds of seedling plants, referred to as test plants. The samples were ground with sterile pestle and mortar (11) or glass plate and glass spatula (12), then rubbed on the surface of three or four leaves of a healthy test plant that had been dusted with a fine (400 grit) carborundum powder for an abrasive (13). If the test plant seedling was particularly small, cotton swabs were used to avoid excessive mechanical injury (14). All inoculations were made on the same day as the samples were taken.

Test Plants used were those listed by K. M. Smith that showed clearly defined virus symptoms (15).

An appropriate number of control plants were inoculated each time with water

Test Plants Used	Virus Sympton Displayed
N. Tabaccum White Burley Jamaica Wrapper Harrow Velvet	Local lesions, vein-clearing, chlorosis, distortion, stunting, blistering
Lycopersicum esculentum Tomato	Yellowing, leaf curl, stunting, distortion
Phaseolus vulgaris French string bean	Puckering and cupping of leaves, chlorosis, stunting
Nicotina glutinosa	Local lesions, vein-clearing, distortion
Physalis	Distortion, chlorosis
Gomphrena globosa	Local lesion
Datura	Mosaic mottle and chlorotic ring patterns
Brassica oleracea var capitata Cabbage	Black ringspot, mosaic mottle
Spinacia oleracea Spinach	Chlorosis, vein-clearing, malformation
Brassica rupa Turnip	Leaf mottling, yellowing, stunting
Chenopodium alba	Local lesions, etel
Petunia hybrida Petunia	Stunted and dwarfed cupped leaves, numerous secondary vein shoots, corolla not developed, dwarfed

Test Plants - cont'd.	Symptoms cont'd.
Brassica oleracea var botrytis Cauliflower	Mosaic mottling of foliage
Brassica oleracea var botrytis Broccoli	Diffuse mottling of small, pale green, roughly circular areas.

TABLE V.

1951 % Infection per Species per Sampling

Plant Sampled	July 30-31,1	Aug. 9-15	Aug. 20-25	Aug. 30-31	Sept. 11	Sept. 16-20
	infected sampled	infected sampled	infected sampled	infected sampled	infected sampled	infected sampled
Impatiens (Jewel weed)	1/5	0/3	1/4	0/3	0/1	0/6
Corylus (Hazel)						
Rubus (Raspberry)	2/6	0/1	0/4	0/2	0/1	2/5
Viola (Violet)	0/0	0/1	1/1	0/0	0/0	0/0
Symplocarpus (Skunk Cabbage)	0/1	0/0	0/1	0/1	0/0	0/0
Solidago (Goldenrod)	0/5	1/4	2/5	1/6	0/1	0/6
Potentilla (Cinquefoil)	0/1	0/1	0/0	0/2	0/1	0/0
Gramineae (Mixed Grasses)	0/3	0/4	0/3	3/6	1/2	0/4
Phleum (Timothy)						
Trifolium (Clover)	1/2	0/1	1/1	0/2	0/0	0/1
Arctium (Burdock)	0/2	0/0	1/1	0/1	0/0	0/2
Smilacina (False Solomon's Seal)						
Podophyllum (Mandrake)	0/1	0/0	0/0	0/1	0/1	0/0
Trillium (Trillium)	0/0	0/1	0/0	0/1	0/0	0/0
Salix (Willow)						
Sparganium (Bur Reed)						

TABLE V Cont'd.

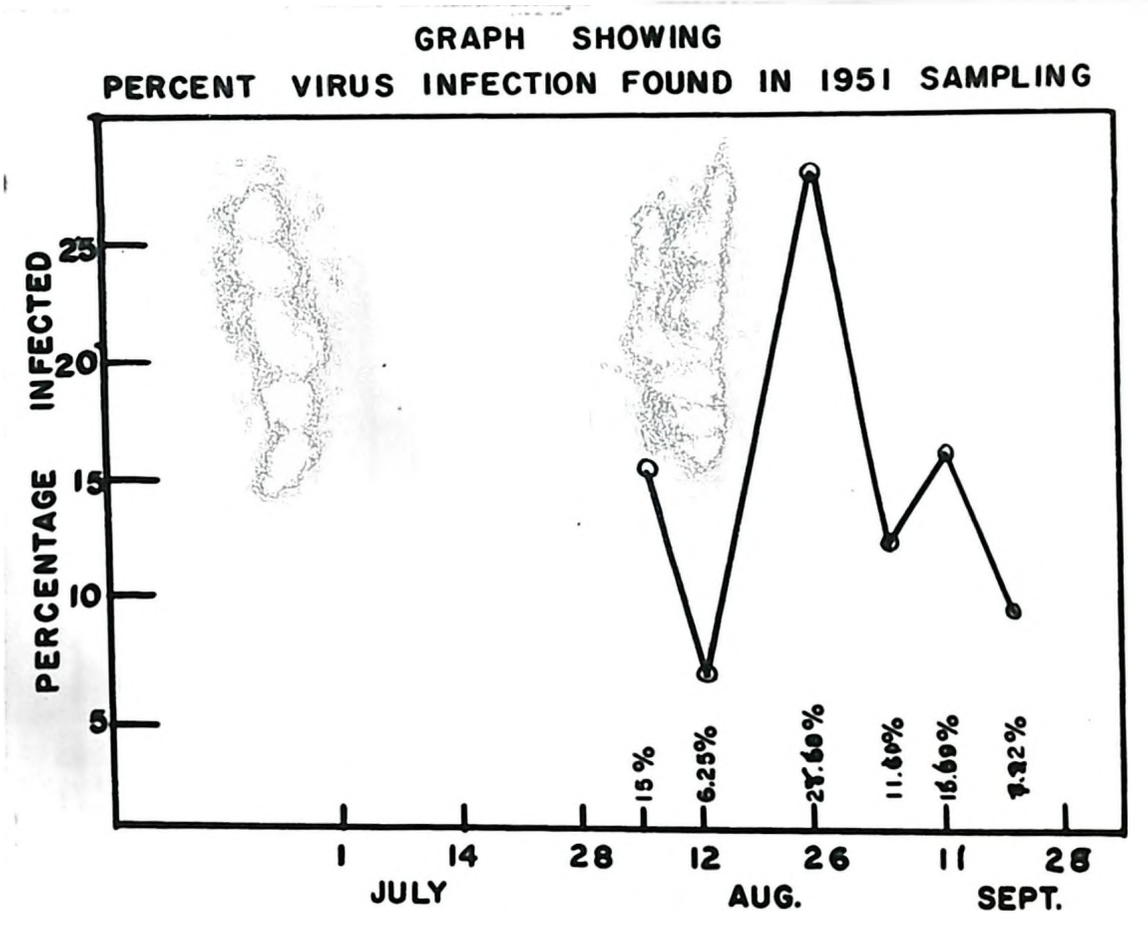
Plant sampled	July 30-31,1	Aug. 9-15	Aug. 20-25	Aug. 30-31	Sept. 11	Sept. 18-20
Nymphaea (Pond Lily)	I/3	I/3	I/3	I/3	I/3	I/3
Lemna minor (Duckweed)	0/0	0/1	0/2	0/1	0/1	0/1
Potamogeton pectinatus	0/0	0/1	0/0	0/2	0/0	0/5
Ceratophyllum	0/0	0/1	0/0	0/1	0/0	0/1
Potamogeton crispus	0/0	0/2	0/0	0/1	0/0	0/2
Medicago (Alfalfa)	0/1	0/1	0/0	0/2	0/1	0/1
Plantago Lanceolate	0/1	0/0	0/0	0/0	0/0	0/1
Plantago major	1/1	0/0	1/1	0/0	0/0	0/1
Stellaria	0/1	0/0	0/0	0/0	0/0	1/2
Chelidonium (Thistle)	0/0	0/1	0/1	0/2	0/1	0/0
Arisaema (Jack in Pulpit)	0/2	0/1	0/1	1/2	1/1	0/0
Solanaceae (Nightshade)	1/1	0/0	0/0	0/1	0/0	1/1
Geranium	0/1	0/1	1/1	1/3	0/0	0/2
Iris	0/0	0/0	0/0	0/2	0/0	0/1
Panicum (Marsh Grass)	0/0	0/0	0/0	0/2	0/0	0/1
Callistophus (Aster)	0/2	0/1	0/1	0/0	0/0	0/4
Psedera (Virginia Creeper)	0/2	0/1	0/0	0/1	0/0	0/3
Boehmeria (Nettle)	0/0	0/0	0/0	0/1	0/0	0/1
Typha	0/1	0/1	0/0	0/1	0/0	0/2
Nymphaea alba	0/0	0/0	0/0	0/2	0/0	0/1

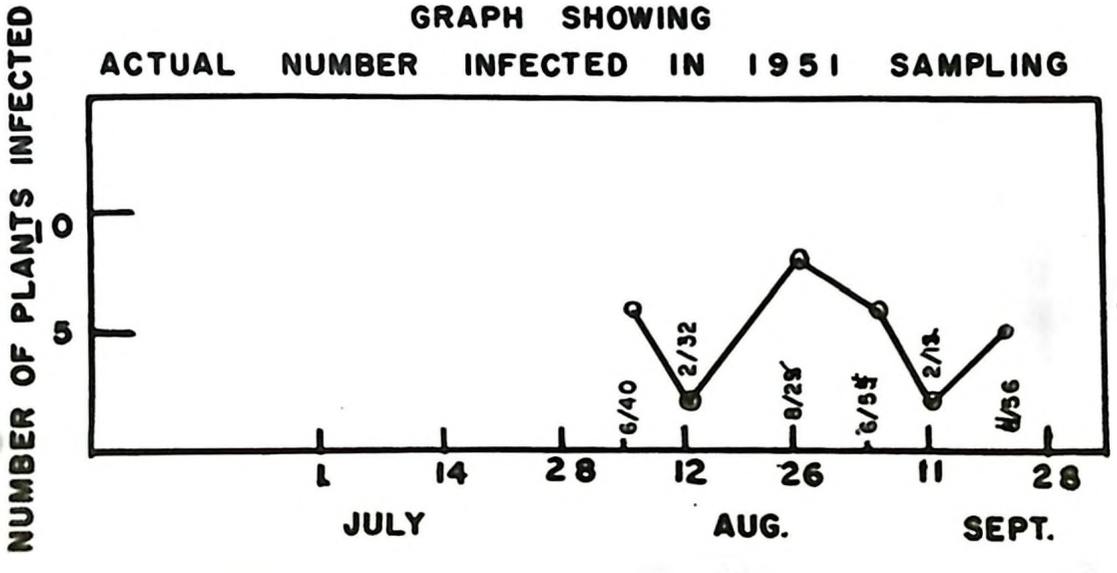
TABLE V. Cont'd.

	July 30-31	Aug. 9-15	Aug. 20-25	Aug. 30-31	Sept. 11	Sept. 18-20
	I/S	I/S	I/S	I/S	I/S	I/S
Scripus	0/0	1/3	0/0	0/2	0/0	0/2
Chelidonium	0/1	0/1	0/1	0/3	0/1	0/0
Total Infection per Collection	6/40= 15%	2/32= 6.25%	8/28= 28.6%	6/54= 11.1%	2/12= 16.6%	4/56= 7.2%

Total Infection 1951: 28/222 = 12.6%

GRAPH 1.





GRAPH 2.

TABLE VI.

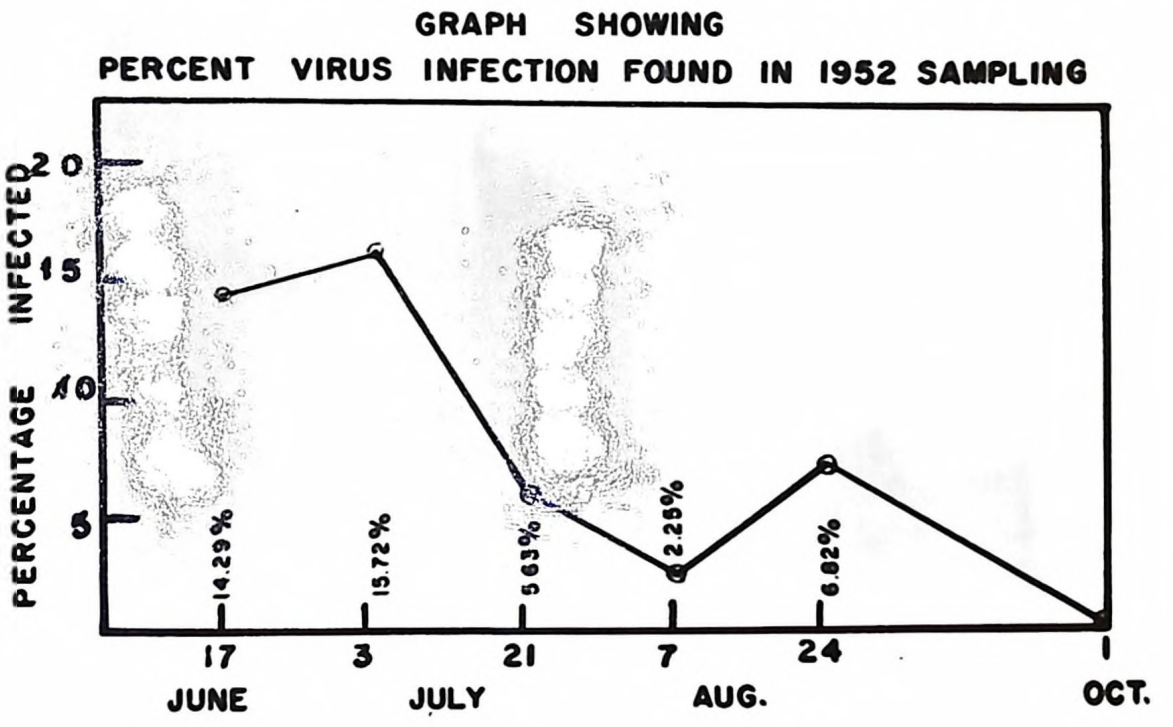
1952 % Infection per Species per Sampling

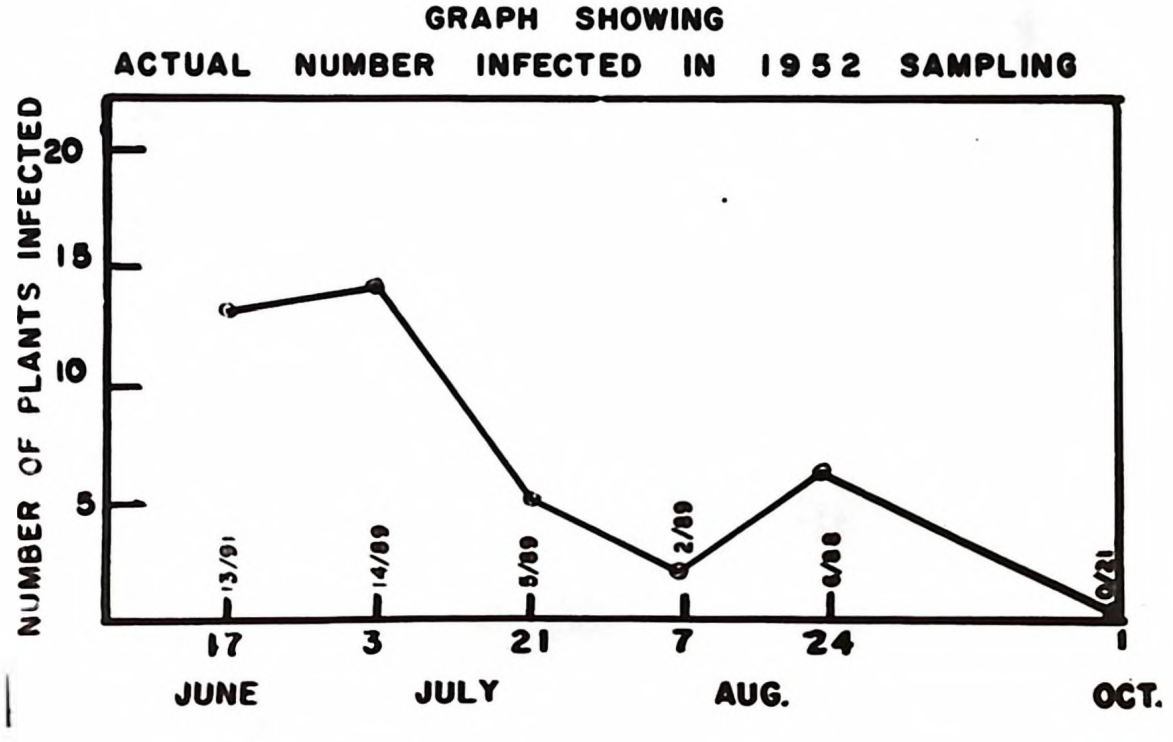
Plant Sampled	June 17	July 3	July 21	Aug. 7	Aug. 24	Sept.	Oct.1 1
	I/S	I/S	I/S	I/S	I/S	I/S	I/S
Impatiens (Jewel wood)	0/9	2/9	0/9	0/9	2/9		
Corylus (Hazel)	1/3	0/3	1/3	0/3	0/3		
Rubus (Raspberry)	1/8	2/8	0/8	0/8	1/8		
Viola (Violet)	1/3	0/3	0/3	0/3	1/3		
Symplocarpus (Skunk Cabbage)	0/3	0/3	0/3	0/3	0/2		
Solidago (Goldenrod)	1/3	0/3	0/3	0/3	1/3		
Potentilla (Cinquefoil)	0/6	2/6	1/6	0/6	0/6		
Gramineae(Grass)	1/4	0/4	0/4	0/4	0/4		
Phleum (Timothy)	1/5	0/3	1/3	0/3	0/3		
Trifolium (Clover)	1/3	0/3	0/3	0/3	0/3		
Arctium (Burdock)	2/2	1/2	0/2	0/2	0/2		
Smilacina (F.Sol.Seal)	0/3	3/3	0/3	1/3	0/3		
Podophyllum (Mandrake)	2/3	1/3	0/3	0/3	0/3		
Trillium	0/3	1/3	0/3	0/3	0/3		
Polypodiaceae (Fern)	0/3	0/3	0/3	0/3	0/3		0/3
Salix (Willow)	0/3	0/3	1/3	0/3	0/3		0/3
Sparganium (Bur Reed)	0/3	1/3	0/3	0/3	0/3		0/3
Nymphaea(Pond Lily)	0/3	1/3	1/3	0/3	0/3		0/3
Lemna minor	0/3	0/3	0/3	1/3	0/3		0/3

TABLE VI. Cont'd.

Plant Sampled	June 17	July 3	July 21	Aug. 7	Aug. 24	Sept.	Oct. 1
	I/S	I/S	I/S	I/S	I/S	I/S	I/S
Potamogeton pectinatus	0/3	0/3	0/3	0/3	0/3		0/3
Ceratophyllum	0/3	0/3	0/3	0/3	0/3		0/3
Potamogeton crispus							
Chrysanthemum (Daisy)	1/6	0/6	0/6	0/6	0/6		
Arisaema (Jack in Pulpit)	1/3	0/3	0/3	0/3	1/3		
Panicum (Marsh Grass)	0/3	0/3	0/3	0/3	0/3		
Total Infection per Collection	13/91= 14.29%	14/89= 15.72%	5/89= 5.63%	2/89= 2.25%	6/88= 6.82%	0/0=	0/21= 0%

GRAPH 3





GRAPH 4

TABLE VII.

1953 % Infection per Species per Sampling

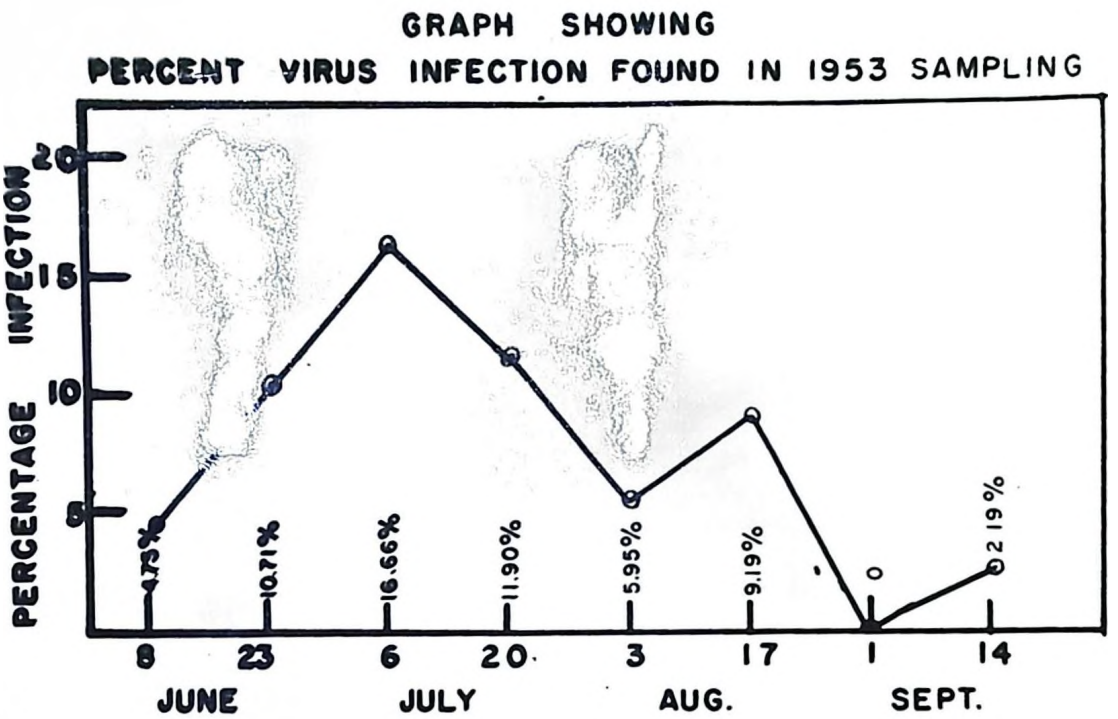
Plant Sampled	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Sept. 1	Sept. 14
	I/S	I/S	I/S	I/S	I/S	I/S	I/S	I/S
Impatiens (Jewel weed)	0/9	2/9	1/9	1/9	0/9	0/9	0/9	0/9
Corylus (Hazel)	1/3	1/3	1/3	0/3	0/3	1/3	0/3	0/3
Rubus (Raspberry)	0/9	2/9	1/9	3/9	0/9	1/9	0/9	0/9
Viola (Violet)	0/3	0/3	0/3	1/3	2/3	0/3	0/3	0/3
Symplocarpus (Skunk Cabbage)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Solidago (Goldenrod)	0/3	1/3	2/3	1/3	1/3	1/3	0/3	0/3
Potentilla (Cinquefoil)	0/6	0/6	3/6	1/6	0/6	0/6	0/6	1/6
Gramineae (Grass)	0/6	2/6	2/6	0/6	1/6	0/6	0/6	0/6
Phleum (Timothy)	0/6	0/6	1/6	0/6	1/6	1/6	0/6	0/6
Trifolium (Clover)	0/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3
Arctium (Burcock)	0/0	0/0	0/0	0/0	0/0	0/3	0/3	1/3
Smilacina (F.Sol.Seal)	1/3	1/3	0/3	1/3	0/3	0/3	0/3	0/3
Podophyllum (Mandrake)	1/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Trillium	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
Polypodiaceae (Fern)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Salix (Willow)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Sparganium (Bur Reed)	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
Nymphaea(Pond Lily)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Lemna minor	0/3	0/3	0/3	1/3	0/3	2/3	0/3	0/3

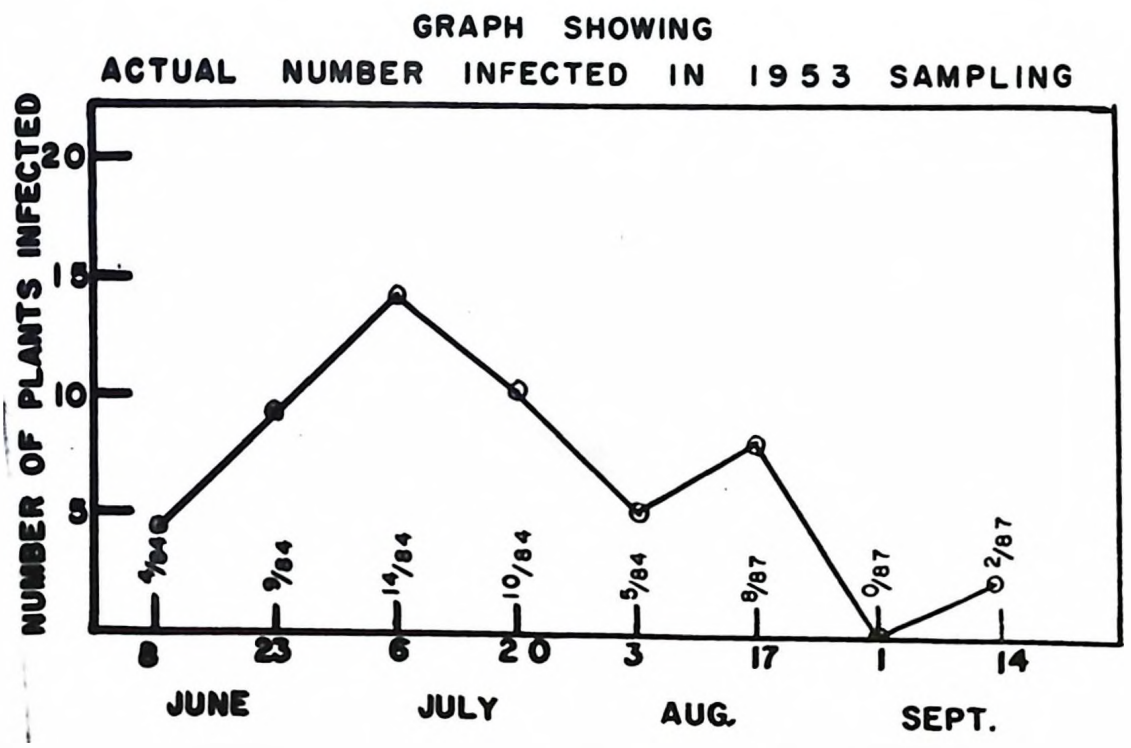
TABLE VII. Cont'd.

Plant Sampled	June 8	June 22	July 6	July 20	Aug. 3	Aug. 17	Sept. 1	Sept. 14
	I/S	I/S	I/S	I/S	I/S	I/S	I/S	I/S
Potamogeton pectinatus	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Ceratophyllum	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
Potamogeton crispus	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Total Infection per Collection	4/84= 4.76%	9/84= 10.71%	14/84= 16.66%	10/84= 11.90%	5/84= 5.95%	8/87= 9.19%	0/87= 0%	2/87= 2.1%

Total Infection 1953: 52/681 = 7.63%

GRAPH 5





GRAPH 6

TABLE VIII.

Total Infection per Species per Annum

Plant Sampled	1951		1952		1953		Total Infection	
Impatiens	2/22	9%	4/45	8.9%	4/72	5.7%	10/139	7.2%
Corylus			2/15	13.0%	4/24	16%	6/39	15.4%
Rubus	4/19	21%	4/40	10.0%	9/72	12.5%	17/131	13 %
Viola	1/2	50%	2/15	13.0%	3/24	12.5%	6/41	14.6%
Symplocarpus	0/3	0%	0/14	0%	0/24	0%	0/40	0 %
Solidago	4/27	14.8%	2/15	13.0%	8/24	33%	14/66	21.2%
Potentillae	0/5	0%	3/30	10.0%	6/48	12.5%	9/83	10.5%
Gramineae	4/22	18.4%	1/20	5.0%	5/48	10.4%	10/90	11.6%
Phleum			1/17	5.8%	3/48	6.0%	4/65	6.3%
Trifolium	2/7	27.6%	1/15	6.6%	2/24	8.2%	5/46	10.8%
Arctium	1/6	16.0%	3/10	33.3%	2/9	22.0%	6/25	24.0%
Smilacina			4/15	27.0%	3/24	12.5%	7/39	18.0%
Podophyllum	0/3	0%	3/15	20.0%	2/24	8.2%	5/42	11.9%
Trillium	0/2	0%	1/15	6.66%	1/24	4%	2/41	4.9%
Polypodiaceae			0/18	0%	0/24	0%	0/42	0%
Salix			1/18	5.55%	0/24	0%	1/42	2.4%
Sparganium			1/18	5.55%	1/24	4%	2/42	4.8%
Nymphaea			2/18	12.0%	0/24	0%	2/42	4.8%
Lemna minor	0/6	0%	1/18	5.55%	3/24	12.5%	4/48	8.3%
Potamogeton pectinatus	0/8	0%	0/18	0%	1/24	4%	1/50	2.0%

TABLE VIII. Cont'd.

Plant Sampled	1951		1952		1953		Total Infection	
Ceratophyllum	0/3	0%	0/18	0%	1/24	4%	1/45	2.2%
Potamogeton crispus	0/5	0%			0/24	0%	0/29	0%
Chrysanthemum			1/30	3.3%			1/30	3.3%
Arisaema	2/7	27.6%	2/15	13%			4/22	18.2%
Panicum	0/3	0%	0/5	0%			0/8	0%
Medicago	1/6	16%						
Plantago major	0/2	0%						
Plantago lanceolata	2/3	66.6%						
Stellaria	1/3	33.3%						
Chelidonium	0/5	0%						
Solanaceae	2/4	50%						
Geranium	2/8	25%						
Iris	0/3	0%						
Callistephus	0/8	0%						
Psedera	0/7	0%						
Boehmeria	0/2	0%						
Typha	0/5	0%						
Nymphaea	0/3	0%						
Scripus	1/7	14.3%						
Chelidonium	0/7	0%						

Total Infection 29/223 = 13% 38/467 = 8.13 52/681 = 7.63

Total Mechanical Inocuable Infection Found Through Random Surveys = 119/1371 = 11.52%

TABLE IX.

% Instance of Virus per Ecological Area

Type of Area	1951	1952	1953
Ravine Bottom	3.1%	11.8%	9.3%
Uncultivated Pasture	16.6%	9.2%	15.8%
Cultivated Pasture	0.33%	18.1%	9.1%
Ravine Slope	4.1%	14.7%	3.9%
Open Marsh	4.1%	4.2%	1.0%
Open Water	0.0%	5.2%	4.1%
Total for Year	13.0%	8.13%	7.63%

TABLE X.

Total % per Plot per Year

Plot	1951	1952	1953
Ravine Bottom			
a.	0.0	16.	10.
b.	8.3	14.8	7.5
c.	1.0	4.1	10.5
Uncultivated Pasture			
a.	50.	4.	17.5
b.		7.6	15.
c.	0.	16.	15.
Cultivated Pasture			
a.	1.[x]	38.4	12.5
b.	0.[x]	4	7.5
c.	0.[x]	12.	7.5[x]
Ravine Slope			
a.	12.5[x]	12.5	4.7
b.	0.	20.0	0.
c.	0.[x]	0	7.1
Open Marsh			
a.	0.[x]	0	3.2
b.	12.5[x]	3.2	0.
c.	0.[x]	9.6	0.
Open Water			
a.	0.[x]	7.6	2.5
b.	0.[x]	8.2	7.5
c.	0.[x]	0.	2.5

[x] not same plot

TABLE XI.

% Diseased per Month

Time	1951	1952	1953
Early June			4.76
Late June		14.29	10.71
Early July		15.72	16.66
Late July	15.00	5.63	11.90
Early Aug.	6.25 27.58	2.25	5.95
Late Aug.	11.61	6.82	9.19
Early Sept.	15.39	----	0.00
Late Sept.	8.92	0.00	2.19
Early Oct.			0.00
Total for year:	13.00%	8.13%	7.63%

GRAPH 7.

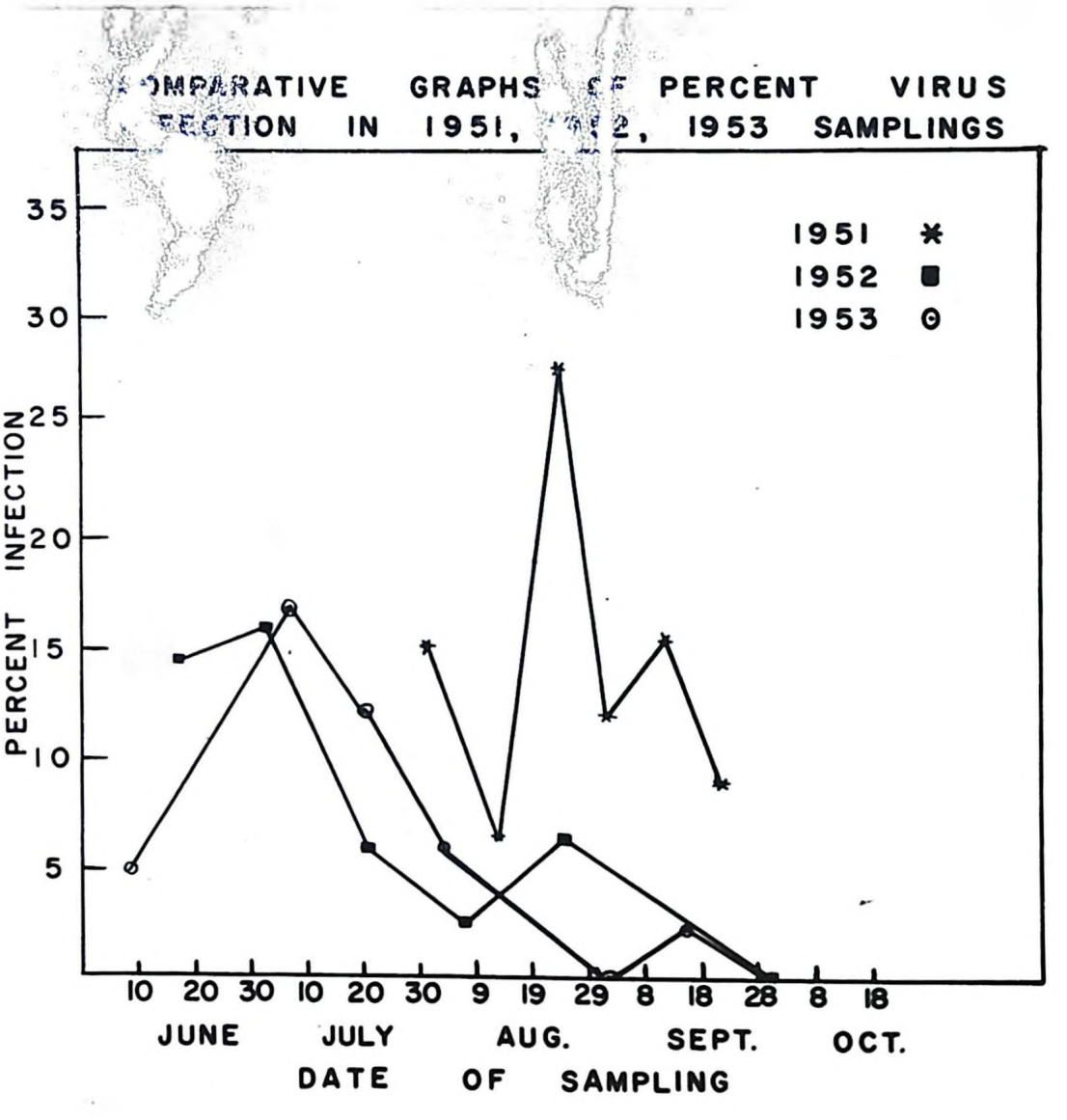

COMPARATIVE GRAPHS OF PERCENT VIRUS
INFECTION IN 1951, 1952, 1953 SAMPLINGS
1951 *
1952 ■
1953 ⊙
PERCENT INFECTION
35
30
25
20
15
10
5
10 20 30 10 20 30 9 19 29 8 18 28 8 18
JUNE JULY AUG. SEPT. OCT.
DATE OF SAMPLING

TABLE XII.

Variations in Per Cent Infection per Species Collected in More than One Ecological Area

	1951		1952		1953	
	#	%	#	%	#	%
Impatiens						
Willow Swamp	1/7	14.28				
Ravine Bottom	1/7	14.28	2/15	13.0	2/24	8.2
Ravine Slope	0/3	0	2/15	13.0	2/24	8.2
Open Marsh			1/18	5.55	0/22	0
Rubus						
Willow Swamp	0/8	0				
Ravine Bottom	1/5	20	3/15	20	2/24	8.2
Ravine Slope	1/3	33	0/10	0	1/24	4.1
Uncultivated Pasture	2/2	100	1/15	6.6	5/24	20.8
Gramineae						
Willow Swamp	0/2	0				
Ravine Bottom	0/1	0				
Uncultivated Pasture	2/7	27.6	1/15	6.6	2/24	8.2
Cultivated Pasture	1/7	14.28	0/6	0	3/24	12.5
Potentilla						
Uncultivated Pasture	0/4	0	2/15	13	4/24	16.4
Cultivated Pasture	---		1/15	6.6	2/24	8.2
Phleum						
Uncultivated pasture	---		0/2	0	3/24	12.5
Cultivated Pasture			2/15	13	0/24	0

GRAPH 8.

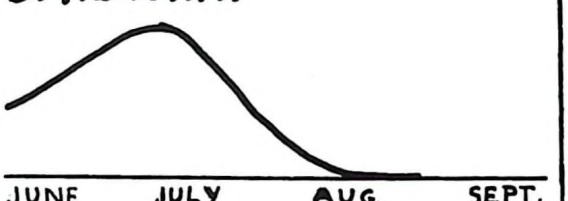
SEASONAL INFECTION CURVES

SPRING FLOWERING

TRILLIUM

PODOPHYLLUM

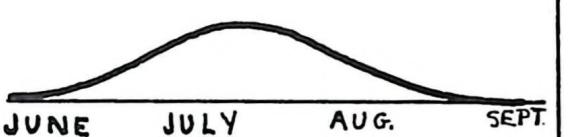
SMILACINA



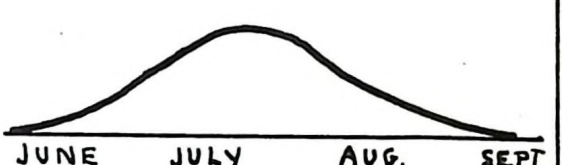
EARLY SUMMER FLOWERS

POTENTILLA

IMPATIENS



GRAMINEAE



LATE SUMMER FLOWERS

SOLIDAGO

ARCTIUM

TABLE XIII.

Prevailing Weather Conditions

Month of May	1950	1951	1952	1953
Max.Temp.	79	87	78	88
Min. Temp.	31	38	36	36
Mean Temp.	54.1	58.4	54.2	56.3
Mean Normal	54.8	54.8	54.8	54.8
D/F/M	-.7	+3.6	-0.6	+1.5
Precipitation	1.48"	1.29"	3.51"	6.66"
Normal	2.32"	2.32"	2.32"	2.32"
D/F/M	-.84"	-1.03"	+1.19"	+4.34"

Month of June	1950	1951	1952	1953
Max. Temp.	90	84	95	92
Min. Temp.	41	43	46	45
Mean Temp.	65.3	65.0	67.5	67.2
Mean Normal	65.2	65.2	65.2	65.2
D/F/M	+ 0.1	-0.2	+ 2.3	+2.0
Precipitation	1.29"	4.34"	1.59"	1.83"
Normal	2.64"	2.64"	2.64"	2.64"
	-1.35"	+1.70"	-0.85"	-0.81"

TABLE XIII Cont'd.

Month of July	1950	1951	1952	1953
Max. Temp.	88	86	96	92
Min. Temp.	47	51	48	47
Mean Temp.	69.0	70.1	74.4	71.5
Mean Normal	71.3	71.3	71.3	71.3
D/F/M	-1.7	-1.2	+3.1	+0.2
Precipitation	4.41"	4.47"	1.90"	1.90"
Normal	3.09"	3.09"	3.09"	3.09"
D/F/M	+1.32"	+1.38"	-1.19"	-1.09"

Month of August	1950	1951	1952	1953
Max. Temp.	93	91	88	98
Min. Temp.	42	46	46	49
Mean Temp.	68.2	67.8	69.0	71.3
Mean Normal	67.7	67.7	67.7	67.7
D/F/M	+0.5	+0.1	+1.3	+3.6
Precipitation	6.73"	1.81"	3.24"	1.50"
Normal	2.32"	2.32"	2.32"	2.32"
D/F/M	+4.41"	-0.51"	+0.92"	-0.82"

RESULTS OF RANDOM SURVEY PROJECT

(1) The total virus infection varies considerably from year to year.

(2) In 1951, 43.7% of the species sampled two or more times proved to be infected at some time. Twenty-nine out of 223 plants sampled or 13% were infected.

(3) In 1952, 83.4% of the species sampled repeatedly were infected. Thirty-eight out of 467 plants sampled or 8.13% had a virus infection.

(4) In 1953, 77.3% of the species repeatedly sampled showed infection. Fifty-two out of 681 plants sampled or 7.63% were infected.

(5) Species consistently showing a high percentage of infection were perennials rather than annuals, indicating a carry-over of infection from one year to the next.

(6) The seasonal graph for 1951 cannot be treated as fully significant, for it covers too short a period. Prior to the end of August the survey was not a random one; hence the figures there were abnormally high. The unusually high peak of infection shown for the sampling period, August 20 to 25, should, I feel, be discounted. During this time only ten species were sampled, and five of these were sampled only once. Each of these five was infected; hence the species was listed as having 100% infection for the period. This type of sampling is too small to be representative of the true picture.

(7) The peak of infection for 1952 and 1953, and probably for 1951, is very early in July. This peak is followed by a steady decline, except for a slight rise in the latter part of August in 1952 and early in September 1953 and September 1951.

(8) The early peak is made up of plants which appear early in the spring. A seasonal graph of infection of these plants shows that they each have a peak infection period late in June and early in July. The plants referred to are Smilacina (False Solomon's Seal); Podophyllum (Mandrake); Trillium (Trillium); Impatiens (Jewel Weed); Gramineae (Grass) and Trifolium (Clover).

(9) Growing conditions should be considered in relation to virus infection. Fast-growing succulent plants are more susceptible to infection than are slower-growing plants (Bawden)[26]. May of both 1952 and 1953 were as warm as, or warmer than, the average month of May (based on 46 years' observation by the Hamilton weather station)./ (p 41) The precipitation for these months was also significantly above average. Therefore, it may be presumed that the early spring plants were brought on quickly and could easily be infected, and this infection would multiply through June to a point where it would be easily transmitted to test plants by the end of June or early July. In both years, June was warmer than usual, which would also favour virus multiplication.

(9)(a) The lower-than-usual rainfall during June and July,/ (p 41, 42) together with higher-than-usual temperatures, would slow down growth through July, and consequently decrease susceptibility to infection. At the same time it would hasten the die-back of spring plants, especially those whose metabolism was disrupted by disease. Hence, a low percentage of virus infection is recorded on the first of August. The weather continued to be warmer and drier than usual in the fall of 1953, and the amount of infection continued to decline.

(10) As well as weather conditions, insect populations are significant in accounting for virus infection. These two factors are related. A mild winter means a higher percent of the insect population is able to overwinter. A mild or warm April and May induce an early beginning to insect life cycles. Hence, by late June there would be a large population of adult insects which spread infection. Smith (15) lists most vectors -- species of thrips and aphids in particular -- as reaching the peak of the first cycle in June. This, then, would help to account for high infection in early July. The average cycle is three to six weeks, depending on weather and species; hence, the second peak is reached late in August and early September. This corresponds to the secondary peaks of infection.

(11) It is an established fact that viruses are sensitive to heat. Changes in temperature, then, can be responsible for the decline in spread of virus during the summer. At about 75° F. leafhoppers readily transmit aster yellows disease, but at 90° F. they lose the power to infect plants. The insects are more sensitive to heat than the plants, because they are much smaller and therefore more quickly warmed.

A fall in temperature will restore the insects' infectivity, if the hot spell has not been too prolonged.

Such conditions occurred in July of both 1952 and 1953.

(12) The aphid population was normal in 1952 and 1953, but above normal in 1951. This fact may account for the higher virus infection in 1951.

(13) Woody perennials such as Corylus (Hazel) and Rubus (Raspberry) showed high infection early, mid and late summer, indicating systemic infection which was probably carried over from year to year.

(14) Water plants which are found in abundance early in the summer, such as Nymphaea (Pond Lily) and Potamogeton pectinatus, show early infection. Water plants which reach the peak of growth later, such as Ceratophyllum and Lemna, show peak infection later in the season.

(15) Symplocarpus (Skunk Cabbage) and Polypodiaceae (Fern) were the only two plants listed (P 33) as "land plants" which were not found to be infected with virus at some time during 1952 or 1953. The former was found in "ravine bottom" areas; the latter was sampled in "open marsh" areas.

(16) There does not appear to be any obvious relation between types of ecological zones and distribution of virus infection. No one type of area was a consistent leader for the three years. The area designated as "uncultivated pasture" showed the highest infection in 1951 and 1953, but this same type of area, while showing a high (9.2%) infection in 1952, was listed near the bottom. The area designated "ravine bottom" showed considerable variation in amount of infection - 3.1%, 11.8%, 9.3% - but was always

listed in second or third place in the order of infection.

(16)(a) The groups listed above (clause 15) as having a significantly high percent of infection in 1953 will be noted to have consistently high infection per quadrat within the group in 1953.

(16)(b) If it were not for the fact that the percent total infection in the "ravine bottom" group was reduced by the lack of infection in Symplocarpus (Skunk Cabbage), this group would probably be the consistent leader. General conditions within these areas are also significantly constant, i.e. temperature, humidity, water table, insect, and plant populations. All of these factors contribute to the spread of infection.

(16)(c) Since so many sampling areas were changed in 1952, it is not advisable to include the figures obtained in 1951 in any comparison of quadrats.

(16)(d) Within an ecological zone, there is considerable variation in the amount of infection. Eg. 1952, cultivated pasture group varied from 38% - 4% - 12% in the three quadrats.

(17) Plants sampled in more than one type of area do not show a constant higher percentage of infection in one type of area over another. Eg. in 1952, Rubus was found to be most highly infected on ravine slopes (20%), while in 1953 it was found most highly infected in uncultivated pastures (20.8%). Gramineae (Grass), Potentillae (Cinquefoil) and Phleum (Timothy) varied in a similar manner, while

Impatiens infection was the same for ravine slope as for ravine bottom in 1952 and 1953.

(18) There seems to be no consistent correlation between any measured amount of available nutrient and the amount of virus infection.

The pH values vary considerably between the three examples of ecological zones which would exert a control on the flora to be found there.

(19) The quantity of microflora varies as much between so-called similar ecological zones as it does between different zones.

A DETAILED STUDY OF VIRUS IN ONE GENUS IN ONE AREA

The plant studied was Solidago, common goldenrod, without regard for species. The virus studied was a sugar beet, curly top type, which was found to be mechanically inocuable to N. glutinosa N. tabacum, and Antirrhinum (snapdragon).

In order to study the prevalence, distribution and seasonal aspect of virus, a twenty-five ft. plot of pasture land was studied in detail. This area included (4c) one of the ten-foot plots regularly sampled to see if the random sampling was giving a true picture of the virus situation. The plot was at the edge of a field ten to twelve ft. from a wooded area and included a footpath across one end. The field had not been cultivated or grazed for twenty-five years.

Once a month for three consecutive months, the location and condition of each goldenrod plant in the plot was charted. Each was classified as healthy, damaged by insects, containing fungal, bacterial or virus diseases. These were classified according to microscopically examined samples.

TABLE XIV.

Date of Sampling	No. of Goldenrod	No. with Virus	No. with Bact. or Fungus	No. with Insect Damage
June 30	412	20=4.85%	15=3.64%	31=7.52%
Aug. 2	406	44=10.8%	48=11.8%	74=18.2%
Sept. 9	402	50=12.4%	53=13.1%	58=14.4%

CHART 1.

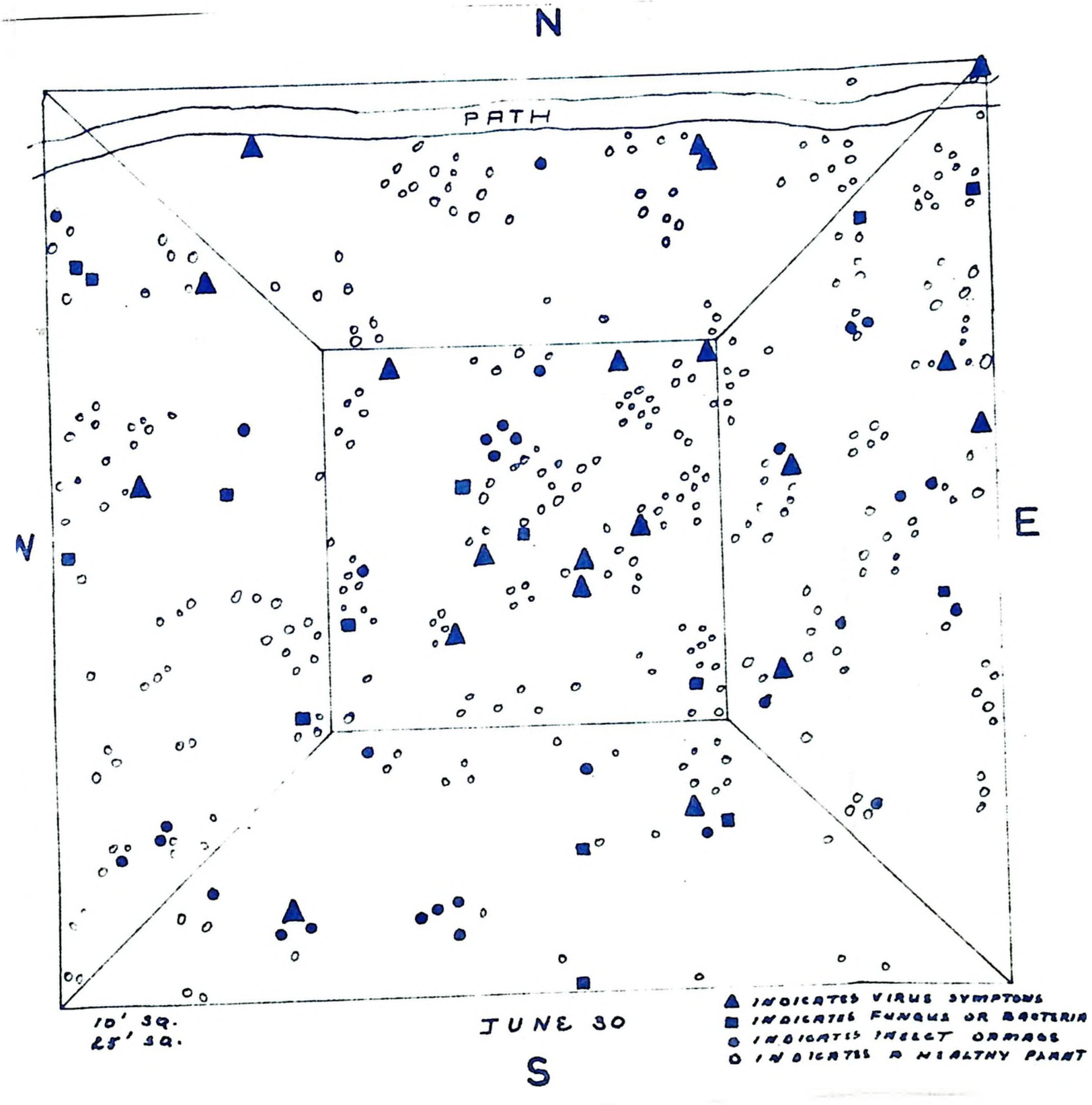
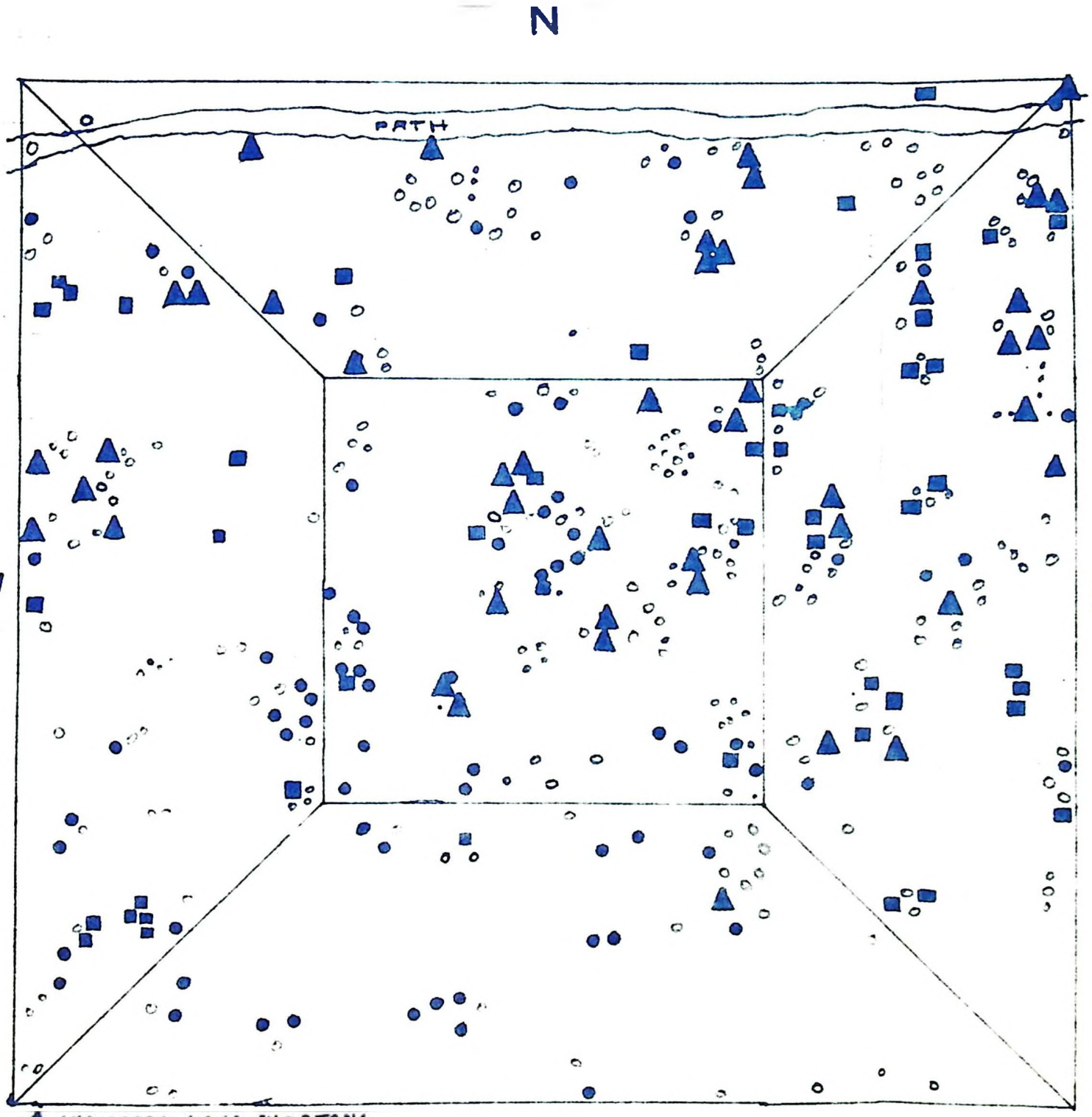

N
PATH
N
E
10' SQ.
25' SQ.
JUNE 30
S
INDICATES VIRUS SYMPTOMS
INDICATES FUNGUS OR BACTERIA
INDICATES INSECT DAMAGE
INDICATES A HEALTHY PLANT

CHART 2.



AUG. 2

10' SQ. 4c
25' SQ

CHART 3.

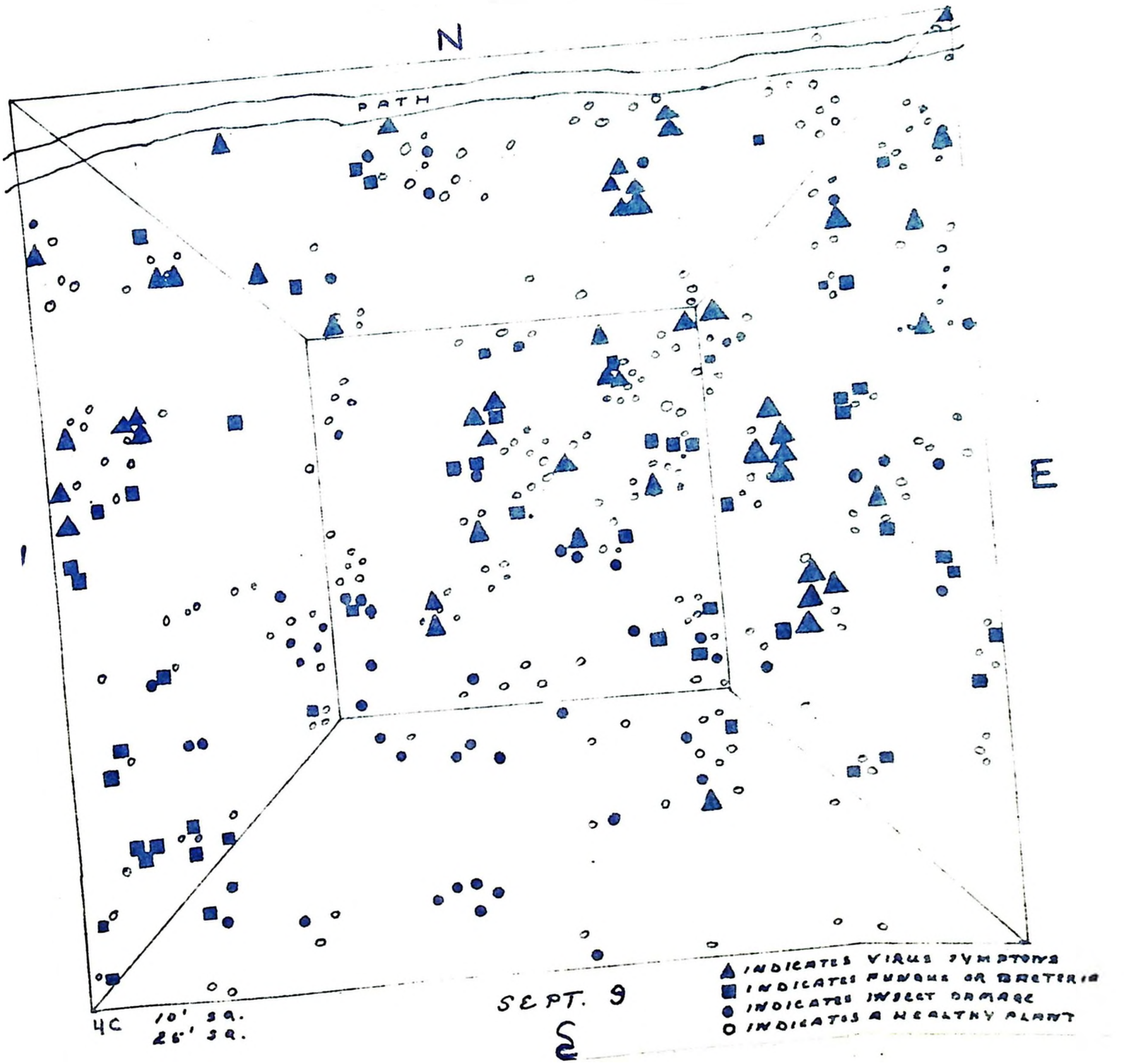
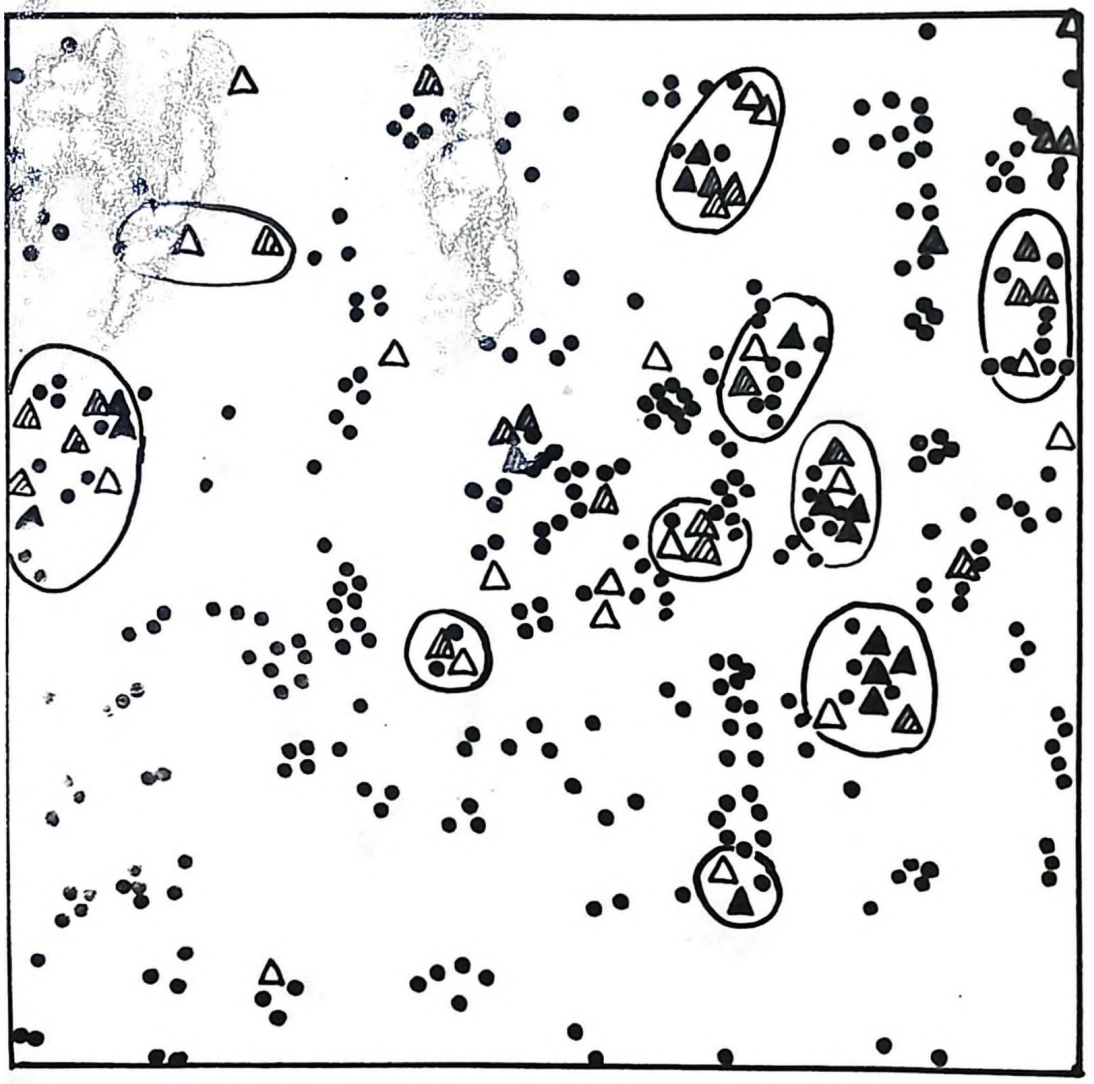

N
PATH
E
S
SEPT. 9
4C 10' SQ.
26' SQ.
▲ INDICATES VIRUS SYMPTOMS
■ INDICATES FUNGUS OR BACTERIA
● INDICATES INSECT DAMAGE
○ INDICATES A HEALTHY PLANT

CHART 4.


△ VIRUS JULY 1
△ VIRUS AUG. 1
▲ VIRUS SEPT. 1
Location of Solidago in 25' sq. pasture plot.

RESULTS OF SOLIDAGO SURVEY

(1) The number of goldenrod plants showing virus symptoms steadily increased through July, August and September. The greatest increase took place in the month of July, i.e. during the main vegetative growing period of goldenrod.

(2) Threequarters of the plants charted remained healthy with respect to all factors counted throughout the season.

(3) The number of virus-infected plants remained less than 13% of the total examined.

(4) The spread of virus was predominantly to plants in close proximity to those previously diseased. In most cases, the leaves of the plants in clusters often were in contact and would rub each other in a slight breeze.

(5) The random sampling of plot (4c) in 1953 showed that three of the 24 plants sampled were infected. This 36.6% is well above the 12% count found in the larger area. The difference, in all probability, is related to the difference in assessing. The larger amount was found from inoculation, the smaller from a count of visible symptoms.

(6) From an examination of these three charts, the spread of virus appears to be related to the density of plants. Sequences of infection are noted in groups of closely associated plants, outlined on the composite fourth chart.

(7) It can be seen that some plants showing insect damage later developed virus. Virus was the primary interest and when it was found, other classifications were omitted. Hence, there is a change in the listing of the number of plants showing a particular symptom.

THE STUDY OF VIRUS IN THREE SPECIES IN EACH OF THREE LINE TRANSECTS

A survey of the Botanical Gardens revealed that the choice of plants for line transect survey would be limited, for few species were found growing in a wide range of conditions. The transects were laid out so as to include a path or roadway in order to assess the effect of traffic on virus distribution. To be of any consequence, it was felt that the transect should extend at least 4 ft. on either side of the path so that the farthest plants would not be touched by traffic. At the same time, it was desirable to have the nearest plants overhanging the path.

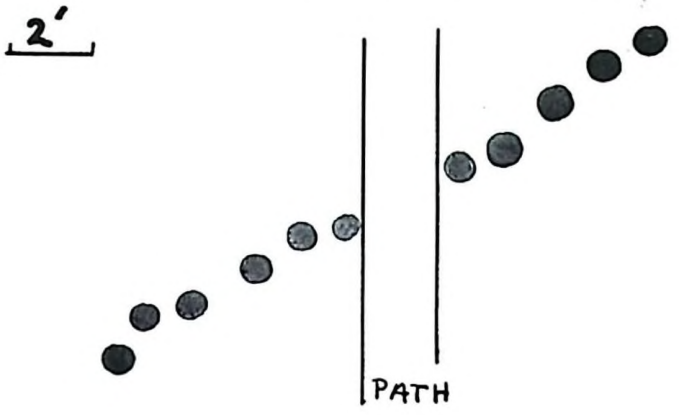
Three such transects were established. The first was a 15 ft. line transect of Desmodium (Tick-Trefoil). The 11 plants in the transect extended 4 to 5 ft. on each side of a path on a ravine slope. The area was heavily shaded by large oak trees. The second transect was a 16 ft. line of Thalictrum (Meadow Rue). The 11 plants of this transect crossed a path and extended 6 to 7 ft. on either side on a steep ravine slope. The area was shaded but not as heavily as number one. The third was a 25 ft. transect of Prunella (Self-Heal). The 21 plants extended from under the shade of the woods out across a roadway and well out into a cultivated pasture field.

The plants in each transect were tagged and charted. Conditions and appearance of each plant were noticed including symptoms of insect damage, fungal or bacterial, and virus disease. Sample leaves were taken from each plant at two-week intervals. These were macerated and inoculated to test plants in the greenhouse in the prescribed method.

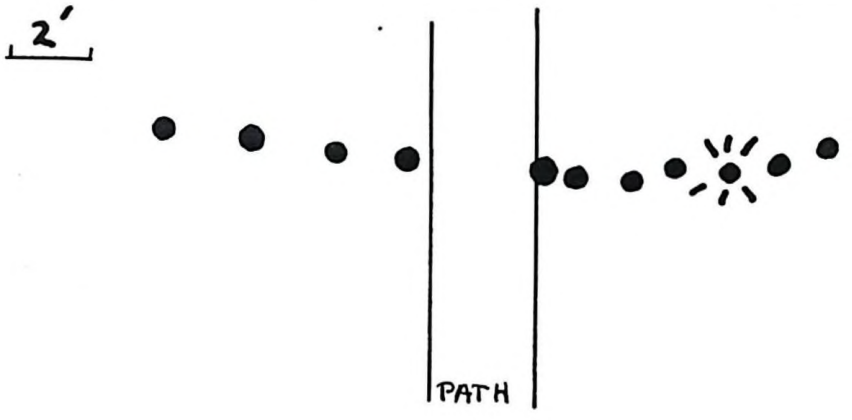
LINE TRANSECTS

CHART 5.

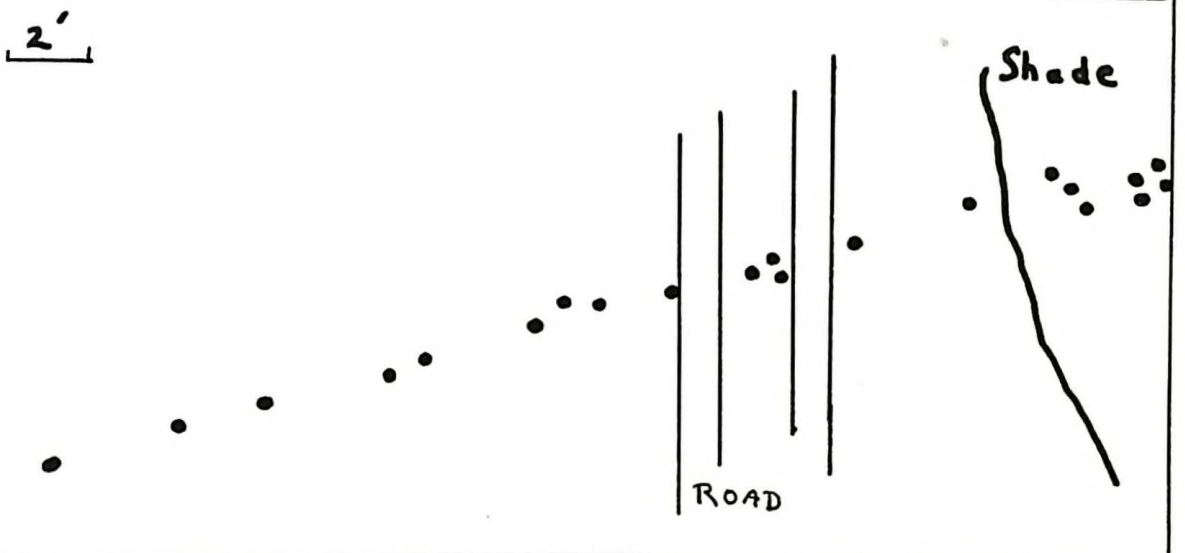
DESMÒDIUM



THALÍCTRUM L.



PRUNÉLLA L.



RESULTS OF LINE TRANSECT SURVEY

(1) None of the sampled plants displayed any virus symptoms in the field.

(2) One out of the forty-three plants sampled contained virus which gave a necrotic ring reaction on N. glutinosa.

(3) This infected Thalictrum was not overhanging the path but rather was approximately four ft. from the path on an upgrade slope.

(4) The survey does not indicate whether traffic plays an important role in the transmission of virus or not.

(5) The amount of infection found in these transects is much less than that found in more densely populated quadrats.

SPREAD OF VIRUS IN LAWN WEEDS

Many plants of Plantago major L. (plantain) in the cut lawns of McMaster campus showed virus symptoms. These included extensive yellow mottling, yellow veins, leaf distortion caused by vein-puckering, and a bright green mottle.

Each symptom was found to be caused by a virus mechanically transmissible to at least one of five test plants, including plantain itself. The symptoms suggest a virus of the cucumber mosaic group. It was assumed that this virus could be spread through the lawns by mechanical means, probably the lawn mowers.

In order to study the virus in plantain, two plots, each 3 ft. by 5 ft. were marked out. These were located in areas where the lawn mower would always pass in the same direction in order to find if there was a predictable progression of the virus. About mid-summer the grounds department changed from a reel-type cutter to a horizontal rotary sickle-type cutter, which spread the sap from infected plants over a broader swath each cutting.

Each plantain was identified by superimposing a grid. The condition of each leaf was noted at two-week intervals. The lawn was cut at least once between observation dates. The symptoms found and studied in these plots were mostly yellow mottles, and a few yellow vein types. This latter symptom was later found to be the early stage of the yellow mottle.

There was a possibility that the virus was transmitted by root contact. This factor was checked by planting diseased and healthy plantains in the same pot, in such a way that the roots were intermingling but the above-ground structures were kept apart by heavy

The following charts represent graphically the arrangement and location of the plantain leaves at six stages of the study.

Sign thus  indicates healthy plantain leaf.

Sign thus  indicates leaf with yellow mottle.

waxed paper.

The virus of plantain was purified by a modification of the method used by Bawden and Pirie (17) for the purification of tobacco mosaic virus. There is no record of plantain virus having been purified.

PREPARATION OF PURIFIED PLANTAIN VIRUS EXTRACT

(1) Plant tissue was ground up in a food chopper.

(2) The sap and masserated tissue were run into one layer of gauze and the juice was expressed by squeezing by hand.

(3) A volume of saturated ammonium sulphate solution equal to half the volume of sap was added.

(4) The mixture was allowed to stand two hours.

(5) The precipitate was centrifuged down and the supernatant was discarded.

(6) The precipitate containing most of the virus was re-suspended in 5 ml. of water (1/20 original volume of sap).

(7) It was dialysed against tap water for thirty hours.

(8) The solution was centrifuged to remove insoluble matter and the remainder bottled and stored in the refrigerator.

This purified virus extract was inoculated to healthy plantain plants in the greenhouse. These plants developed virus symptoms. Three months later some of the extract was inoculated into N. tabacum var Harrow Velvet and N. glutinosa. The tobacco showed green blister symptoms similar to cucumber-type virus within a week. The plantain virus then can be extracted by this simple salting-out process and has a long enough period of viability to make it applicable for making serums for serological virus investigations.

CHART 6.

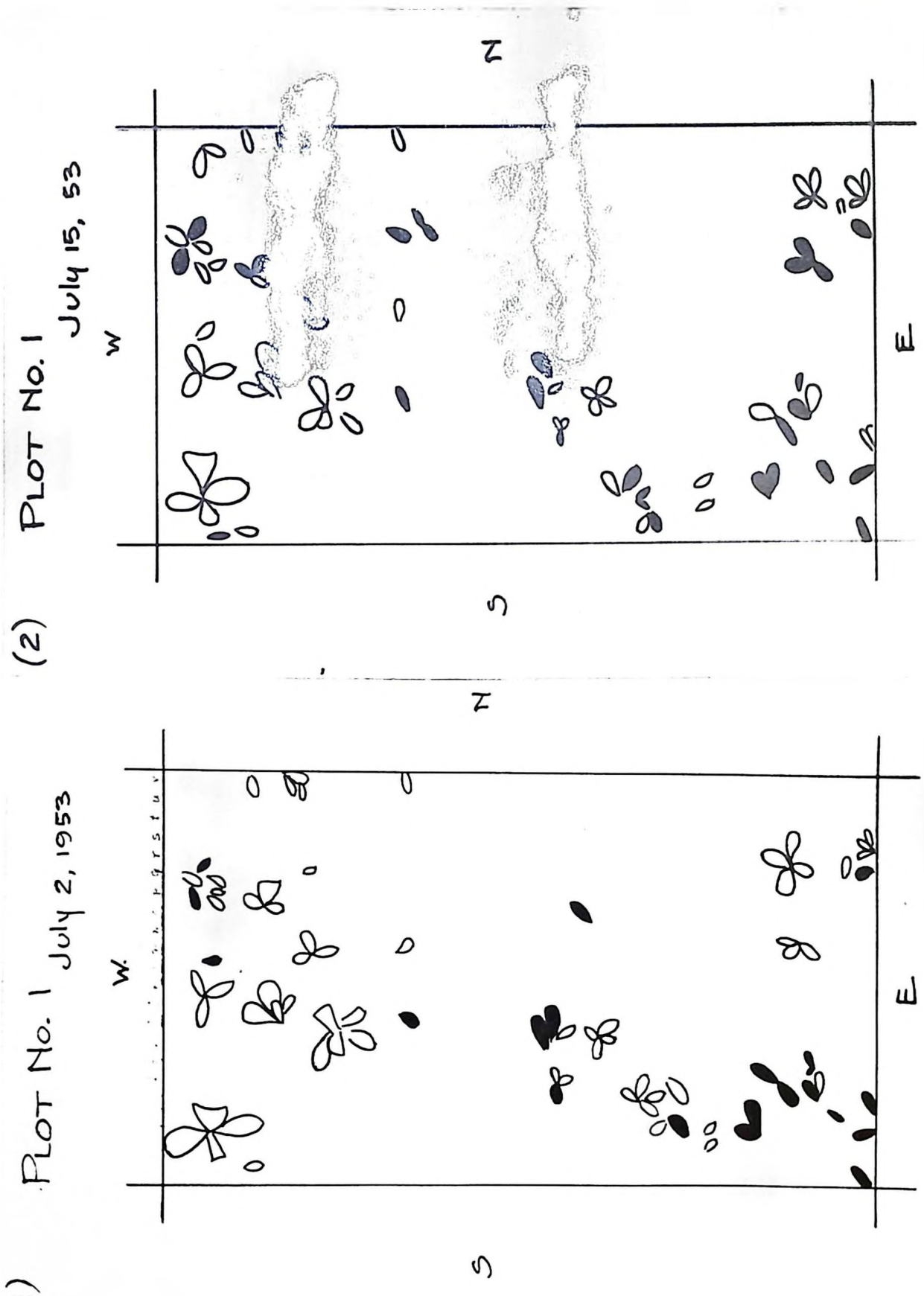
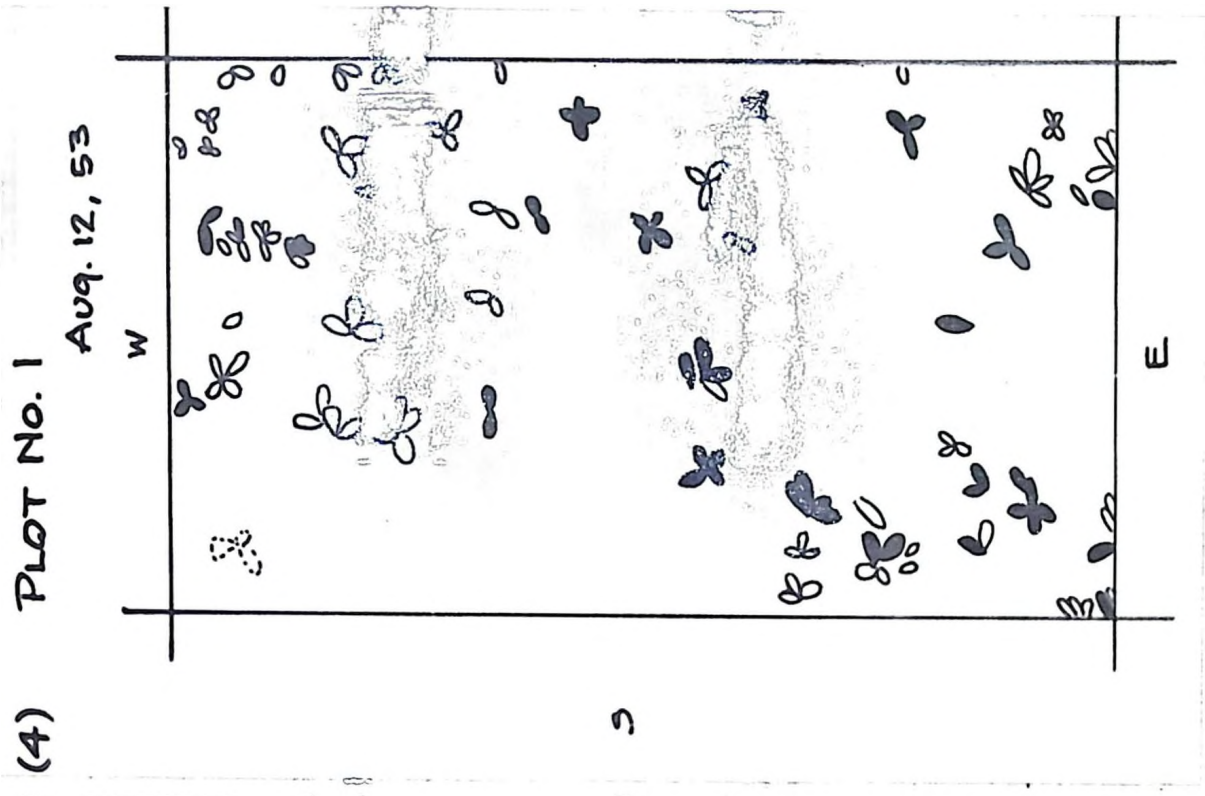
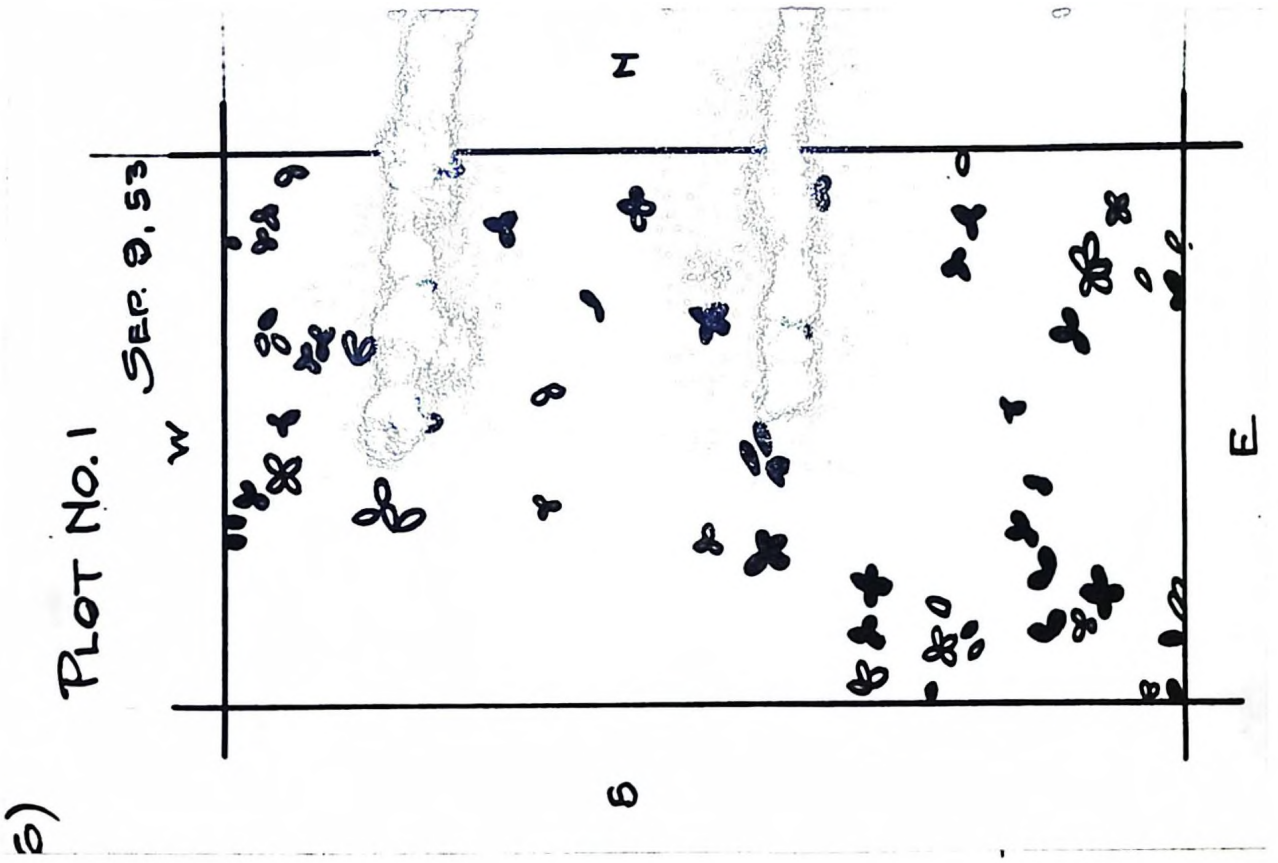
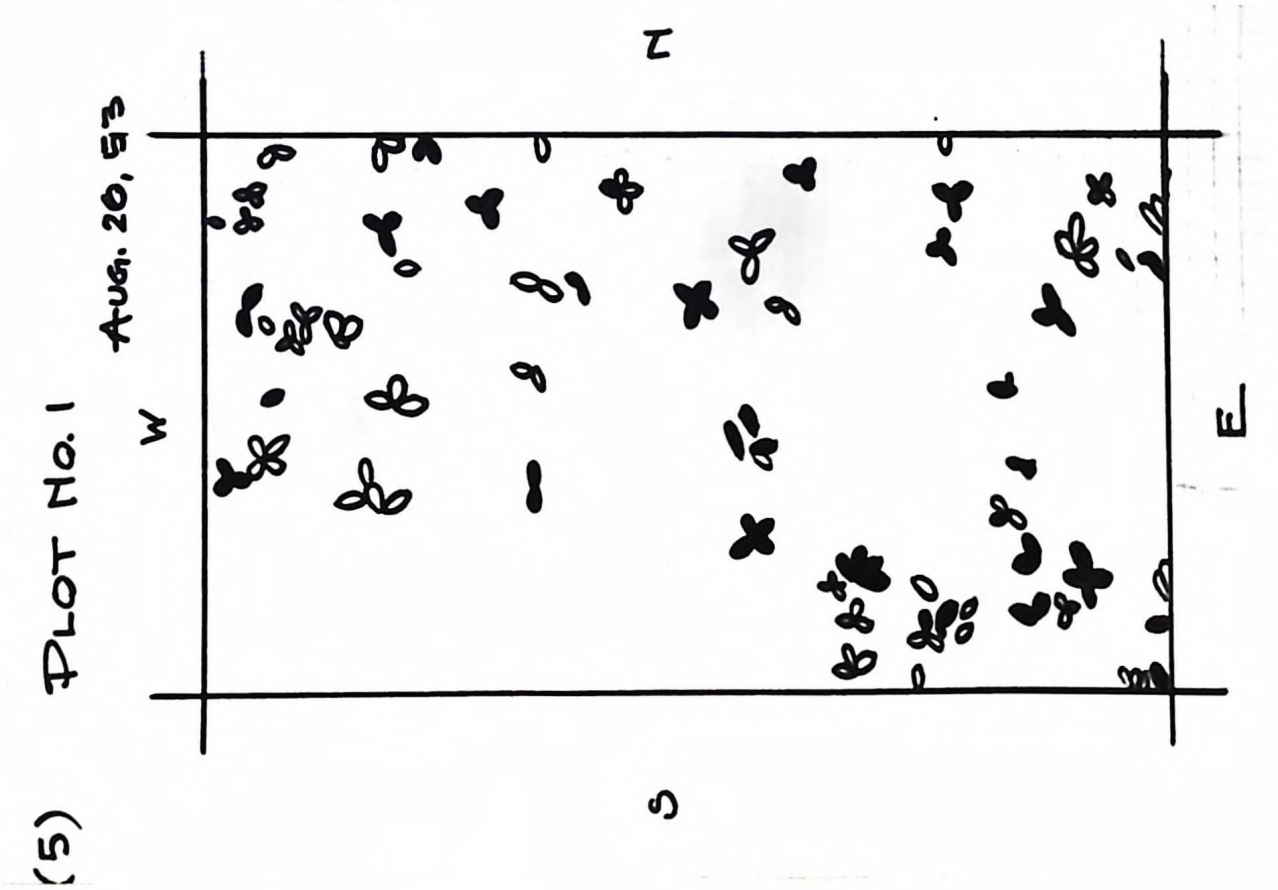


CHART 7.

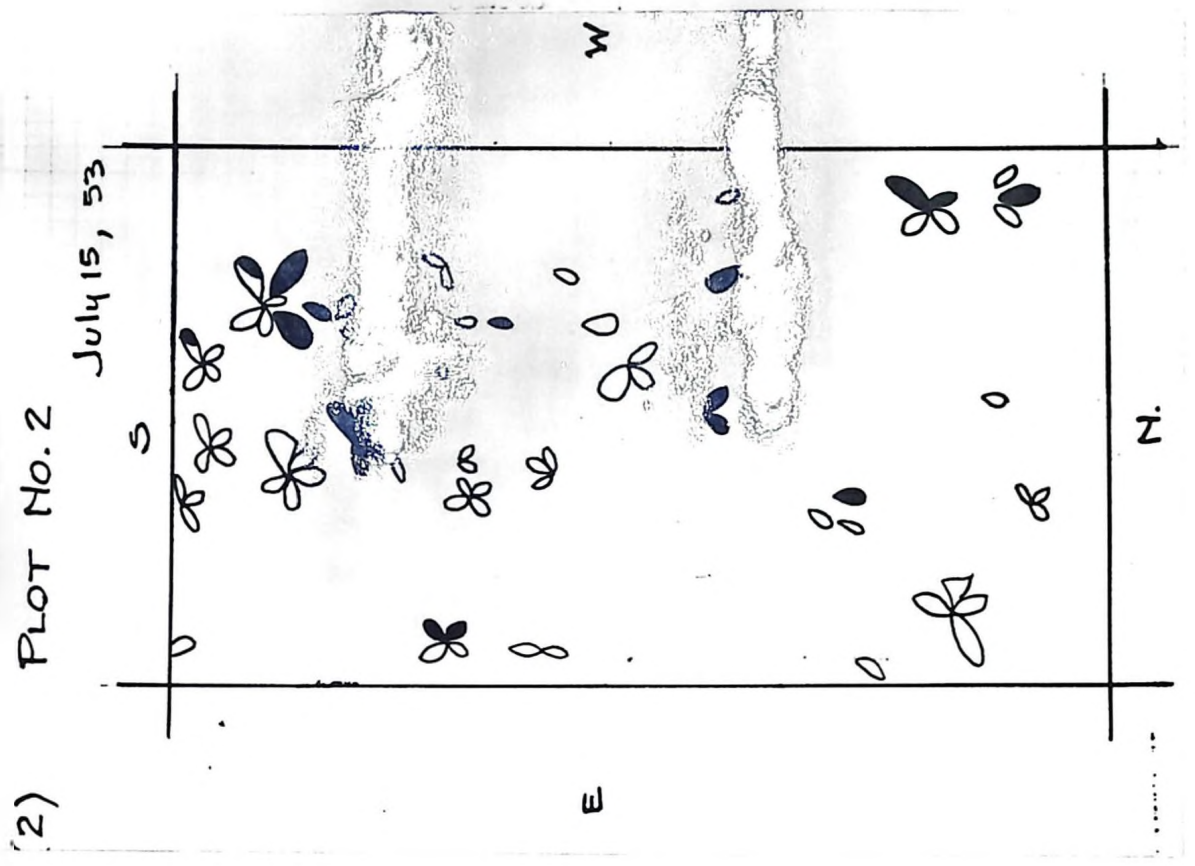
CHART 8 & 9

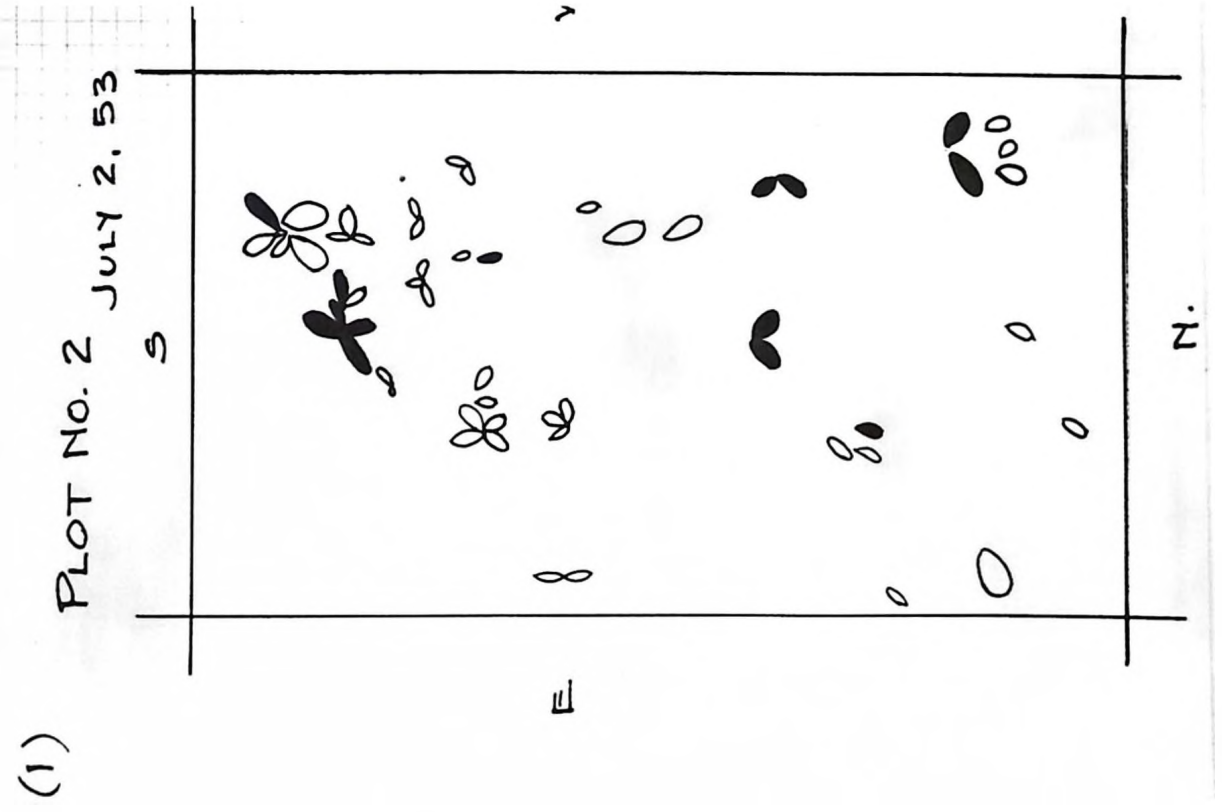



(6)
PLOT NO. 1
SEP. 9, 53
W
N
S
E


(5)
PLOT NO. 1
AUG. 20, 53
W
N
S
E

CHARTS 12 & 13





(4)
PLOT No. 2 Aug. 12, 53
W
S
N
E

(3)
PLOT No. 2 July 26, 53
W
S
N
E

(6)
PLOT No. 2 DEC. 9, 53.
S
W.
N.
E.

(5)
PLOT No. 2 AUG. 26, 53
S
W.
N
E
ym

TABLE XV.

Date	Plot 2 by Tennis Court	Plot 1 by Greenhouse
July 2	8/31 = 25.8%	15/40 = 37.5%
July 15	12/35 = 34.2%	20/41 = 48.7%
July 26	21/60 = 35.0%	29/57 = 50.9%
Aug. 12	19/70 = 27.2%	26/57 = 45.6%
Aug. 26	19/70 = 26.8%	29/63 = 46.0%
Sept. 9	17/67 = 25.4%	32/66 = 48.5%

RESULTS OF PLANTAGO SURVEY

(1) The virus affecting plantain is readily transmitted mechanically to test plants and to plantain.

(2) It could probably be transmitted by a lawn mower.

(3) It is found in not less than 25% of the plants studied and not more than 51%.

(4) The virus of plantain reached its highest proportion in late July, i.e. at the peak of the growing season.

(5) It holds to a fairly constant level of infection.

(6) In the pairs of diseased and healthy potted plants there was no sign of spread of infection from diseased to healthy plants through soil or roots.

(7) It can be purified by an ammonium sulphate method. This extract remained viable for five months. When inoculated to tobacco, it gives a mosaic pattern typical of Doolittle's cucumber mosaic.

(8) Due to the change in cutting method, the probability of regular linear progression of transmission would be reduced.

COMPARISON OF NATURAL VIRUS INFECTION IN CROP AND WILD PLANTS

As has been established in the earlier part of this presentation, there is a high incidence of infection in wild plants. Likewise, virus infection is frequently high in crop plants. To compare the amount of natural virus infection found in uncultivated crop plants with that found in wild plants, two garden plots were established surrounded by natural pasture land.

One plot was cleared out of a weedy area near cultivated gardens and also near a plot used in the random survey (4c).

The second plot was cleared out of an uncultivated field on the north shore of the marsh. It was in a very out-of-the-way place and not likely to be visited by man. It was hoped that in this location any traffic influence or influence due to nearness to civilization might be eliminated.

The plots were seeded in the first week of June with Zea mays L. (corn), Trifolium pratense L. (clover), Pisum sativum (pea), Lactuca savita (lettuce), Raphanus sativus (radish), Phaseolus vulgaris (bean), Daucus carota (carrot) seeds. In addition, eight Turkish tobacco plants, Nicotinum var Turkish, and eight tomato plants, Lycopernicum esculentum, were set out.

These gardens were left to nature for watering and cultivation in order to avoid the possible mechanical spread of virus.

Plants of every type found in the gardens were sampled in mid-July and August. The foliage was masserated and the juice inoculated

The cycle of appearance, growth, and disappearance of leaves was so rapid that some of the sequences were missed. It is, however, evident that the transmission was always to plants in close proximity to those which earlier showed evidence of being diseased.

Nicotinum glutinosa, Physalis a, Nicotinum var Jamaica Wrapper, Gomphrena globosa, in the manner hitherto described.

RESULTS OF CROP AND WILD PLANT COMPARISON SURVEY

(1) No virus symptoms appeared on any of the test plants.

(2) Uncultivated plants in the field adjacent to the south shore plot showed an average of 15% infection in the random survey.

(3) The same area chosen for the graphic survey of virus of Solidago (goldenrod) showed approximately 10% infection.

(4) Agricultural plants in adjacent gardens showed some virus symptoms.

CARRY-OVER OF VIRUS IN OVER-WINTERING PLANT STRUCTURES

In October of both 1952 and 1953 zero percent infection was found in above-ground structures sampled in the random survey. It was suspected that this figure did not represent the true state, for there was probably some reservoir of infection from which the early summer infection was derived. Lack of infection in October could be due to the earlier death of virus-infected above-ground structures.

Three types of investigation were carried out during the winter months.

(a) Underground structures such as roots, rhizomes and bulbs of the species sampled during the summer were tested periodically for virus.

(b) Fifty species of trees were sampled for over-wintering virus.

(c) The root structure of every goldenrod plant in a 7 x 8 ft. plot, adjacent to plot (4c) of the random survey was sampled.

Part (a) was carried out in much the same manner as the summer survey. Root structures were dug up at random in the plot whenever a thaw permitted. The tissue was masserated with a sterile pestle and mortar and diluted to one part in ten with water for inoculation. Some dilution was necessary because of the lack of liquid present in woody-type structures. Each sample was inoculated to N. glutinosa, N. tabacum, and to at least one other of a wide range of garden vegetables known to be susceptible to virus infection.

Results of Part (a) Underground Structure Survey

(1) Virus was found to be present in nineteen of the 241 samples taken.

(2) It was present in the rhizome of Podophyllum, the bulb of Trillium

the roots of Solidago, Potentilla, Phleum, Trifolium, Gramineae and Viola.

(3) No virus was found in the woody underground structure of Corylus or Rubus, nor was there any in the rhizome of Smilacina, or in Symplocarpus.

TABLE XVI.
Infection per Species per Area
Cultivated Pasture

Species	Plot 1	Plot 2	Total
Gramineae	0/6	1/6	1/12
Potentilla	1/6	0/6	1/12
Phleum	1/6	2/6	3/12
Trifolium	1/6	3/6	4/12
Arctium	0/4	0/4	0/8

Ravine Bottom

Species	Plot 1	Plot 2	Total
Viola	2/7	0/7	2/14
Symplocarpus	0/7	0/7	0/14
Impatiens	0/4	0/4	0/8
Corylus	0/6	0/7	0/13
Rubus	0/5	0/7	0/12

TABLE XVI. Cont'd.

Ravine Slope

Species	Plot 1	Plot 2	Total
Smilacina	0/6	0/7	0/13
Podophyllum	2/6	1/7	3/13
Rubus	0/6	0/7	0/13
Impatiens	0/3	0/3	0/6
Trillium	0/6	1/7	1/13
Arisaema	0/2	0/1	0/3

Uncultivated Pasture

Species	Plot 1	Plot 2	Total
Solidago	2/6	1/7	3/13
Potentilla	0/6	1/7	1/13
Rubus	0/7	0/4	0/11
Graminese	0/6	0/7	0/13
Phleum	0/6	0/7	0/13

TABLE XVII.

% Infection per Ecological Area

Cultivated Pasture	9/56 = 16.0%
Uncultivated Pasture	4/63 = 6.4%
Ravine Slope	4/61 = 6.6%
Ravine Bottom	2/61 = 3.3%
TOTAL	19/241 = 7.8%

Part (b): Tree Survey

Fifty species of trees, excluding gymnosperms, found along the wooded south shore of Cootes Paradise, were identified, tagged and sampled. Samples consisted of buds and last year's growth only. This selection was made in order to obtain the largest proportion of living green material, the more likely site of virus. Each sample was masserated in a sterile pestle and mortar, then diluted up to 1:10 parts for inoculation. Test plants used were N. Glutinosa, N. Tabacum, Datura stramonium and Lycopersicum esculentum.

Results of Part (b): Tree Survey

(1) No virus infection was found in any of the tree samples.

Part (c): Solidago Root Survey

The area survey was adjacent to plot (4c) sampled in the random survey in both summer and winter.

Each underground structure, periennial, annual or biennial, of Solidago within an 8 ft. x 7 ft. plot was dug up and sampled for virus. The tissue was masserated in the usual manner and inoculations made to N. Glutinosa, N. Tabacum and Cucumis stativus.

Results of Part (c): Solidago Root Survey

(1) 75 Solidago plants were sampled in the plot.

(2) 5 of the 75 plants were found to contain virus.

(3) Each plant of two pairs of plants with intermingling root structures was infected. The other infected plant was also in a cluster of plants with intertwined roots, but no further infection was found in this clump of plants.

GENERAL DISCUSSION

The virus research in plant pathology has been concerned with particular crop problems. The fundamental problems dealing with the quantity of naturally-occurring virus diseases, the general distribution and frequency pattern have not previously been touched. Therefore, there is no mass of background data with which to compare the findings of this survey. Only isolated references can be found to virus in wild plants (18-25). (27-41) Since a broad basis of existing knowledge is lacking, it is obvious that this one survey cannot solve all the problems. As this is only the beginning of a large field of study, it is appropriate that it should point out many problems which lie ahead. It is only after evaluating such a preliminary survey that the most productive fields of future endeavour can be indicated.

Surveys reported in this thesis account only for viruses which are mechanically inocuable and which show a reaction on the limited number of test plants used. The exact proportion of insect-transmitted virus to those mechanically transmitted is not known. It is, however, inferred by K. M. Smith that this ratio would be four to one. If this is the case, then an infection level of 10% (p 35) of a randomly sampled population could be interpreted as a 50% infection. This is very close to epidemic proportions according to agricultural standards.

The next question to be answered is the detailed identification of viruses in wild plants. These may be the same as those affecting the agricultural crops (p 49, 57, 58). This should be checked by some exact method such as serology.

Another problem, which will be solved at the same time, is whether or not the same viruses which are responsible for the early summer peak infection are also responsible for the late summer peak. If the former, then the host specificity is not limited and the chances of spread and increase throughout the summer are great, including the possibility of spread to agricultural crops. If the latter, then the chance of an epidemic occurring is lessened, depending on the rate of growth of the host.

The rate of increase of amount of above-ground structures of plants can be represented by rate of growth curves, such as the Daily Increment curve (fig. 20, p 116, D'Arcy W. Thompson, Growth and Form). Rate of growth curves for most plants can be divided into three types. The first represents the type of plant which has its main vegetative growing period early in the season. This curve tapers off rapidly by mid season, as the plant dies back by late summer. Trillium is an example of one of the species sampled which has a growth curve of this type.

The second type of curve includes most annuals, such as Impatiens. The peak of the approximately symmetrical vegetative growth curve occurs at mid-summer.

The third case includes the late flowering plants, such as Solidago. The growth curve rises slowly until mid-August before declining.

It is more than a coincidence that the growth curve of Trillium, Impatiens and Solidago suggest the respective curves of virus infection for those species (p40). The total virus infection at any one time as expressed by results on test plants (p 38) should be related to the amount of new above ground structures of plants at that time. The infection found

on spring plants, which suggests the first type of postulated growth curve, plus the infection found on the summer annuals, related to the second type of growth curve, must overlap. This helps to explain the highest amount of infection found in early July of 1952 and 1953 (p 38). The second lesser peak, found in mid-August, would correspond to the peak of rapid vegetative growth found on the third curve, such as the growth of Solidago.

This suggests that not only the rate of spread of infection has been measured, but also the increase of infectious material within a particular species, and probably within an individual plant. It is known that a tobacco plant systemically infected with tobacco mosaic may in time be non-infectious. Such a phenomenon suggests that the virus has become so diluted as to be non-infectious (26), or that, due to host reactions, has become latent, such as the vegetative stage of bacteria-phage (27). This occurrence is found only when the plant has ceased vegetative growth. Hence, the implication would seem to be that what has been measured is the state of virus within the flora, as well as its distribution and frequency. This is a dynamic state, in which the level of infection increases at a rate related to the rate of growth and total growth of the plant, then decreases with maturation and cessation of vegetative growth.

The measure of root system infection shows that viruses are present in many underground structures. It should, however, be noted that the measure was taken from the softest root tissue, i. e. living and slowly growing tissue, rather than woody tissue. Virus was not found in the woody tissue sampled in the survey of trees (p 73). This does not conclusively prove that virus was not present. It may have been too dilute or in such a state as to be non-infectious. It remains to be proven whether or not these structures will harbour

infectious virus units during active growth in the summer.

The fact that the winter survey gave a higher percent infection (p72) than the June sampling in 1953 (p30)i.e. 7.8% as compared with 4.75% needs explanation and could be reasoned this way. Water plants were sampled in the June survey. These plants, due perhaps to their slow growth in early summer, do not show much infection until later in the season. Hence the inclusion of water plants has the effect of reducing the percent infection found. These plants were not included in the winter survey. Another reason for the discropancy lies in the fact that infected plants grow more slowly and hence are less likely to be among those sampled in June, while there is an equal chance of their being sampled in winter, along with healthy structures.

In the beginning it was assumed that all areas similarly situated and having the same cover plants were similar enough to be grouped together as ecological zones. Included in this presumption and grouping was susceptibility to disease. This has not proven to be the case. Far more variables than at first realized enter into the picture of susceptibility to virus infections. Among these variables appear to be all factors which affect plant growth. Hence, it has been found unwise to group widely spaced areas together under one heading, such as "Ravine Slope." Chemical soil analysis and microflora analysis vary in the three plots so grouped. In addition, the steepness of slope and range on the slope differed so that water run off was not the same. One slope was a western exposure, another an eastern and the third a southerly. All of these factors, plus

the shade and type of surrounding plants would affect the rate of growth of the plants tested.

As the peak of infection of individual species seems to approximate the period of fastest growth (p40), it is suggested that the faster-growing succulent plant is more susceptible to virus infection and may provide more infectious virus.

The chances of such a susceptible plant becoming infected with virus is also subject to another series of variables. Since insects are the most important means of transmission, any variable affecting them will be of primary interest. The over-all population of transmitting insects not only varies from year to year, but from month to month within any one area. This population also varies considerably from one area to another at any one time. In addition, the movement of small insects within one area will be influenced by prevailing wind strength, direction, and density of plant population.

These last two factors are of prime importance also in determining the chances of mechanical spread of a narrow-host-range virus. Effective density of suitable host plants includes such factors as height of plants, number of leaves, distance apart of plants and, hence, the amount of leaf abrasion caused by winds.

Dr. G. H. Berkeley stated, when reporting on the rate of spread of two viruses under study in fruit orchards: "Rates of spread are determined by initial incidence and by relative position of affected and healthy trees at planting and there is fundamentally little difference in rates of spread of the two viruses under study. Cherry yellow tends to spread more frequently to adjacent than to remote healthy trees. Its dissemination appears to be influenced to some

extent by prevailing winds." (16).

A similar statement could be made about the spread of infection followed in Solidago (p 54). There is a strong tendency for infection to spread to adjacent plants within a small clump. In this case, as well as the possibility of transmission through leaf abrasion, there is also the possibility of root transmission and systemic transmission through underground stems. Neither of these could be investigated in the preliminary survey. No matter what the means of dissemination, it becomes obvious that the density of the population is the most important influence on the rate of spread.

Spread of virus in a low-growing plant, such as Plantago (p 58), would be less influenced by wind energy than is the spread in taller plants. However, density of population and growing condition of the plant influences the chance of infection. Since mechanical transmission seems to be the likely agent, the amount of mechanical injury would determine the rate of spread of infection.

The problem of traffic influence on virus dissemination was investigated by means of three line transects, chosen to include pathways. None of the three species employed in the transect investigations had been samples in the quadrat surveys. The amount and symptoms of virus in these plants was an unknown. These three turned out to be relatively low in virus content (p 57) when compared to the average of wild plants. This may have disguised any influence of traffic. It does not, however, seem obvious that traffic was significant enough to be of primary importance.

The part of the project (p 68), to compare the amount of natural virus infection found in uncultivated crop plants with that found in wild plants, should be considered a preliminary survey only. Since one plot contained, at the most, only 70-80 plants, the results are not conclusive enough to state that virus could not be found in such an area. Nevertheless, the fact remains that no virus was found in these uncultivated crop plants, while infection was evident in the same crops in adjacent gardens. This may indicate that normally-cultivated crop plants, which are highly susceptible to virus, are less liable to infection when not cultivated. They may be even less liable to infection than wild plants. If this is true, it would indicate that a high percentage of virus infection in crop plants is due to cultivation.

This project should be repeated on a larger scale to further investigate this point. The garden plots should be increased in size, and should contain at least two dozen plants of each species found in the adjacent cultivated gardens. Plants throughout these gardens, as well as the plots, should be sampled to compare infection in cultivated, non-cultivated and wild plants.

The question of proximity to civilization or traffic could be answered through this garden project. No difference in the amount of infection was noted between the plot near to habitation and the isolated plot on the north shore. This is the same story as was found in the three transect surveys.

The garden project is of enough importance to be included in the long-range survey being carried out, so that data will be

collected over several years. It should serve the two-fold purpose of giving comparative data on the amount of natural infection found in wild and uncultivated plants and of answering the question as to whether or not virus dispersion is affected by nearness to civilization.

Since rate of spread of virus appears to be indirectly, but nevertheless significantly, influenced by so many environmental factors such as temperature, humidity, rainfall and, directly, by density of population of suitable hosts and of insect population, any slight variation or coincidence of variables will effect the amount of virus found in samples.

It has already been postulated that in any plant association there is a reservoir of virus. By any change in the above-mentioned factors, this endemic state could be quickly changed to an epidemic state. A virus epidemic, depending on its host range, could, by greatly reducing a species, upset the balance of population of the area. The significance of such a change in wild plant areas would be far reaching in an association of wildlife food and cover plants. Should this epidemic also spread to cultivated plants, it would seriously affect local agriculture.

SUMMARY

From comparison of the results of these experiments, it appears that there are very few species of wild plants which are free from virus infection. In comparison with this, most flowering plants growing under the surface of open water show a low but significant amount of virus. While only those viruses which can be mechanically transmitted and will show a reaction on the range of test plants used could be measured, the total annual infection was approximately 10% of the population sampled. The amount of infection in all plants varies with species and location from zero to 50%. An over-all seasonal trend appears to be related to weather and insect cycles. Total virus infection seems to be related to growth rates of plants. The probability of being infected is measurable in terms of transmissibility of viruses and population density of suitable host plants.

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