A STUDY OF VIRUS DISEASES OF STRAWBERRY IN MICHIGAN

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Robert Harry Fulton

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This is to certify that the

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Donald Cation
Major professor

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A STUDY OF VIRUS DISEASES OF STRAWBERRY IN MICHIGAN

By

Robert Harry Fulton

A DISSERTATION

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Department of Botany and Plant Pathology

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Finally, an expression of thanks to my wife, Lorraine, for her understanding cooperation during the course of studies in attaining this degree and expecially for her help in preparing this manuscript.

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AN ABSTRACT

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Approved Donald Cation
Chairman

Virus investigations in Michigan showed that the Type 2 virus was universally present in all cultivated varieties to the extent of 75 per cent of 575 samples. Type 1 virus was present in 15 per cent and combinations of the two types in 3 per cent. The type 1 virus was found only on plants tracing to out-of-state origin. Only 6.8 per cent of the 575 samples were found virus-free and as a result a virus-free foundation planting was established. The leaf roll and witches'-broom producing viruses were also reported.

Three innovations were developed which improved graft unions. They were enlargement of the stolons, creation of additional grafting surface and prevention of "tongue" curling.

Fragaria virginiana, Fragaria platypetala and Fragaria bracteata were used for the first time in comparison with Fragaria vesca for Type 1 virus and Type 2 virus symptom expression. F. virginiana was symptomless to both virus types. The other two species, F. platypetala and F. bracteata displayed varying degrees of sensitivity to virus infection.

The cultivated varieties Dunlap and Robinson were symptomless to both virus types. Catskill reacted with transient flecking to Type 1 virus and interveinal chlorosis to Type 2 virus infection.

All plants showing leaf roll virus symptoms also carried the Type 2 virus as demonstrated on F. vesca. Reddening of the petioles was a characteristic symptom for the leaf roll disease on F. vesca. Catskill and Dunlap showed leaflet chlorosis and mottling with extreme marginal rolling when infected with the leaf roll virus. Robinson and Gem were mildly affected.

Witches'-broom disease, leaf roll and Type 2 viruses were trans-

mitted by dodder, Cuscuta campestris; the first time this dodder species was used for transmission of these strawberry virus diseases. Through the dodder technique, Potentilla argentea, Potentilla recta and Potentilla anserina were found to be latent carriers of the Type 2 virus.

The viruses investigated were nontransmissible mechanically, through seed, pollen, soil, plant fragments or the root knot nematode in these studies. Transmission tests with Aphis forbesii, Aphrophora sp., Tarsonemus pallidus, Tetranychus sp. and Macropsis trimaculata were negative.

The Type 2 virus was inactivated in vivo with dry-heat ranging between 36° to 40° C. for varying exposure periods, with 38° C. for eight days giving the best results. Zinc salts also inactivated the Type 2 virus in vivo.

Virus-free Robinson strawberry plants showed 4 per cent more total and amino nitrogen than Type 2 virus-infected plants. The Type 2 virus-infected plants showed a 58 per cent reduction in transpiration rate.

It was demonstrated in these studies that Thornberry's * variegated clones with viruslike particles in the plant extracts were also infected with the Type 1 virus, which may have been responsible for his results with electron microscopy.

*Thornberry, H. H., A. E. Vatter and D. M. Beeson. 1951. Viruslike particles in strawberry plants with foliar variegation. Phytopath. 41: 35 (Abst.).

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CHAPTER I

INTRODUCTION

The weakening of strains in varieties of strawberries accompanied by reduced yield and runner production have been reported through the past years in Michigan. Many reasons, mostly unsatisfactory, have been proposed for this varietal "running out". The possibility that these disorders were of a virus nature was not discounted. However, determination of virus contamination was considered a lengthy and difficult task, but in recent years development of facilities and methods has made this determination feasible.

The importance of this problem was not realized until the announcement by workers in the United States Department of Agriculture (17) in 1950 that viruses were isolated from strawberry plants in Michigan. Furthermore, importation of the Marshall strawberry from the Pacific Northwest implicated a virus disease unknown to Michigan. These findings were met with concern for Michigan is considered a leading state in strawberry production.

In 1950, through the encouragement and support of Mr. C. A. Boyer, Michigan Bureau of Plant Industry, the writer was sent to the Plant Industry Station of the United States Department of Agriculture in Beltsville, Maryland, to learn the techniques of virus determination. This was accomplished under the skillful guidance of Mr. J. B. Demaree, Emeritus, Senior Plant Pathologist.

Soon thereafter a program was initiated to establish primarily a foundation in strawberry virus research which would ultimately serve the strawberry industry in Michigan. The first objective was to sample

cultivated strawberries for virus content and distribution in the state, which in turn would serve to establish a foundation stock of virus-free strawberry varieties to be made available to cooperating nurseries for future grower use.

Other problems considered were modes of virus transmission in the field, amount of virus content in wild strawberry and method of inactivating the virus in vivo. Physiological comparisons between infected and healthy plants which have a bearing on plant productivity have been carried out.

The results of the investigations on the viruses affecting Michigan strawberries are recorded herewith.

CHAPTER II

REVIEW OF LITERATURE

The development of strawberry virus research has developed independently in the British Isles and in North America. An account of the existing literature on the various virus diseases present in these areas is recorded in the following sections.

A. Strawberry Virus Diseases in the British Isles

In 1933, Harris (34) reported that degeneration of the Royal Sovereign variety was of a virus nature. Infected plants were dwarfed and the leaves showed a pronounced marginal chlorosis. The name yellow-edge was given to this disease. Subsequently, Massee (52) demonstrated that the strawberry leaf aphis, Capitophorus fragaefollii Ckll. transmitted the strawberry virus yellow-edge.

Massee (53), working with mild and severe crinkle, obtained transmission by using the strawberry leaf aphis. In 1947, Wood and Whitehead (102) reported that plants infected with severe crinkle were suffering from two virus entities, one persistent in the aphid while the non-persistent virus is transmitted when the acquisition feeding period is shortened.

Recently, Prentice and Harris (74) and Prentice (75 and 76) presented evidence that yellow-edge was the result of multiple infection by two or more viruses which were separated by varying the feeding periods of the vector.

In 1951, Prentice and Woollcombe (77) showed that a period of 10 to 19 days elapsed before C. fragaefollii became crinkle infective. This is recorded as the longest "latent period" for an aphid-transmitted virus.

The complexity of strawberry virus diseases was reported by Prentice (78) in 1952. He showed that by varying the acquisition and transfer feeding periods of the vector the yellow-edge and crinkle viruses could be separated into four entities; namely, strawberry mottle virus, mild yellow-edge virus, crinkle virus, and vein chlorosis virus.

In 1953, Posnette (73) reported on a new strawberry virus disease which he named "green-petal". The infected plants were dwarfed and the flower petals were green in color. This virus was considered to be of a killer type for the infected plants never survived.

The question of whether any of the viruses isolated in the British Isles are identical with any present in North America still awaits confirmation.

B. Strawberry Virus Diseases in Western United States and British Columbia, Canada

In 1922, Horne (39) reported on a disorder of strawberry in California. He presented a pathological description and a list of conditions which supposedly contributed to its cause. In 1926, Plakidas (69) presented a preliminary report on this disorder covering symptomatic and cytological studies. At that time he tentatively applied the term "yellows" to this disorder. A year later he (70) reported on the successful transmission of the disease by means of the strawberry leaf aphid, Capitophorus fragaefollii Ckll., thus proving its virus nature. This is the first known report in the literature on the verification that strawberry may be infected by a virus. The typical symptoms of this disease were yellowing of leaf margins and a marked stunting of the entire plant. The damage caused by yellows was estimated to be a 50 per cent decrease in productiveness.

In 1927, Zeller (104) found a witches-broom producing virus infecting the Marshall strawberry that was also transmitted by Capitophorus fragaefollii. The symptoms recognized in Oregon were long petioles, small leaflets and a multiple crown which gave the plant a bushy appearance.

Another disease reported in Oregon on the Marshall strawberry was crinkle. Zeller and Vaughn (105) presented a brief description of the disease in 1932. The presence of chlorotic areas in the leaflets caused a wrinkled condition and an uneven margin. It was not proved to be of virus origin until Vaughn (97) in 1933 reported transmission by the strawberry leaf aphid. In the same year, Zeller (106) further found that seven cultivated varieties and two wild species of Fragaria were susceptible to crinkle. The yield in these infected plants was reduced more than 50 per cent.

In 1951, Fitzpatrick and Mellor (23) found British Sovereign, the commercial strawberry variety of British Columbia, to be a latent carrier of yellows. When Fragaria vesca was grafted to yellows-infected British Sovereign the reaction was rapid and severe. The young runner tips hooked back, the newly developed leaves were minute and yellow at the margins, and the plants eventually died. Later that year, the same investigators (56) showed that yellows was a complex of at least two component viruses. They described the general symptoms of these individual components on F. vesca.

In 1953, Frazier (30) presented evidence on a graft transmissible latent virus found in Fragaria vesca. The symptoms were expressed on the leaves as a mild mottle. Information concerning the distribution of this virus in indicator and cultivated strawberries is not known.

Recent investigations by Frazier and Thomas (31) show that western aster yellows produces phylloidy of flowers on the Lassen strawberry variety in California. This is the first record of a virus that infects host plants other than Fragaria occurring naturally in strawberries.

C. Strawberry Virus Diseases in Eastern United States and Ontario, Canada

The presence of virus diseases in strawberry fields in eastern United States was not recorded until 1931. At that time Mook (66) found witches'-broom to be quite abundant in plantings in New York, Minnesota, Wisconsin and Illinois. In 1942, Berkeley and Plakidas (8) reported on a new strawberry virus disease found in New York and Ontario which they named "leaf roll". They considered leaf roll to be of minor importance in both localities.

In 1946, Demaree (16) stated that a virus disease of the yellows type was present east of the Rocky Mountains. He considered it to be the most prevalent and destructive type common in eastern strawberry plantings. Later Demaree and Marcus (19) reported on a survey of eastern cultivated strawberries. Their data showed that most of the strawberry plants tested were infected with one or more viruses. They described the viruses without given names but designated them as numbered types according to the symptoms expressed on indicator plants.

In 1952, Fulton (24) showed that one of the tobacco necrosis viruses was transmitted mechanically from contaminated F. vesca roots. This represents one of the few cases of virus transmission from strawberry by mechanical means.

CHAPTER III

MATERIALS AND METHODS

Increased investigations of strawberry virus diseases have disclosed that various Fragaria species differ markedly in their reaction to such diseases. Four Fragaria species were used by the writer in these studies to differentiate between virus types and to determine which species could be used as a standard indicator plant in all subsequent work. A brief history of each is given.

A. Virus-Sensitive Indicator Species

a. Fragaria virginiana L. The first report on the use of a Fragaria species for virus determination was that of Harris and Hildebrand (35). Their data, published in 1937, proved that Fragaria virginiana was extremely susceptible to the yellow-edge complex. In 1941, working with leaf roll, Berkeley and Plakidas (8) demonstrated that this species could be successfully used as an indicator for this virus disease.

b. Fragaria vesca L. (East Malling Strain). Progress in determining virus infection in the cultivated strawberry has been greatly increased in recent years due to the discovery of Harris and King (36). In 1942, they reported that a selection of Fragaria vesca collected near East Malling, England, was highly sensitive to strawberry virus diseases. In 1950, Demaree (18) showed that this selection would be useful in the United States as a virus-sensitive indicator plant. As a result of these findings this species strain has been used throughout North America as the principal indicator for determining the strawberry viruses.

c. Fragaria platypetala Rydb. (Seneca Strain). Methods of transmitting strawberry viruses have improved and consequently there has been an increase in the isolation of new virus-sensitive Fragaria species. In 1951,

Miller (59) showed that a variant of F. vesca (now identified as F. platypetala Rydb. by C. L. Gilly, Michigan State College) was extremely susceptible to the yellows virus. In addition, this species has a stolon of large diameter which is conducive to successful grafting techniques.

d. Fragaria bracteata Heller. While investigating insect transmission of strawberry viruses, Frazier (28) also found the bracted strawberry, F. bracteata, to be an excellent indicator plant for yellows. This species appeared to show marked sensitivity to infection the year-round under greenhouse conditions.

B. Culture of Indicator Species and Cultivated Varieties Under Test

The various indicator species used in this study were grown in four-inch pots. These plants were placed in a greenhouse bench filled six inches deep with sand. During the summer months the plants received partial shade to produce succulent growth. A 16 hour day is essential for the development of stolons. During the winter months it was necessary to furnish additional artificial light and this was accomplished by suspending dual fluorescent units (40 watt std. cool white) two feet above the greenhouse benches. Every 14 days each plant received 50 milliliters of a stock solution of fertilizer. This solution was prepared by adding two ounces of a 10-52-17 water soluble fertilizer to one gallon of water. Under these conditions the plants tended to develop at a faster rate than those under general maintenance.

As the cultivated plants were received from the cooperators they were dipped in a 1:400 solution of nicotine sulfate to kill any aphis present. This is a general practice for control of root aphis. At least

four of the most vigorous plants of each lot were set in four-inch pots and given the standard growing conditions described previously.

C. Method of Indexing

The idea of stolon-grafting cultivated strawberry varieties to indicator species to test for the presence of viruses was first reported by Harris in 1932 (33). A similar method was described by Demaree and Marcus (19) in 1951. Miller (63) described these methods in greater detail summarizing them as the "tongue" graft and the approach graft.

The "tongue" stolon-graft method was used throughout these investigations. However, certain modifications that further enhance positive transmission of the virus were developed in this study. These modifications were: covering the stolons with sand to enlarge their diameters, scraping the epidermis for additional surface union, and cutting off the tips of each "tongue" to prevent curling. Details of the entire method follow.

"Tongue" Stolon-Graft Technique. During the initial phases of these virus studies, the writer used the primary stolon of small diameter for grafting. This resulted in many graft union failures. Subsequently, it was observed that when the stolon tips were buried in moist sand their diameters increased from 0.026 to 0.057 inches. Stolons which had been thus treated were thereafter used for grafting, thereby solving the main objection to most virus-sensitive species of Fragaria.

Another point considered was the stage of stolon growth best adapted for grafting. Harris (33) showed that successful unions can be made by

using stolons which have completed their primary elongation with the terminal leaf bud beginning to unfold. At this time the stolon has grown past the stolon node about four inches.

The equipment required for grafting consisted of a single-edge razor blade, one-quarter inch tape (Polyken Industrial, Bauer & Black Co.) cut into one and four inch lengths to be readily available for wrapping the joined stolons, and a coded pot label for identification purposes.

Procedure

1. The actual graft is made in the terminal two inches of the stolon. Carefully scraping the epidermis in this section with a razor blade creates additional wound surface that may form callus and so unite with a similarly treated surface of the other stolon when the graft is made. (Figure 1).
2. The first cut is made on the side of the test plant stolon in the direction of the plant. This cut is made longitudinally about one-half inch in length slanting towards the center of the stolon. A similar cut is made on the indicator stolon away from the plant. This gives two similar tongue-like slivers of stolon tissue that will be fitted together. Care should be taken in making these cuts, as too deep an incision may result in death of the stolon. (Figure 2).
3. Using the thumb-nail as a backstop, the tip of each "tongue" is cut off with the razor blade (Figure 3) thus preventing curling under of the tips when actual insertions are made. This modification was found to eliminate much time in making the graft and to further insure better contact of the cut surfaces.
4. The "tongue" of the indicator is inserted into the cut of the variety stolon. (Figure 4).

5. While the joined stolons are held in place with one hand, each end of the graft is bound with one inch length of tape. (Figures 5 and 6).
6. Starting one inch beyond the graft at the end nearest the plants, a four inch strip of tape is wrapped spirally around the entire graft. (Figure 7). This is to prevent the "tongues" from twisting out of position and further reduces strain on the union.
7. The completed "tongue" stolon-graft is presented in Figure 8. The pair of grafted plants is placed in line on a sand bench with the grafted stolons lying on the sand and extending toward the outer side of the bench. (Figure 9). The sand is kept damp to prevent grafts from drying out and to facilitate rooting of the runner plants.
8. Inspection may be made after a ten day period by removing the tape to examine for callus formation which indicates probable union. If union is not evident then the pair of plants are regrafted later when new stolons develop.
9. The successfully grafted stolons are allowed to remain intact for 60 days to permit establishment of runner plants from each of the grafted plants and for possible symptom expression of virus on the indicator. Variety plants found to be infected were discarded. If no symptoms of virus were present after a 60 day period the variety plant was retested by grafting to another indicator plant for confirmation.

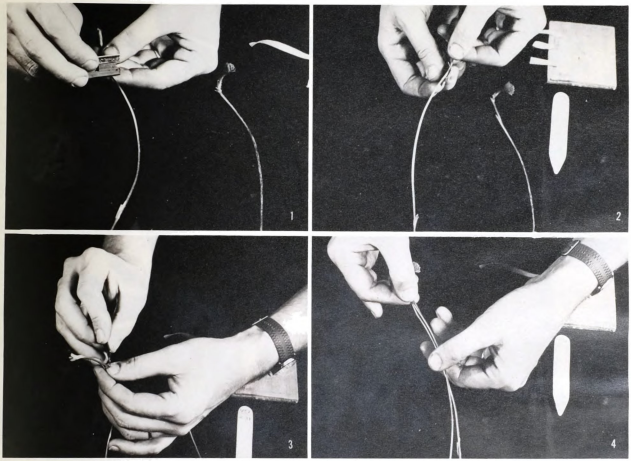


Figure 1. Scraping Epidermis of Stolon with Razor Blade to Create New Callus Surface.

Figure 2. Method of Making Longitudinal Incision in Indicator Stolon (Left); Completed "Tongue" Incision (Right).

Figure 3. Cutting off Tip of "Tongue" to Prevent Curling.

Figure 4. Inserting Indicator "Tongue" into Stolon of Cultivated Variety.

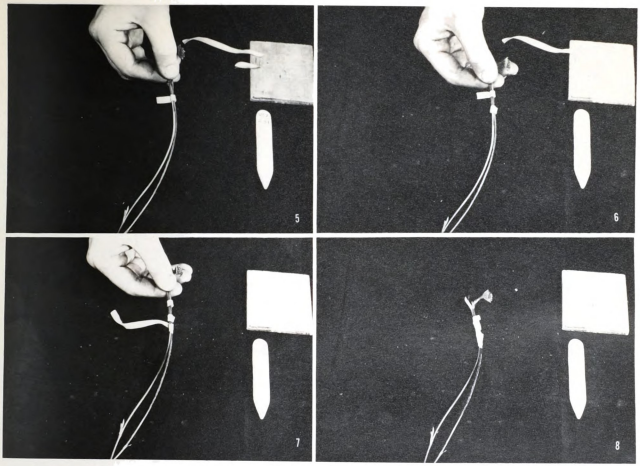


Figure 5. Binding of Tape around Upper Portion of Graft.

Figure 6. Binding of Tape around Lower Portion of Graft.

Figure 7. Binding of Four Inch Tape around Entire Graft to Prevent "Tongues" from Twisting.

Figure 8. Completed "Tongue" Stolon-Graft.

CHAPTER IV

SYMPTOMATOLOGY OF TYPE 1 AND TYPE 2 VIRUSES ON INDICATOR SPECIES AND CULTIVATED VARIETIES

Demaree and Marcus (19) showed that two distinct and easily distinguishable types of virus symptoms were expressed on plants of the East Malling strain of Fragaria vesca; namely, Type 1 and Type 2 viruses. They indicated that Type 1 virus is similar to the crinkle virus predominant west of the Rocky Mountains while Type 2 virus is prevalent only in the eastern section of the United States. They briefly described the general symptom expression of these two virus types on Fragaria vesca.

A preliminary sampling of Michigan strawberry varieties for virus content by the writer indicated that Type 1 and Type 2 viruses were evident on Fragaria vesca stolon-grafted plants. This was the first indication of Type 1 virus in Michigan. Since symptom expression of these virus types had been described on F. vesca only, it was concluded that a study of symptomatology of Types 1 and 2 on other Fragaria species and cultivated varieties would be desirable. (Figures 10 and 11).

Stock plants from the preliminary sampling, showing the described symptoms of Types 1 and 2 viruses were maintained and used for inoculation to the virus-free test species and varieties. In tests where the inoculated plant did not express symptoms it was regrafted to Fragaria vesca for confirmation of virus transmission.

A. Type 1 Virus Symptoms

a. Fragaria virginiana L. This species when stolon-grafted to Type 1 virus-infected plants did not exhibit any marked symptoms, with the exception of a moderate dwarfing in 60 days when compared with healthy plants. Therefore, F. virginiana may be regarded as a symptomless

carrier of the Type 1 virus.

b. Fragaria vesca L. Within 25 days after grafting a healthy plant of this species to the Type 1 virus-infected plant, the distal runner showed chlorotic mottling of young leaflets. The chlorotic spots averaged one millimeter in diameter. These leaflets differ from healthy ones in the following respects: 1) one or two are about 1/3 normal size or entirely absent, 2) their color is deeper green than normal.

Type 1 virus-infected F. vesca plants produce flowers approximately 1/2 normal size and develop a few thin stolons after infection is evident. The symptoms of the Type 1 virus appear on the parent indicator plant about 45 days after grafting. This indicates that the virus entity involved can move in either direction through the stolon.

Infected plants maintained for 16 months did not display any new symptoms.

c. Fragaria platypetala Rydb. The initial reaction of this species to the Type 1 virus was evident in about 30 days on the distal runner by chlorotic mottling of the young leaflets. The chlorotic areas on the leaflets were large, averaging 2.5 millimeters in diameter. The leaf symptomatology consisted of the following: 1) leaflets reduced to 1/8 normal size, 2) the entire leaf is deeper green than normal.

Type 1 virus-infected F. platypetala plants produce flowers approximately 1/3 normal size and do not form stolons after infection is evident.

F. platypetala plants infected with the Type 1 virus were usually dead after a period of one year, thus showing this species to be more sensitive to this virus entity than Fragaria vesca.

d. Fragaria bracteata Heller. This species reacted to the Type 1 virus

in approximately 40 days. The mottling of the leaflets was most pronounced on the distal plant of each grafted stolon. The chlorotic spots averaged 1.5 millimeters in diameter. The leaflet became severely asymmetrical because of the presence of only one leaflet per petiole.

Type 1 virus-infected F. bracteata plants produced normal flowers and developed normal stolons after infection was evident.

The reaction of F. bracteata to the Type 1 virus is manifested by the severe asymmetry of the leaf. The deep green color and abnormal flowers and stolons which develop in F. vesca and F. platypetala are absent in F. bracteata.

e. Cultivated Varieties. Three virus-free cultivated strawberry varieties were used in this study, namely; Catskill, Dunlap and Robinson. These plants were stolon-grafted to plants infected with the Type 1 virus. The varieties, Dunlap and Robinson, displayed no marked symptoms of Type 1 virus infection. Catskill exhibited only slight flecking on young leaflets which disappeared as the leaves matured. These results showed that the varieites used in this test are latent carriers of the Type 1 virus.

A comparison of indicator sensitivity when infected with the Type 1 virus is presented in Table 1.

TABLE I

COMPARISON OF TYPE 1 VIRUS SYMPTOMATOLOGY ON INDICATOR SPECIES

Species	Incubation Period Days	Leaflet Reaction	Leaflet Color	Stolon Development	Flower Size
Fragaria virginiana	0	Regarded as a symptomless carrier.	Normal	Normal	Normal
Fragaria vesca	25	Variation in size and shape--1/3 normal size; chlorotic mottling, 1 mm. in diameter; plants slightly dwarfed.	Deep Green	Few, small diameters	One-half normal size
Fragaria platypetala	30	Size differential more severe--1/8 normal size; chlorotic mottling, 2.5 mm. in diameter; plants slightly dwarfed.	Deep Green	None	One-third normal size
Fragaria bracteata	40	Asymmetry very severe, generally one leaflet per petiole; chlorotic mottling 1.5 mm. in diameter, plants extremely dwarfed.	Normal	Normal	Normal

B. Type 2 Virus Symptoms

a. Fragaria virginiana L. In these studies F. virginiana did not display any symptoms to represent infection by the Type 2 virus. Some dwarfing was noted but could not be used as a diagnostic tool.

b. Fragaria vesca L. Interveinal chlorosis and epinasty of young leaflets, the first sign of Type 2 virus infection, was noted 21 days after inoculation. Two days later epinasty of the petioles was evident (Figure 12). After a period of 16 days both petioles and leaflets assumed an upright position. In length the petioles were 1/4 normal size and bore 1/16 normal size light green leaflets (Figure 13).

Bud proliferation of the crown was quite evident as the infected plants became more mature. A one year old plant had a multi-crown that was at least two inches in diameter. Upon sectioning, it was apparent that the central crown divided into three to five secondary crowns from which arose many small individual crowns. This gave the plant a tussock-like appearance.

Type 2 virus-infected F. vesca plants produce flowers 1/2 normal size borne on pedicels that are 1/8 the length of healthy stalks (Figure 14). Small stolons were evident on Type 2 virus-infected plants.

Parent indicator plants became infected demonstrating that the Type 2 virus can move in either direction in the stolon.

c. Fragaria platypetala Rydb. This species had a longer incubation period that F. vesca for the expression of the Type 2 virus; namely, 25 days. The general epinastic symptoms were more severe on this species and individual leaflets were dwarfed and varied in size and shape (Figure 15).

Crown proliferation was not as severe as shown in F. vesca. A one year old plant of F. vesca had over 100 small crowns compared with only 30 estimated for F. platypetala.

Type 2 virus-infected F. platypetala plants produced flowers 1/2 normal size borne on pedicels 1/8 the length of healthy ones. Small, spindly stolons were evident on Type 2 virus-infected plants.

d. Fragaria bracteata Heller. This species had the longest incubation period for Type 2 virus expression. The symptoms of interveinal chlorosis were evident in 35 days on the distal runner plant. The leaflets were reduced to 1/4 normal size (Figure 16). The typical epinastic symptoms observed in the other two species tested were not expressed on this indicator.

Type 2 virus-infected F. bracteata plants produced solitary flowers instead of a cymose inflorescence found on healthy plants. No stolons were produced after infection was evident.

e. Cultivated Varieties. The Type 2 virus was inoculated into virus-free plants of the varieties Catskill, Dunlap and Robinson. Interveinal chlorosis was evident in the Catskill variety within 40 days. This symptom became more pronounced and ultimately all the Type 2 virus-infected Catskill plants were severely dwarfed. Interveinal chlorosis and dwarfing may now be considered as diagnostic for Type 2 virus infection on Catskill.

The Dunlap and Robinson varieties displayed no characteristic sign of Type 2 virus infection, showing they are symptomless carriers.

A comparison of indicator symptom expression when infected with the Type 2 virus is presented in Table II.

TABLE II

COMPARISON OF TYPE 2 VIRUS SYMPTOMATOLOGY ON INDICATOR SPECIES

Species	Incubation Period Days	Leaflet Reaction	Crown Proliferation	Stolon Development	Flower Size
Fragaria virginiana	0	Regarded as a symptomless carrier.	None	Normal	Normal
Fragaria vesca	21	Interveinal chlorosis; epinasty on leaflet and petiole; leaves dwarfed and symmetrical--1/16 normal size.	Average 100	Few, small diameters	1/2 normal, pedicels 1/8 normal
Fragaria platypetala	25	Interveinal chlorosis; severe epinasty on leaflet and petioles; leaflet size differential severe.	Average 30	Few, small diameters	1/2 normal, pedicels 1/8 normal
Fragaria bracteata	35	Interveinal chlorsis; no epinasty present; leaflets 1/4 normal size.	None	None	Normal, inflorescence solitary instead of cymose

C. Combinations of Virus Types 1 and 2

Inter-grafting of F. vesca plants individually infected with Type 1 and Type 2 viruses resulted in a combination of the general symptoms of both types. This is demonstrated in Figure 17 which shows the effect of both viruses on a leaf of F. vesca. Asymmetry and a deep green color which are characteristic of the Type 1 virus infection and leaflet reduction produced by the Type 2 virus are evident.

This intermingling of both virus types with no decided change in over-all symptomatology indicates that infection by one virus type will not immunize against the other.

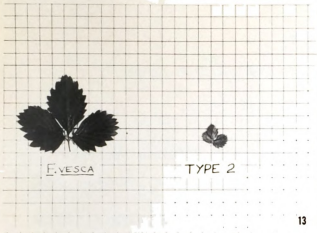
In summation, these investigations involving the sensitivity of various Fragaria species to Types 1 and 2 viruses show that Fragaria vesca would be the standard indicator plant for subsequent studies for the following reasons: 1) lowest incubation period, 2) leaflet reaction to the virus types, 3) stolons develop after virus infection which are desired for inter-grafting species studies.

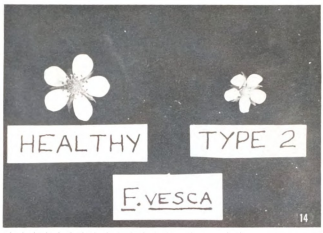
Figure 9. Grafted Plants Lined Out in Greenhouse Sand Bench for Virus Observation.

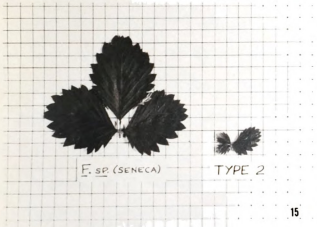
Figure 10. Blakemore, Symptomless Carrier of Type 1 Virus (Left); Expression of Type 1 Virus on Fragaria vesca (Right).

Figure 11. Expression of Type 2 Virus on Fragaria vesca (Left); Dunlap, Symptomless Carrier of Type 2 Virus (Right).

Figure 12. Type 2 Virus Symptoms of Epinasty on Petioles and Leaflets of Fragaria vesca.







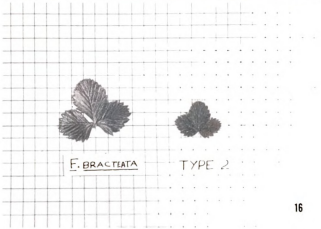


Figure 13. **Comparison of Leaf Size between Healthy and Type 2 Virus-Infected Fragaria vesca (one-quarter inch grid).**

Figure 14. **Comparison of Size between Healthy and Type 2 Virus-Infected Fragaria vesca Flowers.**

Figure 15. **Comparison of Leaf Size between Healthy and Type 2 Virus-Infected Fragaria platypetala, Seneca Strain, (one-quarter inch grid).**

Figure 16. **Comparison of Leaf Size between Healthy and Type 2 Virus-Infected Fragaria bracteata (one-quarter inch grid).**

CHAPTER V

VIRUS DISEASES AND OTHER VIRUSLIKE ABNORMALITIES OF THE STRAWBERRY IN MICHIGAN

In a survey of 16 eastern and central states, Demaree and Marcus (19) indicated that the majority of the strawberry varieties tested were infected with viruses. Included in the preliminary survey were 42 plants from Michigan and all proved to be virus-infected. This work by Demaree and Marcus constituted the first screening of Michigan strawberries for virus content and indicated the need for a more thorough investigation of strawberry viruses within the state itself.

Keeping in mind the findings of Demaree and Marcus, it was hoped that a sampling of Michigan strawberries for virus content would serve in locating virus-free varieties from which disease-free plants could be produced. When a stock of disease-free plants was obtained comparative studies on plant and fruit production between infected and virus-free stocks could be made and the establishment of a virus-free foundation stock for distribution to Michigan nurserymen would be possible.

A. Virus Content in Cultivated Varieties

A form letter stating the purposes of this study was sent to plant inspectors, county agents, nurserymen and various growers throughout the state. In brief, it was intended for growers who had established plantings which have been intra-propagated rather than by propagation from outside sources. Through these cooperators it was hoped that disease-free plants would be isolated.

This state-wide request for strawberry plants was well received. Since 1950, 257 lots of plants including 15 varieties have been

received by the writer to be tested for virus content. All of the plants received were from the lower peninsula of Michigan. The results obtained by indexing on Fragaria vesca were tabulated according to virus type. The results of the sampling for virus types and virus-free plants covering a four year period are presented in Table III.

TABLE III

SAMPLE OF CULTIVATED STRAWBERRY VARIETIES

IN MICHIGAN FOR VIRUS CONTENT 1950-53

Variety	Plants		Virus Types		
	Indexed	Virus-Free	1	2	1 & 2
Blakemore	24	0	18	5	1
Brilliant	12	7	0	5	0
Catskill	56	4	3	49	0
Dunlap	62	16	6	36	4
Dorsett	14	0	2	12	0
Fairfax	23	0	4	16	3
Gem	29	2	0	27	0
Midland	18	0	7	8	3
Premier	94	0	13	81	0
Red Crop	13	0	0	13	0
Robinson	141	5	21	110	5
Sparkle	40	2	7	29	2
Superfection	27	3	0	24	0
Temple	22	0	4	16	2
Totals	575	39	85	431	20

The data given in Table III show that of 575 plants indexed, only 6.8 per cent were found to be virus-free. However these virus-free plants have been found in varieties which consist of about 55 per cent of the strawberry acreage in Michigan. Of the 536 plants found to be virus infected, Type 1 virus was found in 16 per cent, Type 2 virus in 81 per cent and the combination of both types in 3 per cent of the plants.

The history of the plant lots showing Type 1 virus infection indicated that the original stock of plants was obtained from nurseries in either Illinois or the East, implying that the Type 1 virus in not indigenous to Michigan. However, this was not the case for the 3 per cent of plants infected with both Type 1 and Type 2 viruses. In each instance, the original planting stock had a history of being grown exclusively in Michigan. This information suggests there is spread of the Type 1 virus within the state.

The highest per cent of infection was that of the Type 2 virus. All of the varieties tested had plants infected with this virus type, indicating that the Type 2 virus is most prevalent in Michigan strawberries. Corroboration of this is noted in Table III for the variety Brilliant, originated in central Michigan seven years ago, revealed infection by the Type 2 virus. It would seem apparent that the virus infection acquired in the Brilliant variety was a further indication of spread of this virus in the state.

In studies on seedling selections used in the Michigan strawberry breeding program, the 20 selections tested showed that five contained the Type 2 virus. If all seedlings are inherently disease-free, these data not only indicate that the Type 2 virus is common in Michigan but field spread by insects is also suggested.

From the foregoing data it is concluded that the dissemination of

these virus types through the state may be due to inter and intra-state shipment of infected plants.

As the virus types were isolated, their locations in the state were mapped. This information is presented on a map of Michigan. (Figure 21). It is evident from this map that the Type 2 virus is commonly found throughout the lower peninsula. The majority of the Type 1 virus sites was located in southern Michigan with one exception, Alpena County. Combinations of Types 1 and 2 viruses were isolated from Alpena and Berrien Counties where Type 1 virus and Type 2 virus are known to occur separately. This combination was never isolated from a field infected with both types individually.

In conjunction with the project for virus content the isolation of virus-free plants was desired. Variety plants which did not give a reaction on the indicator plant were re-indexed to confirm the first test. Generally only one or two runner plants of a virus-free variety were available for propagation from the indexing tests; hence, increase was very slow in the beginning.

This initial propagation of a given virus-free variety was accomplished under specific conditions. Stolons from these clones were placed on steam-sterilized soil in greenhouse benches. As the runner plants became established they were severed from their parents. This precaution precluded the chance of contamination with the red stele fungus (Phytophthora fragariae) in the greenhouse propagating benches and later in the foundation stock field plots which were established in soil free from the fungus.

Near the end of the growing season in 1951, several plants of Dunlap were isolated as virus-free and subsequent indexing early in 1952 verified these findings. In 1952 certain plants of the varieties Catskill, Gem,

Dunlap and Robinson were considered virus-free through the process of indexing.

Continued indexing in 1953 resulted in finding virus-free plants of the everbearing varieties Brilliant and Superfection. The varieties Brilliant and Dunlap were found to have a higher per cent of virus-free plants than the other varieties tested. This is of interest since Brilliant is a new variety originating in Michigan and Dunlap is one of the oldest known in the midwestern United States. Dunlap is considered to be a variety very closely related to the species, Fragaria virginiana. Since the numbers of virus infected plants in this native species are relatively few as indicated in Section B. (Page 30), it is possible that Dunlap inherited a character that makes it repellent to insect vectors. Also, there is a possibility that the chemical composition of the plant sap inactivates the virus entities.

In 1953, through the kind cooperation of the Michigan Bureau of Plant Industry, what is believed to be the first virus-free strawberry foundation planting in the midwest was established at the Michigan Agricultural Experiment Station. Virus-free plants of the varieties Catskill, Dunlap and Robinson were planted in an isolated field plot for mass propagation. Plants of these varieties should be available to cooperating nurseries in the Spring of 1955. In addition, the writer has proposed a certification program for the distribution of these virus-free plants, which is presented in the Appendix.

B. Virus Content in Fragaria virginiana in Southern Michigan

The species Fragaria virginiana, when infected with either the Type 1 or Type 2 viruses does not show any visible symptoms of disease as indicated in Chapter IV (Page 18). These wild infected plants would constitute a

possible source of contamination for nearby virus-free plantings. Knowledge as to the extent and distribution of these virus types in wild strawberries would be important for the location of cultivated and foundation stock planting sites.

Other workers have reported on the presence of virus in wild strawberry species. Miller (60) showed that plants of F. chiloensis and F. ovalis growing in the wild in Oregon were infected. The percentage of infected plants of both species was relatively low. The type of virus infection was not revealed. In 1952, Marcus (51) reported in a limited survey on the amount of infected F. virginiana in seven eastern states. His studies indicated that 21 out of 73 plants tested were virus-infected. The Type 2 virus was common throughout the seven states, with the Type 1 virus common on the Atlantic seaboard and in Illinois. The presence of the Type 1 virus on F. virginiana in Illinois is in accord with the findings in this study on cultivated strawberries received from Illinois.

In 1952 and 1953 a virus sampling of wild strawberry was made in seven counties located in southern Michigan. It is in these areas that the majority of the nursery and commercial strawberry plantings are located. Plants were collected at random from various sections of each county. They were potted, labeled according to county and location, then placed in the greenhouse for testing. When the runners were of proper size they were indexed for virus content. The Type 2 virus was the only type isolated for the wild strawberry in this study. The results of these findings are given in Table IV.

TABLE IV

TYPE 2 VIRUS CONTENT IN FRAGARIA VIRGINIANA IN SOUTHERN MICHIGAN

County	Number of Plants		
	Indexed	Infected	Virus-Free
Allegan	11	0	11
Berrien	21	4	17
Cass	17	2	15
Hillsdale	5	0	5
Kalamazoo	10	1	9
St. Joseph	18	3	15
Van Buren	19	0	19
Totals	101	10	91

As shown by the data in Table IV, virus-infected wild strawberries are present in some of the strawberry growing areas in southern Michigan. Only 10 per cent of the plants tested were infected and of these 8 per cent were from plants collected growing near cultivated strawberries. It is obvious that these infected plants will constitute a reservoir of virus for nearby commercial strawberry plantings. The presence of an insect vector is implied from this infection of wild plants.

From the results of this investigation, it seems likely that isolation from wild strawberries should be considered in a certification program for future virus-free foundation stock and nursery plantings.

C. Other Strawberry Virus Diseases and Abnormalities with Viruslike Symptoms

In the field sample for determination of viruses present in Michigan other disorders than those mentioned formerly were diagnosed and are presented in this section.

a. Leaf Roll Complex. In 1952, several plants of the Dunlap and Premier varieties were observed to have long petioles, small leaves and a definite downward rolling of the leaflet margins. This general symptom suggested these plants may be infected with the leaf roll virus. (Figure 18). Indexing on Fragaria vesca indicated the presence of the Type 2 virus. Rolling of leaflet margins was not expressed in this species. Subsequent grafting to virus-free clones of Fragaria virginiana, as suggested by the limited work of Berkeley and Plakidas (8), verified the original diagnosis of leaf roll.

A survey of the literature revealed that the original paper by Berkeley and Plakidas was the only report on this disease. Their data showed transmission of leaf roll symptoms by grafting to three Premier plants and one of F. virginiana. Although the evidence was small it did indicate that this disorder was of a virus nature.

Due to the limited experimental evidence concerning this disease, additional studies on varietal susceptibility and symptomatology were desired.

As determined by reciprocal indexing, i.e. transferring the virus both ways between the two species of indicator plants, these plants of Dunlap and Premier carried both leaf roll and the Type 2 viruses. Continued surveys in the state resulted in the isolation of 41 individual plants infected with leaf roll as confirmed on F. virginiana. All of these plants expressed Type 2 virus symptoms when indexed on F. vesca. Initial reciprocal passage through the two indicators showed no deletion of the virus entities involved. These data suggest the possibility that leaf roll is initiated by a virus complex that includes the Type 2 virus.

Using the stolon-graft technique, plants of Fragaria virginiana infected with the leaf roll complex were indexed to three virus-free plants each of Catskill, Dunlap, Gem and Robinson. Periodical observations were made on the daughter clones for symptoms of transmission. The evidence obtained is presented in Table V.

TABLE V

SYMPTOMATOLOGY OF THE LEAF ROLL COMPLEX ON SEVERAL CULTIVATED STRAWBERRY VARIETIES

Varieties	Incubation Period* Days	Leaflet Symptoms			Severity Rating**
		Rolling	Coloration	Surface	
Catskill	31	Margins of leaflet overlap in basal portion of leaflet.	Interveinal and marginal chlorosis	Slightly ruffled	2
Dunlap	27	Extreme, opposite margins of the leaflet touch, forming a funnel-shaped tube.	Greenish-yellow mottling	Rugose	1
Gem	40	Margins of leaflets slightly rolled at basal portion only	Pale green	Smooth	4
Robinson	27	Opposite margins of the leaflet touch at basal portion only. Midveins of the leaflets arch downward.	Pale green	Smooth	3

*Count from grafting date to first visual symptom of leaflet rolling.
**Scale of 1 to 4, most severe rated as 1.

The evidence given in Table V shows that symptoms of the leaf roll complex vary with the variety, but the rolling of the leaflet margin appears to be the consistent character of this disease. Dunlap was the most severely affected, as demonstrated by mottled leaflets forming funnel-shaped tubes. The interveinal and marginal chlorosis of Catskill leaflets are probably due to the Type 2 virus component and not leaf roll. This is also presented in Chapter IV, Page 19. Even though Robinson presented the same incubation period as Dunlap, the general symptoms were not as severe. The everbearing variety Gem had the longest incubation period and appeared to exhibit the most tolerance to the leaf roll complex.

During the observation period, virus-free F. virginiana plants were grafted to the inoculated cultivated varieties. Leaf roll symptoms were noted on the daughter plants of the indicator species, proving that the virus complex was present in the cultivated plants. In addition, the parent plants of F. virginiana became infected, indicating that the leaf roll complex can move in either direction in the stolon.

During observations on the transmission of this virus complex several plants of indexed F. vesca exhibited red petioles while other F. vesca plants exhibited the typical green color. In checking the records for the original indexings, it was noted that these infected plants were from the leaf roll series. To determine whether reddening of the petioles of F. vesca may be due to the leaf roll complex another series of grafts were made.

Six leaf roll infected Dunlap plants were indexed on F. vesca plants. Initial symptoms of the Type 2 virus were observed. Approximately two weeks later reddening of the older petioles was evident. One week later this symptom had progressed to the point of infolding the young petioles

emerging from the crown. This reddening of petioles was evident in five of the six indicator plants used in the test. Transmittal of this new symptom, through indexing infected to healthy F. vesca plants, substantiated the data. From these original data it would appear that Fragaria vesca is a fairly good indicator plant for the leaf roll complex and that reddening of the petioles found on this indicator species is probably a result of leaf roll contamination.

Leaf Roll Complex Summary. These studies constitute the first report on the presence of leaf roll in Michigan. Reciprocal indexings indicated that all leaf roll infected plants were carrying the Type 2 virus. Using the stolon-graft indexing method the host range of this virus complex was increased to include four additional cultivated varieties; namely, Catskill, Dunlap, Gem and Robinson. Symptomatology of Fragaria vesca indicated absence of leaflet rolling, a symptom that is distinct in the species F. virginiana. Reddening of petioles of F. vesca appears to be a definite indication for the presence of the leaf roll complex.

b. Leaf Variegation. The first known report on variegation was as early as 1719 (92). It is found in both parent species of the cultivated strawberry; namely, Fragaria chiloensis and Fragaria virginiana (13). Through the years there have been numerous reports (1, 15, and 71) on the negative transmission of this anomaly. The disorder was first observed in the Blakemore variety, but through the years many varieties have been found affected (15). This anomaly was commonly known as Blakemore Yellows and June Yellows but more recently has been termed leaf variegation. Although it has been observed by State Plant Inspectors on the Blakemore variety in Michigan (48), the only variety in the state observed by the writer to be affected by variegation was Premier. Several affected plants

of the Premier variety collected by the writer were grown in the greenhouse for study.

The typical symptoms of leaf variegation are a streaking of the new leaves with yellow or cream and green. Later these leaves lose most of their green color and finally become golden or light yellow in color. Severely affected plants become stunted and unproductive.

The symptoms of variegation vary with the air temperature. Plants maintained at 55° F. showed strong yellow interveinal streaking as compared with leaves merely lighter green than normal on plants grown at 75° F. This indicates that symptoms are influenced by the environment.

It is believed that the apparent increase of leaf variegation in strawberry plantings is merely a transmission of variegation to daughter plants through stolons. Attempts to transmit leaf variegation to normal Premier plants by the stolon-graft method failed. Similar negative results were incurred by the writer when pollen from affected plants was used to fertilize flowers on healthy plants. The stolon-graft and pollen attempts were a repetition of Demaree's (15) unsuccessful attempt to transmit variegation by these means.

In 1951, Thornberry (95) reported that viruslike particles were demonstrated by electron microscopy to be in the juice of strawberry plants having leaf variegation. These particles were sparse in the juice of plants of a clone not showing variegation. Attempts were made at mechanical transmission using juice extracts. Thornberry implied that these viruslike particles might well be the leaf variegation entity.

In 1952, the writer was privileged to index the plants used by Thornberry and found both normal and variegated clones to be infected with the Type 1 virus. Therefore, the viruslike particles reported by Thornberry

may possibly be those of the Type 1 virus.

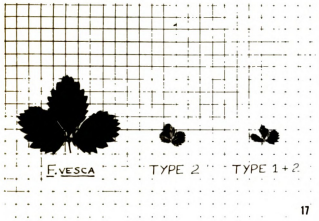
Selfing flowers on variegated plants and growing the resulting seedlings produced approximately 68 per cent variegated F_1 progeny (Figure 19). This evidence indicates that leaf variegation is not due to a single gene.

c. Witches'-broom. During the course of these investigations several plants showing a "bushy" appearance were received for diagnosis. The infected plants had multi-branched crowns supporting spindly petioles with small leaves. This is illustrated in Figure 20. The midveins of the leaflets tended to arch downward and were broader and lighter in color than normal. These symptoms agree with those reported by Zeller in Oregon (104) and from New York, Minnesota, Wisconsin and northern Illinois by Mook (66) for the virus disease known as witches'-broom.

General field surveys by the writer and state plant inspectors revealed that this anomaly of strawberry is present only in the northern portion of the lower peninsula of Michigan. Catskill, Premier, Robinson, Red Crop and Sparkle were observed as the varieties infected. However, this disease was not general or abundant in these northern areas.

Under greenhouse conditions the witches'-broom plants never formed stolons. Attempts at petiole stolon-grafts to healthy F. vesca failed to form a union. Petiole to petiole inarch grafts were attempted between infected and healthy plants of the same variety. These also failed.

This limited study shows by general indications that these plants were infected with the witches'-broom virus, for so far as is known no other strawberry disease shows similar symptoms.



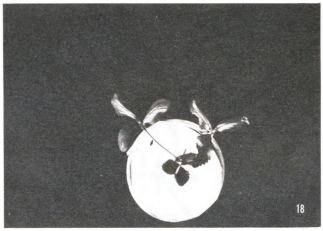
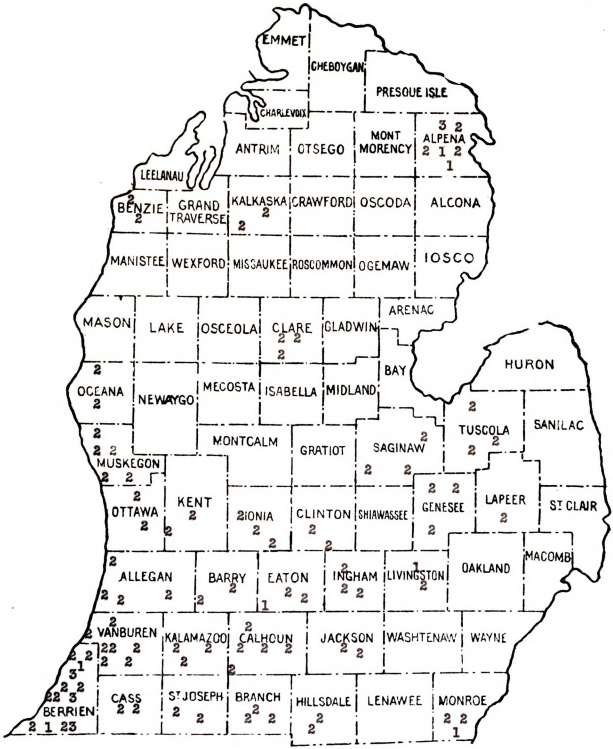


Figure 17. Comparisons of Fragaria vesca Leaf Sizes between Healthy, Type 2 Virus-Infected and Combination of Type 1 and Type 2 Viruses.

Figure 18. Dunlap Variety Infected with the Leaf Roll Virus Complex.

Figure 19. Seedlings Grown from a Variegated Plant Showing Symptoms of Leaf Variegation.

Figure 20. Premier Variety Infected with a Witches'-Broom Producing Virus (Left); Normal Premier Plant (Right).



Key: 1-Type 1 Virus; 2-Type 2 Virus; 3-Combination of Type 1 and Type 2 Viruses

Figure 21. Map of Lower Michigan Showing the Location of the Strawberry Virus Types Found in a Limited Sampling from Certain Counties.

CHAPTER VI

TRANSMISSION OF STRAWBERRY VIRUSES

The methods of transmission of plant viruses are considered in this chapter. The individual sections include both natural and artificial means of virus spread with the exception of transmission by grafting which was fully explained in Chapter III. The purpose of these studies was twofold, for it was desired to determine how the various strawberry viruses may be disseminated and to find a quicker method of determining virus content by possible mechanical transmission to another host.

A. Dodder Transmission Studies

Perhaps the most ingenious method of virus transmission involves the use of the plant parasite, commonly known as dodder (Cuscuta sp.). The genus Cuscuta is characterized by twining, scale-leaf plants, mostly devoid of chlorophyll and capable of parasitizing many plants. This parasite obtains its water and elaborated food by the production of haustoria, which penetrate and become attached to the conducting tissues of the host. Plants which are not easily inter-grafted for virus study can become connected by a direct union through dodder, offering a course for possible virus transfer from one plant to another. This method of transmission offers the possibility of determining the host range of a virus, when other means of transmission cannot be used.

The development of virus transmission by means of dodder was first shown by Bennett (7) and later elaborated by Johnson (44). Their individual studies describe the various methods of transmitting viruses through the use of several Cuscuta species. Demaree and Marcus (19) were the first to report dodder transmission of a strawberry virus. They were able to infect one Fragaria vesca plant out of seven with

the Type 1 virus using Cuscuta campestris. In 1952, Smith and Moore (88) reported the transmission of Type 1 and Type 2 viruses by Cuscuta subinclusa to healthy F. vesca. These two reports on dodder transmission of strawberry virus types indicated that dodder transmission was worthy of further investigation.

Method:

Healthy stock of Cuscuta campestris Yuncker (obtained from L. O. Kunkel, Rockefeller Institute, New York, New York) was maintained on Vinca rosea for use in the various tests. The apical end of a branch of dodder approximately ten inches long was trained around the youngest leaf of a healthy strawberry species. An eight day period was allowed for the dodder to establish itself on the healthy host plant. The other free end then was trained to a virus-infected strawberry plant. The dodder connection between infected and healthy plants was severed at the end of fourteen days. The receptor host plants were maintained for symptoms of transmission.

The following series of studies were made to determine if the dodder species, Cuscuta campestris, would transmit the strawberry viruses found in Michigan to other Fragaria species. This is the first use of C. campestris for transmission of strawberry viruses other than the Type 1 virus reported by Demaree and Marcus (19).

a. Type 2 Virus Transmission. Dodder branches were established independently on healthy F. vesca and F. virginiana plants. Then, one apical end of the dodder branch was twined to a Type 2 virus-infected plant and allowed to become attached by means of haustoria. Periodical observations for symptoms of virus transmission were made on the receptor host species.

Epinasty of the youngest leaflet, the first visual symptom of transmission of the Type 2 virus, was evident on the F. vesca plants within 35 days after the dodder union was evident. Twelve F. vesca plants were included in this test and nine of these showed typical Type 2 virus symptoms. Two facts are borne out in this test. The average incubation period for symptom expression by dodder is two weeks longer than by the stolon-graft technique. Second, there was 29 per cent more infection with this virus type by using Cuscuta campestris than Smith and Moore (88) achieved with Cuscuta sub-inclusa.

No symptoms of virus transmission could be distinguished on Fragaria virginiana after a 50 day incubation period. Dodder, still actively growing on these plants, was trained to healthy F. vesca plants. Symptoms of the Type 2 virus were observed on the F. vesca indicator within 38 days, further corroborating that F. virginiana is a symptomless carrier of the Type 2 virus.

b. Leaf Roll Transmission. A similar study of dodder transmission was made using leaf roll virus-infected plants. The leaf roll virus was transmitted from infected Dunlap plants to four plants of F. virginiana by dodder in 48 days.

Since the stolon-graft technique did not transmit the characteristic leaf roll symptoms to F. vesca it seemed advisable to try transmission by C. campestris. Dodder from these four infected F. virginiana plants was trained to healthy F. vesca. Symptoms of the Type 2 virus (usually found in the leaf roll complex) were observed on three of the four receptor plants. Two weeks after initial symptoms, the petioles of the epinastic leaves turned red in color. This reddening of the petioles is characteristic of leaf roll infection in F. vesca as determined previously in the

stolon-graft technique. These studies substantiate that reddening of petioles is a typical symptom of the leaf roll virus complex in F. vesca.

c. Witches'-Broom Transmission. Using C. campestris, the virus from a witches'-broom infected plant of Premier was transmitted to a healthy plant of Dunlap. This agrees with Zeller (104) and Demaree (19) who transmitted the disease by viruliferous aphids and stolon-grafting showing that witches'-broom is of a virus nature. Dodder from a witches'-broom infected plant was trained to healthy F. vesca to determine the symptom expression on this sensitive indicator. After 45 days incubation, symptoms of epinasty, asymmetry and a slight chlorosis of leaflet margins was evident on the young emerging leaves. At this time it was apparent that the symptoms of witches'-broom on F. vesca were quite similar to those of the Type 2 virus. It is possible but not definite that the witches'-broom plant used had a complex of a witches'-broom producing virus plus the Type 2 virus. Since dodder transmission studies of both types were underway a critical comparison was made over a six month period and is presented in Table VI.

The table of comparison between the virus or complex from the witches'-broom plants used and the Type 2 virus shows that witches'-broom virus is characterized by marginal chlorosis, slight crown proliferation, absence of either flowers or stolons and severe dwarfing.

At that time the writer believed this to be the first description of witches'-broom on Fragaria vesca. However, in the fall of 1952 Skiles and King (87) reported on the transmission of strawberry stunt to F. vesca. The description of their symptoms is similar to those obtained in these studies on witches'-broom.

The original papers on witches'-broom (104) and stunt (107) were

described by Zeller on the Marshall strawberry variety. The general symptoms of both diseases are similar. Infected plants are dwarfed with crown proliferation and the stolon internodes are quite shortened. The only difference statedby Zeller is that the midveins of the leaflets on plants infected with witches'-broom are arched downward while marginal leaflet cupping is characteristic of stunt. Skiles and King (87) diagnosed their dwarfed plants of Robinson as stunt due to leaflet cupping but leaflet cupping is characteristic and a mark of identification in the normal Robinson plant. The Premier plant used in these studies exhibited leaflet inarching in addition to the general symptoms of multibranched crowns, spindly petioles and small leaves, and was diagnosed as infected with witches'-broom virus.

One would consider that the symptoms described in this study and those of Skiles and King on Fragaria vesca are the same. This may indicate that witches'-broom and stunt viruses described by Zeller were the same virus.

TABLE VI

COMPARISON OF WITCHES'-BROOM AND TYPE 2 VIRUSES

AS EXPRESSED BY C. CAMPESTRIS TRANSMISSION TO F. VESCA

	Type 2 Virus	Witches'-Broom
Incubation Period	35 days	45 days
Initial Symptoms	Young petioles and leaflets epinastic; Leaflets symmetrical; Slight interveinal chlorosis.	Young petioles and leaflets epinastic; Leaflets asymmetric; Chlorosis prominent on leaflet margin.
20 Days	Petioles of old leaves horizontal; Leaflets still green.	Petioles of old leaves horizontal; Leaflets turning yellow.
30 Days	Emerging leaves dwarfed, 1/4 natural size; Light green and erect; Crown proliferation approximately 20; Large old leaves turning yellow.	Emerging leaves dwarfed, 1/8 natural size; Chlorotic and erect; Crown proliferation approximately 5; Large old leaves dead.
90 Days	Leaves dwarfed, 1/8 natural size; Crown proliferation approximately 65; Large old leaves dead; Flowers evident and 1/2 natural size.	Dense group of erect dwarfed leaves, 1/16 natural size; 12 crowns evident on original stem; Flowering not evident.
180 Days	Extreme crown proliferation, estimated 125; Small stolons evident.	30 individual crowns evident; No stolons nor flowers present.

d. Studies on the Host Range of the Type 2 Virus by Dodder. Previous tests showed that Cuscuta campestris is capable of transmitting various strawberry viruses. Through the use of dodder the host range of a strawberry virus may be extended beyond the genus Fragaria. Demaree and Marcus (19) reported that Potentilla canadensis may be infected with either the Type 1 or Type 2 virus and yet not exhibit any definite symptoms. Although this is the only report on the host range of strawberry viruses it does indicate that P. canadensis if infected is a symptomless carrier. Since the genus, Potentilla, is quite common throughout the strawberry growing areas of the state, it was advisable to test some of the species for virus content and transmission.

Two parent plants each of the following species were brought in from the wild and grown in the greenhouse; namely, Potentilla argentea L., P. recta L., and P. anserina L.. In subsequent testing with dodder all parent plants appeared to be virus-free as indicated by F. vesca. Seeds collected from these plants were used in transmission studies. Dodder branches actively growing on Type 2 virus-infected plants were trained to the Potentilla seedlings. Two weeks later the connections were broken and dodder established on healthy F. vesca was twined to these seedlings for evidence of virus transmission. At no time during these studies did any of the various species under test exhibit any definite symptoms. However, all of the species used in this study became infected with the Type 2 virus as ascertained by subsequent dodder transmission to F. vesca.

In 1947, Wood and Whitehead (102) reported that Potentilla anserina was a virus-immune plant. These studies show that P. anserina is not virus-immune but possesses the same inherent resistance to symptom express-

ion that is common in most cultivated strawberry varieties. The findings of Demaree and Marcus (19) indicating that Potentilla canadensis is a latent carrier of the Type 2 virus are verified. Finally, the results obtained extend the host range of the Type 2 virus to include three new species of Potentilla; namely, P. argentea, P. recta, and P. anserina.

Dodder Transmission Summary: These studies show the first successful transmission of the Type 2 virus, leaf roll and witches'-broom by the use of Cuscuta campestris Yuncker. They also indicate that C. campestris is better than C. sub-inclusa for transmission of the Type 2 virus. The incubation period for Type 2 virus and leaf roll complex is 14 and 21 days longer respectively by dodder than by the stolon-graft technique. As the witches'-broom virus expression on F. vesca is similar to the stunt virus expression described by Skiles and King (87), it is possible that these are one and the same disease. Dodder studies on the genus Potentilla extended the known host range of the Type 2 virus to include three new species; namely, P. argentea, P. recta, and P. anserina.

B. Seed Transmission Studies

There are less than a dozen plant viruses which are considered to be transmitted through the seed. Various hypotheses have been proposed through the years to explain the lack of virus transmission through seed but none have been generally accepted.

Seed transmission studies in strawberry have not been too extensive. Plakidas (70) grew 2,000 seedlings from plants infected with xanthosis and all appeared to be healthy. In research work on crinkle, Zeller (106) raised 122 seedlings from seed obtained from infected Marshall plants. Crinkle was transmitted to ten of these seedlings by viruliferous aphids.

This demonstrated they were originally virus-free. He concluded, "The infective principle is probably not transmitted through the seed." Studies on seed transmission using the Type 2 virus have not been reported. This virus type is widely distributed through varieties used as parent stocks in a current Michigan strawberry breeding project. Information on such transmission would be an important factor for this program.

a. Attempted Transmission of the Type 2 Virus Through Seed. Three cultivated varieties, Catskill, Dunlap and Robinson, infected with the Type 2 virus were maintained in the greenhouse until they began to flower. Then each flower was self-pollinated and bagged to prevent contamination by foreign pollen grains. The berries were allowed to ripen and were ground separately in a Waring Blender with 100 mls. of water for one minute. This mash was then poured onto moist sphagnum moss in flower pots. The resulting seedlings were transferred to four-inch pots and as stolons developed were indexed on F. vesca. Thirty-eight Catskill, 33 Dunlap and 187 Robinson seedlings, a total 258 seedlings were indexed and found to be virus-free.

b. Attempted Transmission of the Type 2 Virus by Pollen. Since strawberries flower readily and develop large anther sacs on exposure to short days this test was completed in the greenhouse during the winter months when the days were short. Twelve virus-free plants of the Robinson variety were used. As these plants flowered they were emasculated, pollinated and bagged. Pollen was obtained from Type 2 virus-infected Robinson plants. The fertilized berries remained on the plants until the pedicels dried up giving ample opportunity for transmission to occur to the pollinated plant. These plants were then indexed for virus content and found free from virus.

The seeds resulting from pollination with pollen collected from Type 2 virus-infected plants were planted and grown into new plants. A total of 622 seedlings developed but only 93, or 15 per cent, were selected at random for indexing. None of the 93 seedlings thus indexed showed evidence of virus transmission by pollen.

The evidence presented here indicates that the Type 2 virus is not present in pollen.

c. Attempted Transmission of Leaf Roll and Witches'-Broom Viruses Through Seed. A sampling from strawberries from different areas in the state showed that the viruses leaf roll and witches'-broom were present in Michigan. However, no studies on seed transmission with these virus diseases have been reported. Thus, infected plants of these diseases were maintained in the greenhouse for study. It may be assumed that if transmission occurred the seedlings would show visual symptoms of disease. However, progeny from leaf roll infected plants were further indexed on F. virginiana, the indicator for leaf roll, for seedlings could be infected with a mild form of the leaf roll virus and display no visual symptoms.

Similar pollenizing and seed techniques previously described were used. No transmission was indicated for 53 seedlings from Dunlap infected with the leaf roll virus or for 211 seedlings from Robinson infected with the witches'-broom virus.

These negative results further substantiate the lack of seed transmission of viruses in strawberry.

d. Attempted Transmission of the Type 2 Virus in Seed of the Indicator, Fragaria sp.. In the preceeding experiments, seed transmission of the various strawberry viruses was not shown for certain cultivated varieties. Since these viruses are not seed borne in one host one should not assume

that they will not be in another. Henderson (37) found this to be the case with tobacco ringspot virus which is seed borne in Petunia but not in tobacco. Working with cucumber mosaic Doolittle and Gilbert (20) found the virus to be seed transmitted in wild cucumber and not by seed of cultivated varieties. The question thus presented was whether or not strawberry viruses might be seed borne in the wild indicator plants, F. vesca, F. bracteata and F. virginiana.

Flowers from plants of F. vesca and F. bracteata infected with the Type 2 virus were self-pollinated and bagged. Similar methods were used for leaf roll virus-infected plants of F. virginiana. The resulting seedlings were potted and received daily care. Forty seedlings of each of the Fragaria species mentioned were maintained for study. Observations on these seedlings extended over a period of seven months.

The final analysis by observation for visual symptoms indicated that all 120 seedlings were apparently healthy. It appears quite evident that the strawberry Type 2 and leaf roll viruses are not seed borne in the wild species used in this test. These experiments show that infected Fragaria species growing in the wild would not perpetuate the virus through seedlings.

e. Attempted Transmission of an Anomalous Characteristic Found in Seedlings. Certain seedlings from the preceding experiments were observed to have leaflets that were thick and leathery with a mild mottled pattern. The affected seedlings were indexed since these symptoms might constitute a virus transmission. Eighteen seedlings were checked for virus content on the indicator F. vesca with negative results. The virus-free condition of these seedlings indicates that the given morphological differences are probably due to an inherited factor.

Seed Transmission Summary: These studies are the first to report that the Type 2, leaf roll and witches'-broom viruses are nontransmissible through seed in several cultivated varieties. Leaf roll and the Type 2 viruses were not found to be seed borne in the wild Fragaria species that were being used as indicator plants. Pollen from infected plants did not transmit the Type 2 virus to healthy plants or the seedlings resulting from this pollination. The results of these studies are encouraging for the strawberry plant breeder for he is practically assured that seedling selections will be virus-free.

C. Soil and Nematode Transmission Studies

A virus being soil borne may imply nothing more than mere survival on infected host tissues. These plant fragments transmit the disease when brought in contact with healthy tissue as is the case with tobacco mosaic (89). Other soil borne viruses such as "big vein" of lettuce (93) and "rattle" of tobacco (83) appear to have an active phase of their existence in soil. Rosette mosaic of peach, a soil transmitted virus disease, is even more closely related to strawberry. Cation (98) found that healthy trees planted in soil from the root environment of infected trees became diseased. However, this did not preclude the possibility of plant fragment or insect transmission.

In the early work by Plakidas (70) healthy strawberry plants were potted in soil and root fragments from the root environment of xanthotic plants. The plants were still healthy at the end of an 11 month period, giving evidence that transmission by soil or infected host tissue in the soil is not involved. This is the only report in the literature on soil studies involving a strawberry virus disease.

a. Attempted Transmission of the Type 2 Virus Through the Soil. Twelve

runners from healthy Robinson clones were allowed to root in six-inch pots filled with soil collected from around roots of Type 2 virus-infected plants. The plants remained in these pots for 16 months and became quite "pot-bound". Stolon-grafts were then made on the indicator plant for virus determinations. All grafts were successful and the indicator plants remained healthy, indicating non-transmission through soil.

b. Attempted Transmission of the Type 2 Virus by Infected Plant Fragments. Using similar methods as described in the preceeding test, 12 healthy runners were rooted in steam sterilized soil inoculated with root and crown fragments from the Type 2 virus-infected plants. These plants were also allowed a 16 month incubation period. The usual indexings for virus content were made and indicated that infected plant fragments in the soil will not transmit the virus. The findings of this test and Section a. are the same as found in the strawberry yellows studies (70). These studies further corroborate that certain virus diseases of strawberry are not transmitted through the soil.

c. Attempted Transmission of the Type 2 Virus by Nematodes. Johnson (45) found that soil treated with insecticides controlled the soil borne virus disease, wheat mosaic. He suggested that the insecticides act on a vector rather than on the virus and the vector may be a nematode. R. H. Fulton and Cation (25) showed that soil from around a rosette mosaic peach tree will not transmit the virus if treated with Chlordane indicating that a living organism may be the vector. Since there are no reports on the role of nematodes in strawberry virus transmission, studies on this phase were projected.

In 1952, it was noticed that a sandbench used in the virus screening program was contaminated with a root knot nematode, probably Meloidogyne

hapla. The Type 2 virus-infected Robinson plants were set in this inoculated nematode sand. Later examinations showed the roots of these plants to be seriously infested. Individual clones of these plants were then set in six-inch pots with single plants of healthy F. vesca. Another set was potted using these infested plants and healthy Robinson strawberry plants. The two lots of plants then received the regular cultural maintainance for 14 months.

At the end of the 14 month period, roots of the F. vesca plants were heavily infested with the root knot nematode. However, no visual symptoms of virus infection were evident. The originally healthy Robinson plants in the second lot were stolon-grafted for virus content. All of these indexings indicated the plants were still virus-free. Root examinations of these plants also showed heavy infestation of Meloidogyne hapla.

These negative results show that the root knot nematode, which is common in Michigan strawberry plantings, did not transmit the Type 2 virus under the conditions of this test.

D. Insect Transmission Studies

The strawberry aphid, Capitophorus fragariae Theob. (now called C. fragaefollii Ckll.) is considered the common natural vector of strawberry viruses. This was made evident by Plakidas (70), Vaughn (97) and Zeller (104) who proved that the viruses, crinkle, yellows and witches'-broom, are transmitted by this aphid. Later studies in the British Isles by Massee (51 and 53), Prentice (74) and Whitehead (101) further confirmed that this aphid is a vector for strawberry viruses in their locality.

In 1951, Frazier (29) reported on the transmission of the crinkle virus by four other species of aphids; namely, Myzus porosus Sand., Myzus ornatus Laing., Macrosiphum pelargonii Kalt. and Amphorophora

rubi Kalt.. Three other species of aphids which he tested failed to transmit the virus; namely, Aphis forbesii Weed., Macrosiphum solanifolii Ashmead and Myzus solani Kalt..

The aphid, Capitophorus fragaefolii is widely distributed in the three Pacific Coast states and British Columbia, Canada. In the East, it is considered to be of minor importance in strawberry virus transmission. The aphid has been found only in Geneva, New York, and Madison and Sturgeon Bay, Wisconsin. The species common in the East is C. minor Forbes. Using this species, Demaree and Marcus (19) readily transmitted Type 1 and Type 2 viruses.

A literature survey of the nine known insect vectors for strawberry viruses in the United States indicates that four have been reported in surrounding states and are presumed to be in Michigan. Since 1950 the writer has continually made surveys in the strawberry areas of the state for the genus Capitophorus but to no avail.

During the course of the writer's investigations on strawberry virus diseases, sucking pest species were found colonizing on plant species in the family Rosaceae. These were Aphis forbesii Weed., Aphrophora sp., Tarsonemus pallidus Banks, Tetranychus sp. found on Fragaria species and varieties. In addition, Macropsis trimaculata Fitch. was isolated from Prunus persicae. These pest species were identified by the writer.

It was believed worthy of investigating these species as possible vectors for the Type 2 virus since all of these species are common in or near strawberry plantings in Michigan. Finally, there are no reports on Type 2 virus transmission studies using these pest species.

Miller (62) found that maximum transmission was achieved by using

five viruliferous strawberry aphids per plant with a minimum feeding period of three hours. In these studies the feeding period and number of pests per plant were enlarged to increase the likelihood of their becoming viruliferous. Colonies of the individual pest species were maintained for five days on Type 2 virus infected strawberry plants. Eight pests of a given species were transferred to each of six healthy F. vesca plants and allowed to feed thereon for five days. This feeding period was terminated by spraying the indicator plants with Parathion, except in the case of plants infested with Tarsonemus pallidus which received hot-water bath treatment. These plants of F. vesca were maintained for observations of Type 2 virus transmission.

The five pest species used in this test did not transmit the Type 2 virus from infected plants to Fragaria vesca. The negative results with Macropsis trimaculata are noteworthy since this insect is common in southwestern Michigan and responsible for the spread of two peach viruses, little peach and peach yellows. The first four species listed are general throughout the state on both wild and cultivated strawberries. It is indeed important to know they are probably not vectors of the Type 2 virus.

E. Mechanical Transmission Studies

Many virus diseases attacking rosaceous plants have been described, but only a few have been transmitted mechanically. The failure to transmit these diseases by mechanical means may be due not only to the properties intrinsic to the virus but also to those of the host. Bawden and Kleczkowski (2) reported that tannins present in strawberry leaf extracts precipitated the protein in the extracts. It may be assumed that normal proteins as well as the virus will be brought into contact with these

protein precipitants in extracted strawberry leaf juice. These precipitants are probably present in sufficient amounts to precipitate the virus.

Attempts to transmit strawberry viruses by various mechanical methods have been reported (19 and 70). In 1951, Miller (61) reported transmission of strawberry yellows to F. vesca plants by extracted leaf juice. The percentage of plants infected was so small that the method was considered undesirable as a routine procedure for transmitting strawberry yellows. In 1952, Cornuet (12) reported on a mechanical technique used to transmit a mild crinkle virus. Again the results were unsatisfactory for only two of five plants inoculated became infected. These reports, although limited, did indicate that mechanical transmission was possible.

Individual tests were initiated on the factors effecting mechanical transmission. The methods used and final results are presented in the following sections.

a. Host Plants. All of the plants used in this study were grown in a greenhouse maintained at 70° F. The following 11 plant families, including 22 species, were used as local-lesion hosts for mechanical transmission studies on the Type 2 virus of strawberry.

CHENOPODIACEAE
Beta vulgaris L. var. saccharifera Lange

COMPOSITAE
Helianthus annuus L.
Lactuca sativa L.

CRUCIFERAE
Brassica nigra L.
Raphanus sativus L.

CUCURBITACEAE
Cucumis sativus L.
Cucurbita maxima Duchesne

LEGUMINOSAE
Phaseolus vulgaris L. var. Michelite, Pinto
Pisum sativum L. var. Alderman
Vicia faba L.
Glycine max (L) Merrill

MALVACEAE
Abutilon theophrasti Medic.
Althaea rosea Cav.

PHYTOLACCACEAE
Phytolacca americana L.

PORTULACACEAE
Portulaca oleracea L.

ROSACEAE
Fragaria vesca L.
Potentilla canadensis L.

SOLANACEAE
Datura stramonium L.
Lycopersicon esculentum Mill.
Nicotiana glutinosa L.

UMBELLIFERAE
Daucus carota L. var *sativa* DC.

b. Pre-conditioning of Host Plants. Viruses are known to combine readily with many substances, some of which are efficient inhibitors of infectivity. Bawden and Roberts (3 and 4) have reported on this phase of virus infection. Their data show that plants placed in a darkened chamber 24 hours prior to inoculation increased their susceptibility to virus infection. They postulate that plants treated in this manner have a low titre of labile products of photosynthesis which may be able to combine with virus particles and render them non-infective. Yarwood (103), Sill and Walker (85) and others have reported that susceptibility of local-lesion host plants was greatly increased when they received preinoculation darkening.

Therefore, the local-lesion host species used in the various tests were placed in a darkened chamber 24 hours prior to actual inoculation.

c. Preparation of Inoculum. It may be assumed that tannins or other protein precipitants are present in extracted strawberry leaf juices. A method to avoid the detrimental effects of these chemicals in the extracts was desired. Studies by Best and Samuel (9), Best (10), Thornberry (94) and Thung and Want (96) have shown that the addition of buffering mixtures of phosphate salts, reducing agents and various adsorbents to extracted leaf juice increase virus infectivity.

The chemical solutions considered to increase virus infectivity which were used independently in these tests were sodium sulfite (0.01 M), cysteine hydrochloride (0.01 M), lead acetate (1%), nicotine sulfate (40%) and gelatin (1%). A 0.1 M sodium phosphate buffer (pH 7.0) was used in combination with all these chemical solutions. Following the findings of R. W. Fulton (27) on rose mosaic virus, the buffer chemical solution equal in amount to 1/2 the weight of infected leaf tissue was added before grinding.

d. Leaf Inoculum. The inoculum used was obtained from the Type 2 virus-infected Robinson plants. The tissue was punched from young leaflets with a 5/16 inch cork borer. The resulting disks were placed in a mortar with a buffer chemical solution and immediately ground with a pestle. The juice extract was decanted into a four-inch watch glass and used immediately as inoculum.

e. Corolla Inoculum. Various investigators (54 and 99) have reported that the tissues of petals of some flowers possess a high virus titre. Studies on cucumber mosaic virus 1 by Sill and Walker (86) showed the highest lesion counts were obtained from the corollas of infected cucumber. Work by McWhorter (55) and Milbrath (58) on the cherry latent virus complex showed a higher mechanical transmission to cowpea by

using cherry flower petals than infected leaf tissue. They conclude that flower tissues contain less of the material that inhibits transfer of virus. With these facts in mind, a similar test was inititated using corollas of Type 2 virus-infected Robinson plants.

The studies were made during the winter months since the strawberry plant flowers readily on exposure to short days. A clonal line of Type 2 virus-infected plants was allowed to flower and the individual petals were carefully removed from each flower. All of the petals from a single picking were placed in a mortar with a small volume of buffer and triturated. The amended juice was used immediately as inoculum.

f. Methods of Inoculation.

1. In 1936, Rawlins and Tompkins (79) showed that by using carborundum the efficiency of virus inoculations was increased. Histological studies demonstrated that carborundum pierced the epidermal cells and increased the number of entry points for virus particles. The primary leaves of the host species were dusted with carborundum (600 mesh) and then wiped with gauze pads dipped in the amended inoculum. The treated leaves were rinsed immediately with distilled water. The plants were placed back in the 70° F. greenhouse and maintained for observations of virus transmission.

2. In 1940, Bennett (6) cited that rubbing of leaves with a virus solution may introduce the virus into the surface parenchyma cells, but needle inoculations may carry the virus into the phloem where it could spread throughout the plant. Therefore, a similar series of inoculations were made using a hypodermic syringe (No. 16 needle).

3. The method reported by Cornuet (12) for successful transmission of mild crinkle was also attempted. His technique employed an alcohol

extraction of leaves followed by centrifugation and inoculation with the supernatent solution.

g. Results of Mechanical Transmission Tests. It is evident in the preceding sections that care was executed to attain the best conditions for mechanical transmission of the strawberry Type 2 virus. However, the data obtained on the 22 plant species covering a period of 14 months indicated that transmission did not occur. This corroborates the findings of others (19 and 70) who have made attempts at mechanical transmission.

These negative results should not be considered as final. Indeed, there are many plant species yet to be tested as local-lesion hosts and other chemicals to be evaluated as agents for increasing virus infectivity. These studies point to the need for additional work on various chemicals and techniques to perfect a method of mechanical transmission of strawberry viruses.

CHAPTER VII

STUDIES ON THE INACTIVATION OF THE TYPE 2 VIRUS IN VIVO

Over a two year period, 238 strawberry plants were obtained from sources throughout the lower peninsula of Michigan and were indexed in an attempt to find virus-free plants of strawberry varieties. Virus-free sources were obtained for only three varieties from this extensive screening program.

Since it was evident that the stolon-graft indexing procedure involved much time and space, a shorter approach to the problem of obtaining strawberry plants free from the Type 2 virus prevalent in Michigan plantings was desired. This prompted an investigation to determine whether the virus could be eliminated from the plants by either heat or chemical treatments. Furthermore, information on thermal or chemical inactivation points would be of value in the classification of the virus type.

A. Heat Treatment Studies

It has been stated that the most successful way to cure plants infected with a virus is by heat treatment (89). In 1936, Kunkel (46 and 47) found that peach yellows, rosette, red suture and little peach could be inactivated by exposure to 50° C. for 10 minutes and other stated time and temperature relationships. Working with the phony peach virus, Hutchins and Rue (41) reported the apparent inactivation of the virus by immersing infected trees in a water bath for 40 minutes at 48° C. In 1941, Hildebrand (38) presented data on the successful inactivation of eastern "X" disease of peach by treating infected budwood for five minutes in a hot-water bath at 50° C. Moore (67) in his studies on sour cherry infected with yellows and necrotic ring spot was unable to inactivate the viruses

present by heat treatments of either budwood or potted trees. These reports indicate that some viruses in the family Rosaceae, of which strawberry is a member, are thermo-labile.

Demaree and Marcus (19) presented the findings of their research on strawberry viruses in 1951. In a survey of 16 eastern and central states their results indicated that strawberry plants from southern states are less likely to have viruses than those from northern states. The reasons for this general rule are not readily explained but may be connected with the recent work of Miller (64). His studies showed that the strawberry yellows virus complex persisted over a longer period of time within the aphid at cooler temperatures than at room temperature. The possibility that the virus entity itself may have a thermal inactivation point within a feasible range is suggested.

In 1952, R. H. Fulton (26) presented a preliminary report on attempts to inactivate the Type 2 virus in strawberry by hot-water treatment. The data showed that the virus was not inactivated by the maximum temperature range for the host plant, 48° C. for 10 minutes. Then in 1953, Posnette (72) of England reported positive inactivation of mild crinkle and the crinkle virus when plants were subjected to dry-heat at 37° C. for eight days. The same year, Miller (65) presented his results on the inactivation of strawberry viruses occurring in Oregon by use of the hot-water treatment. Inactivation evaluations of temperatures from 43° C. to 51° C. at varying time intervals were given. Immersion of infected plants at 43° C. for 30 minutes appeared to give the best results. Miller failed to indicate the virus types involved.

As indicated previously the writer attempted to inactivate the Type 2 virus by use of hot-water and it was noted in this study that dormant plants

survived treatment better than plants in vigorous growth. Since actively growing plants did survive the writer was prompted to test the possibility of a dry-heat treatment to potted plants. The work on this phase was well underway when Posnette (72) reported from England on similar methods but involving different virus types.

a. Survival of Strawberry Plants Grown in Different Media at a Temperature of 35° C. The first test to determine what type of planting medium would be easily penetrated by heat and yet maintain enough moisture to prevent the plants from drying out. Runners from Type 2 virus-infected Robinson plants were allowed to root in four-inch pots individually containing the following: 1) sand, 2) one-half sand and one-half loam, 3) loam, 4) vermiculite (fine), and 5) vermiculite (medium). The established plants were then defoliated and placed in a Cenco incubator (Cat. No. 95105A) maintained at 35° C. The various soil media were moistened daily to prevent wilting. At the end of six days the plants were removed from the incubator for observation. The surviving plants were maintained and indexed for virus content.

All of the plants grown in the sand, sand-loam mixture and loam failed to survive the treatment. One-third of the plants established in fine vermiculite and all of the plants in the medium grade vermiculite survived the test. Therefore, medium vermiculite was the most advantageous media to use for the studies. Results of the indexings on these remaining plants indicated that the virus was still thermo-stable at 35° C. for six days.

b. The Determination of the Thermal Inactivation Points of the Type 2 Virus in vivo. Since an ideal type of soil media was determined the next step was an attempt to find the temperature at which this virus was

thermo-labile. The temperatures selected were 34°, 36°, 38°, 40° and 42° C. Eight well-rooted Robinson plants were used for each exposure period; namely, four, six and eight days. The surviving plants received daily care and as stolons developed they were indexed to ascertain the effect of the individual treatments on the virus. The indexing data obtained in this test are presented in Table VII.

TABLE VII

EFFECT OF DRY-HEAT TREATMENT ON THE TYPE 2 VIRUS IN VIVO

Number of Days	Temperatures in Centigrade				
	34°	36°	38°	40°	42°
4	0/8	0/8	0/8	2/5	0/0
6	0/8	0/8	2/8	1/3	0/0
8	0/8	3/8	5/8	0/0	0/0

Denominator - Number of surviving plants for each treatment.
Numerator - Number of plants virus-free.

Table VII shows that inactivation of the Type 2 virus by dry-heat treatment is indeed possible. This virus type appears to have a thermal inactivation range from 36° to 40° C. for various exposure periods. The lowest temperature time for inactivation was 36° C. for eight days. However, inactivation was most assured when infected plants were exposed to 38° C. for eight days. Only one-third of the plants survived at 40° C. and all succumbed to the effects of the heat at 42° C. The 40° C. treatment was not as efficient as the 38° C., even though one-half of the plants were found to be virus-free. The reason for this is that plants treated at 40° C. remained in an apparent dormant condition for six weeks before growing normally. However, the plants treated at 38° C. resumed normal growth shortly following the exposure to heat and were indexed for

virus content at the end of the six week period required for higher heat treated plants to resume runner production.

The heat therapy work by Posnette (72) indicated the presence of at least two virus components. In the writer's studies, daily observations of indexed F. vesca plants revealed no change in symptom expression. The possible absence of other components within this virus type was suspected.

Previous work by the writer using hot-water methods were negative (26). Continued studies now show that the Type 2 virus, prevalent in Michigan plantings, can be successfully inactivated by dry-heat treatment. This is the first known report on the inactivation of the Type 2 virus. There appears to be a close correlation of thermal inactivation points of mild crinkle, crinkle and the Type 2 virus. These viruses are considered distinct from one another but due to the findings of Posnette (72) and the writer they may be classed into a given group for thermal inactivation.

Dry-heat treatments may prove the means of obtaining virus-free varieties which are considered to be 100 per cent infected. This will alleviate the problem of extensive screening and reduce to a minimum the time of obtaining healthy plants.

B. Chemical Treatment Studies

a. Inorganic Compounds. Many investigators have reported on the use of chemicals as inhibitors of plant virus infection in vitro, but very little work has been directed toward inactivating the virus in vivo. In 1942, Stoddard (90) reported inactivation of "X" disease of peach by soaking diseased buds in water solutions of quinhydrone, urea and sodium thiosulphate. Further work by Stoddard (91) indicated that "X" diseased seedling trees were cured by soil treatments of various chemicals. He considered in this study that calcium chloride and zinc sulfate were

the most efficient chemotherapeutants. Studies by Rumley and Thomas (84) verified the findings of Stoddard through inactivation of tobacco mosaic virus by floating infected Nicotiana glutinosa leaves on water solutions of zinc chloride and zinc sulfate. It may be concluded from these reports that calcium chloride, zinc chloride and zinc sulfate may serve as effective chemotherapeutic agents.

Chemical inactivation studies were initiated for the purpose of gaining additional information which would be of value for the classification of the Type 2 virus. All of the treatments used in this study were made on Type 2 virus-infected Robinson plants growing in sand in four-inch flower pots. Three inorganic chemicals; namely, calcium chloride, zinc chloride and zinc sulfate were used at concentrations of one and two per cent. Four plants were watered individually with 50 mls. of a given chemical concentration every four days for a period of 40 days. Supplementary maintainance watering was made with tap water direct from college wells without further softening or chemical treatment. As stolons developed after the chemical treatment the plants were indexed onto Fragaria vesca for virus content. The graft unions were not broken for 60 days to ascertain if the effect of chemical treatment on the virus was only of a temporary nature. The results of these studies are presented in Table VIII.

TABLE VIII

INORGANIC CHEMICAL EFFECTS ON THE TYPE 2 VIRUS IN VIVO

Chemical	Concentration	Effect on Type 2 Virus
Calcium chloride	1%	0/8
" "	2%	0/8
Zinc chloride	1%	0/8
" "	2%	3/8
Zinc sulfate	1%	0/8
" "	2%	4/8

Numerator - Number of plants virus-free.
Denominator - Number of infected plants treated.

Table VIII indicates that the chemical calcium chloride is not effective against the Type 2 virus at the concentrations used in this study; whereas, zinc chloride and zinc sulfate were effective at the two per cent concentration. This indicates that the zinc ion, rather than the chloride ion or sulfate radical is responsible for the inhibitory action. These findings further corroborate existing evidence that the zinc salts possess chemotherapeutic properties for inactivation of viruses.

It is evident in these investigations that the Type 2 virus can be inactivated by heat or water soluble forms of zinc. If one was to choose between treatments, the writer would suggest dry-heat for it requires approximately 60 days to complete as compared to 80 days for a chemical treatment. It is believed that these tests are the first record of direct eradication of the Type 2 virus in strawberry plants by chemicals. Furthermore, it gives the plant pathologist another means of controlling a

strawberry virus in vivo.

b. Organic Compounds. Reports on the use of organic compounds as chemotherapeutants is a relatively recent development in plant pathology. Probably experimental compound 4-chloro-3, 5-dimethyl-phenoxyethanol (Compound 1182 - Carbide and Carbon Chemical Division) received the most attention within the past few years as a fungal and viral chemotherapeutant (40). Davis (14) found that tobacco mosaic virus infecting Nicotiana glutinosa was reduced to one-third of the check by daily soil applications of Compound 1182. He suggests the activity of Compound 1182 as that of a modification of the host's metabolism.

Other organic compounds which have been tested extensively against viruses are the antibotics but the majority of these tests have been in vitro. Manil (50), the first investigator to report on in vivo studies was unable to inactivate tobacco mosaic virus by soil drenchings of various antibiotics such as streptomycin. Weinkling et al (100) reported there was no inhibition of tobacco mosaic or potato yellow dwarf viruses by aureomycin, streptomycin and other antibiotics when these materials were used systemically in the plant. These limited studies on organic compounds in vivo show that virus inactivation by this means may not be feasible.

Similar experimental design as given in the inorganic compound section was used. The materials furnished for testing were Compound 1182 (Carbide and Carbon Chem. Div, New York, N.Y.), actidione, neomycin and streptomycin sulfate (Upjohn Co., Kalamazoo, Mich.), aureomycin hydrochloride (Lederle Lab. Div., American Cyanamid Co., New York, N. Y.) and P. A.-96 (Chas Pfizer and Co., Inc., New York, N. Y.). The

latter five compounds are classified as antibiotics. The concentrations used were: 1182 - 16 ppm. and 32 ppm.; actidione, aureomycin hydrochloride, neomycin, PA-96, and streptomycin sulfate were all used at 250 ppm. and 500 ppm.

Four days after the termination of the ten 50 mls. waterings with the chemical solutions the treated plants were indexed to determine the effects of these organic compounds on the Type 2 virus. None of the materials tested exhibited any inhibitory action against the Type 2 virus in vivo. A dwarfing phytotoxic effect was noted on plants treated with neomycin and PA-96.

CHAPTER VIII

PHYSIOLOGICAL STUDIES ON THE TYPE 2 VIRUS

Many investigations (5 and 89) have been conducted on the metabolism of virus-infected plants for it is contended that a knowledge of physiological disturbances which accompany virus infection may contribute to a better understanding of the nature and cause of the disease itself. However, very little attention has been directed toward fruit virus diseases. In fact, in the field of small fruit virology there has been only one paper on the physiological phase of a virus disease, namely red raspberry mosaic (32). The data showed that even latent virus infection will reduce the production of carbohydrates and photosynthetic activity. Therefore, a study was initiated to determine the effect of the Type 2 virus upon certain metabolic processes of the respective host plant.

Healthy and Type 2 virus-infected plants of the Robinson variety were grown side by side in the greenhouse and received equal cultural treatment. Leaf samples for analyses were collected simultaneously using care to select leaves of the same age from corresponding positions on comparable plants.

A. Hydrogen-Ion Concentration

In 1928, Iyengar (42) reported that sap of spike infected sandal leaves were more acid than normal. Similar findings on tomato yellow mosaic were made by Bolas and Bewley (11). Working with potato leaf roll virus, Robertson and Smith (81), indicated that normal plants are more acid than the infected plants. They contend that a mixed virus infection is responsible for an increase in acidity.

Hydrogen-ion concentrations were determined on sample extracts of

mature leaflets and petioles, and ripened berries over a three month period. The average pH of sample leaf and petiole extracts indicated that the healthy plant material pH was 5.80 while that of infected plants was 6.10. Similar data were recorded for ripened berries. The berries collected from healthy plants had an average pH of 3.85 while those from infected plants was 4.10.

Due to the findings of Robertson and Smith (81) these results may well indicate that the Type 2 virus is caused by a single entity.

B. Nitrogen Metabolism

In 1918, Jodidi et al (43) reported a decrease of total nitrogen in blighted leaves of spinach. Rosa (82) found that leaves of tomato infected with "western yellow blight" showed a decrease of total nitrogen. In 1929, Dunlap (21) showed that yellows of spinach and aster lowered the nitrogen content of the leaves. Further studies by Dunlap (22) indicated that mosaic virus diseases increased total nitrogen in leaves whereas yellows type viruses lessened total nitrogen. These reports indicate that the yellows type virus diseases are accompanied by a reduction in total nitrogen as compared with healthy leaf tissues.

This recalled to mind the statement of Demaree and Marcus (19), "The most prevalent and destructive virus diseases in strawberries east of the Rocky Mountains are now thought to be of the yellows type.". Previous studies as indicated in Chapter IV (Page 19) showed that virus-free Catskill plants exhibited a yellows type reaction when infected with the Type 2 virus. It was concluded that an investigation of nitrogen metabolism would help to verify that the Type 2 virus is of a yellows nature.

The total nitrogen was determined by the methods described by Ma and Zuazaga (49) and Pepkowitz and Shive (68). The method is presented in the Appendix.

Table IX presents the percentage of total nitrogen obtained in healthy and Type 2 virus-infected leaves.

TABLE IX

TOTAL NITROGEN IN HEALTHY AND TYPE 2 VIRUS-INFECTED LEAVES, EXPRESSED AS PERCENTAGES OF DRY WEIGHT

Determination*	Type 2 Virus-Infected Leaves	Healthy Leaves
1	4.748	4.922
2	4.746	4.963
3	4.736	4.945
4	4.742	4.957
5	4.743	4.924
6	4.741	4.922
7	4.747	4.927
8	4.745	4.920
Average	4.744	4.940

*Determination - Represents the average value of four samples.

The healthy leaves contained 4.940 per cent total nitrogen as compared to 4.744 in diseased leaves. If the per cent nitrogen in Type 2 virus-infected leaves is calculated as per cent nitrogen in healthy leaves then the increase is considered to be 4 per cent. This difference is considerably greater than the error of determination.

The amino nitrogen was determined by the same method as described

in the Appendix for total nitrogen, except for the reagents used in the digestion. In these determinations the writer added a pinch of copper sulfate, pinch of selenium and 2 mls. of sulfuric acid to the digestion flask.

The percentages of amino nitrogen obtained in healthy and Type 2 virus-infected leaves are presented in Table X.

TABLE X

AMINO NITROGEN IN HEALTHY AND TYPE 2 VIRUS-INFECTED LEAVES, EXPRESSED AS PERCENTAGES OF DRY WEIGHT

Determination*	Type 2 Virus-Infected Leaves	Healthy Leaves
1	4.623	4.804
2	4.616	4.810
3	4.609	4.801
4	4.615	4.805
Average	4.616	4.805

*Determination - Represents the average value of four samples.

Table X shows that the reduction of nitrogen in Type 2 virus-infected leaves is largely in the amino fraction. The reduction is considered to be approximately the same as recorded for total nitrogen, namely, 4 per cent.

The findings in this section on nitrogen metabolism indicate that Type 2 virus infection reduces the total and amino nitrogen fractions. This reduction is in accord with the data presented on other yellows virus diseases and may indicate that the Type 2 virus should be classed in the yellows group.

C. Transpiration Rate

Various investigators (5, 42, 82 and 89) have presented the data which indicate that virus infection greatly decreases the transpiration rate. Data on the effect of the strawberry Type 2 virus on the transpiration rate were obtained by the standardized hygrometric paper method of Meyer (57) as described in the Appendix. The data obtained were recorded and are presented in Table XI.

TABLE XI

GRAMS OF WATER VAPOR TRANSPIRED FROM HEALTHY AND TYPE 2 VIRUS-INFECTED LEAVES*

Determination**	Type 2 Virus-Infected Leaves	Healthy Leaves
1	2.13	3.29
2	2.26	3.77
3	2.26	3.77
4	2.17	3.40
5	2.13	3.92
6	2.17	3.92
7	2.26	3.77
8	2.26	3.40
Average	2.21	3.66

* Recorded as grams of water vapor lost in one hour per 100 square centimeters of leaf area.
**Determination - Represents the average of four samples.

As shown by the data in Table XI, the transpiration rate in Type 2 virus-infected leaves was less than that recorded in healthy leaves. This was calculated to be 58 per cent of the normal value.

CHAPTER IX

SUMMARY

These investigations were conducted to determine the extent of virus infection and distribution in cultivated and wild strawberries in Michigan, to determine methods of differentiation of virus entities, to find and increase virus-free clones of desirable varieties, and to explore therapeutic measures for destruction of the viruses in vivo.

Three modifications were developed that improved previous techniques and enhanced the success of graft unions. These were covering the stolons with moist sand to increase their diameters, scraping the epidermis to give additional surface union, and cutting off the tips of each "tongue" to avoid awkward curling when joining the two prepared stolons while making the graft.

The reactions of Fragaria virginiana L., Fragaria bracteata Heller and Fragaria platypetala Rydb. to Type 1 and Type 2 viruses were investigated and compared with the standard indicator Fragaria vesca L.

F. virginiana was a symptomless carrier when infected with either virus type.

For the Type 1 virus, F. platypetala showed greater leaflet size reduction with darker green coloring while F. bracteata merely showed severe asymmetry. When compared with F. vesca, F. platypetala and F. bracteata showed slightly larger mottled areas. F. platypetala showed the smallest leaflets with only slight plant dwarfing while F. bracteata showed more asymmetry and extreme plant dwarfing. Leaflet coloration was deep green for F. vesca and F. platypetala but normal for F. bracteata.

Finally, the symptoms of Type 1 virus infection showed F. platypetala

to be characterized by the greatest reduction in leaf and flower size and lack of stolon formation when compared to F. vesca. F. bracteata was characterized by severe leaflet asymmetry and extreme dwarfing of the plant with normal stolon development and flower size.

For the Type 2 virus, interveinal chlorosis was common to all three reacting species, with varying leaflet size differential. F. bracteata showed no epinasty, while epinasty was more severe in F. platypetala than in F. vesca. F. vesca showed intense crown proliferation, with one-third less for F. platypetala and none for F. bracteata. Stolon development was reduced only for F. vesca and F. platypetala while flower symptoms on F. bracteata were differentiated mainly by solitary inflorescence.

Symptom expression was five days earlier for the Type 2 virus than for the Type 1 virus. F. vesca showed symptoms first for both types, followed about five days later by F. platypetala and about ten days later by F. bracteata.

When both viruses were present the general symptoms of both virus diseases were discernible. The cultivated varieties Dunlap and Robinson were symptomless carriers for both virus types. Catskill reacted with interveinal chlorosis and stunting to Type 2 virus but merely displayed a transient leaf flecking for the Type 1 virus.

A previous limited sampling of strawberry plants from Michigan conducted by Demaree and Marcus (19) showed only infection by the Type 2 virus. In these studies, a sampling of 575 cultivated strawberries from scattered locations in Michigan revealed that 15 per cent were infected with the Type 1 virus, 75 per cent with the Type 2 virus and 3 per cent with a combination of both types. Plants infected with the Type 1 virus were

traced to original sources outside of Michigan. This indicates Type 1 virus was imported and that Type 2 virus is possibly indigenous to Michigan. Cultivated plants free from Type 2 virus were exceedingly rare. Only a few plants of six varieties obtained in 575 samples were found free from viruses by the established techniques. These consisted of Brilliant, Catskill, Dunlap, Gem, Robinson and Superfection. The disease-free selections of the varieties Catskill, Dunlap and Robinson were increased by mass propagation for eventual distribution. This constitutes the first virus-free strawberry foundation planting in Michigan.

A sampling of 101 wild strawberries, F. virginiana, collected in southern Michigan revealed infection by the Type 2 virus only. Only 10 per cent of the plants tested were infected and of these 8 per cent were from plants collected near cultivated strawberries. From these results the isolation of virus-free foundation stock and nursery plantings from wild strawberries should be considered.

Another strawberry virus disease characterized by rolling of the leaflet margins and know as leaf roll was isolated. This was the first report of this disease for Michigan. These studies verified the investigations of Berkeley and Plakidas (8) that F. virginiana expressed symptoms of this disease. Reciprocal indexings to F. vesca with 41 isolates of leaf roll indicated that all of the plants were carrying the Type 2 virus in addition to the leaf roll virus. The F. vesca plants inoculated with the combined leaf roll and Type 2 viruses first expressed the typical Type 2 virus symptoms of epinasty and crown proliferation. These plants later showed a reddening of petioles. This petiole reddening was found characteristic for leaf roll infection on F. vesca. From

this finding it is apparent that F. vesca can be used as an indicator for the leaf roll complex. Inoculation by grafting of this leaf roll complex into several cultivated varieties revealed that Catskill and Dunlap displayed leaflet chlorosis and mottling accompanied by extreme marginal rolling, while Robinson and Gem were mildly affected.

It was indicated in this study that the viruslike particles shown in variegated plants by Thornberry (95) might well result from Type 1 virus contamination.

A virus disease demonstrated by witches'-broom symptoms was found in the northern portions of the Michigan lower peninsula. In these studies it was first shown to be transmissable by dodder, Cuscuta campestris.

Continued studies with dodder recorded the first use of C. campestris in the virus transmission of Type 2 and leaf roll viruses. The incubation period for the Type 2 and leaf roll viruses were respectively 14 and 21 days longer than by the stolon-graft technique.

By use of dodder the host range of the Type 2 virus was increased to include three new species of Potentilla; namely, P. argentea, P. recta and P. anserina. These species were found to be latent carriers of the Type 2 virus.

Investigations on seed transmission of various strawberry viruses showed that Type 2, leaf roll and witches'-broom viruses were nontransmissable through seed in cultivated strawberry varieties. Leaf roll and Type 2 viruses were further found nontransmissable through seed in F. vesca and F. virginiana. Pollen from Type 2 virus-infected plants did not transmit the virus to healthy plants or their F_1 progeny. These

studies suggest that virus-infected parent stocks may be safely used in a breeding program.

Soil and plant fragments collected from the root environment and roots of Type 2 virus-infected plants were placed around the root systems of healthy plants without transmission during a 16 month period. Experiments with the transfer of the root knot nematode, Meloidogyne hapla, from Type 2 virus-infected roots to virus-free stock also indicated non-transmission.

Transmission tests with the Type 2 virus using five sucking pest species common in or near strawberry plantings were negative. The pest species used were Aphis forbesii, Aphrophora sp., Tarsonemus pallidus, Tetranychus sp. and Macropsis trimaculata. This is noteworthy for the first four species are general throughout the state on both wild and cultivated strawberries.

Unsuccessful attempts were made to transmit the strawberry Type 2 virus mechanically. These mechanical transmission tests were made on 11 plant families covering 22 species, as possible local-lesion indicator plants. Pre-conditioning of indicator plants, leaf and corolla inoculum, inoculations by rubbing and syringe were included.

Investigations on the inactivation of the Type 2 virus in vivo revealed that this virus type exposed to dry-heat has a thermo-labile range of 36° to 40° C. varying with exposure periods. Inactivation was most assured when infected plants were exposed to a temperature of 38° C. for eight days.

Treatments of the Type 2 virus-infected plants with various chemicals revealed that a 2 per cent solution of either zinc chloride or zinc sulfate

will inactivate the Type 2 virus *in vivo*. Chemical inactivation tests using several other chemicals including antibiotics were negative.

These tests on the inactivation of the strawberry Type 2 virus by heat and chemical treatment show new methods for obtaining virus-free plants of varieties which are considered to be 100 per cent infected with this virus type.

A comparison of certain metabolic processes was made between Type 2 virus-infected plants and healthy plants. The average pH of extracts from healthy plants was lower than that from infected plants. Healthy plants showed 4 per cent more total and amino nitrogen than Type 2 virus-infected plants. Further evidence of a retarding influence of a virus in a symptomless carrier was demonstrated when Robinson plants infected with the Type 2 virus showed a 58 per cent reduction in the transpiration rate.

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APPENDIX

SUMMARY OF THE VIRUS-FREE STRAWBERRY CERTIFICATION PROGRAM IN MICHIGAN

A. Foundation Stock

Varieties known to be virus-free as determined by indexing onto Fragaria vesca will be maintained by the Department of Botany and Plant Pathology, Michigan State College, under the direction of the writer.

a. Varieties. Under the present conditions of this program, the following varieties will be propagated for distribution: Catskill, Dunlap, Marshall, Premier and Robinson. The main emphasis of production will be placed on Premier and Robinson which consist of 90 per cent of the strawberry acreage in Michigan.

b. Greenhouse. The screening of new varieties for virus content will be done in the greenhouse. At least six plants of each virus-free variety will be maintained.

c. Field Plots. Plantings of virus-free stock will be isolated at least 3,000 feet from either cultivated or wild strawberries. Plants set in these open field plots will be dug the following spring for distribution.

d. Control Program. Field plots will be dusted with one per cent Parathion at ten day intervals during the growing season. Leaf diseases of strawberry will be controlled by fungicides if the need arises.

B. Distribution of Stock

Notice of the availability of virus-free plants for propagation and the requirements for growing them will be made state-wide. This notification can be handled by the Bureau of Plant Industry or College Information Services. Upon notification by a grower that he will be able to comply with regulations, the tentative plot and history should be

thoroughly checked by a certification representative for confirmation.

It is contended that less than a dozen individuals will be able to comply with this program. Thus, the release of foundation stock to such growers will be set at not less than 500 plants per variety per season.

C. Grower Requirements

a. Isolation. The virus-free propagation plot should be located at least 3,000 feet from strawberries, either cultivated or wild. Subsequent plantings of foundation stock may be placed next to the original planting provided that the same isolation distance is maintained.

b. Soil History. The land to be used for this program is not to have been cropped to potato, tomato or eggplant within the past four years to avoid infection by Verticillium wilt. No tolerance will be shown to land which has a previous history of red stele. It is suggested that the grower use soil fumigation practices to reduce nematode problems.

c. Insecticide Program. In order to keep the insect vector at a minimum the grower is expected to comply with the following: the isolated virus-free strawberry plot is to be dusted at ten day intervals with one per cent Parathion. This material will also serve as a general insecticide for other insect pests found on strawberry.

d. Continuance of Certification. Certified growers will be expected to return to foundation stock at least once every four years. Failure to comply with the existing regulations will result in nullification of virus-free certification.

ANALYTICAL PROCEDURES

A. Total Nitrogen in Plant Material (micro method)

a. Reagents.

Hydrochloric acid. Standard 0.02 N solution

Boric Acid. 2 per cent solution. Dissolve 10 grams of acid in 500 ml. of hot water.

Methyl purple indicator. Commercially prepared solution. Add two drops to the boric acid solution per 100 mls.

Sodium hydroxide. 30 per cent solution. Dissolve 150 grams of sodium hydroxide pellets in 350 ml. of water.

Ranker's solution. Dissolve 32 grams of salicylic acid in one liter of concentrated sulphuric acid.

Sodium thiosulphate. Dissolve 50 grams of sodium thiosulphate in 100 ml. of water.

Selenium. Powdered metal.

Potassium sulphate-Copper sulphate mixture. Pulverize in a mortar 3 parts of copper sulphate with one part of potassium sulphate.

b. Procedure.

1. Weigh from 40 to 50 milligrams of the finely ground dry plant material into a 50 milliliters micro Kjeldahl digestion flask. A long handled micro weighing tube will be found very convenient.
2. Add 3 ml. of Ranker's solution, mix with the tissue and allow to stand in cold for 30 minutes.
3. Add 5 drops of the thiosulphate solution to reduce the nitro group and warm gently over a micro-burner.
4. After several minutes, add about 3 mg. of powdered selenium and 5 mg. of potassium sulphate-copper sulphate mixture.

5. Place the flask on digestion rack and boil the mixture gently until clear. The reaction is complete usually in 10 minutes.
6. After cooling, add 2 ml. of distilled water, mix, and cool again.
7. Transfer the contents of the digestion flask to the micro Kjeldahl still. Rinse the digestion flask twice with about 2 ml. of distilled water.
8. Add 10 ml. of 30 per cent sodium hydroxide, then wash funnel with distilled water.
9. Distill the ammonia into a 50 ml. Erlenmeyer flask containing about 5 ml. of 2 per cent boric acid.
10. Add 4 drops of the mixed indicator and titrate to a pink color with standard 0.02 N hydrochloric acid.
11. Run a blank determination using all the reagents as in the determination of the unknown.

c. Calculations.

$$\text{Per cent N} = \frac{(\text{mls. HCl used} - \text{mls. HCl blank}) \times 0.028}{\text{wgt. of sample}}$$

B. Determination of the Transpiration Rate

a. Materials.

Filter Paper - Whatman No. 1

Cobalt Chloride - 3 per cent solution

Celluloid - 1 x 4 centimeter strips

Cloth Reinforcement Labels - 9/16 inch diameter, 1/4 inch hole

b. Procedure.

1. Using a cork bore, cut disks from the filter paper approximately one centimeter in diameter.
2. Immerse disks in a 3 per cent solution of cobalt chloride for one minute while stirring constantly.
3. Dry the cobalt chloride impregnated disks in an oven maintained at 40° C. for a period of 30 minutes.
4. Complete drying of disks in a descicator over calcium chloride. A full blue color should be attained in two days.
5. Transfer 20 disks to a weighing bottle and determine their dry weight.
6. Remove disks from the bottle and spread out in a moist chamber until a full pink color develops.
7. Transfer these disks to the weighing bottle and reweigh to determine exact amount of water absorbed.
8. Attach the paper disks to celluloid strips with cloth reinforcement labels. This transpiration equipment should be kept in a descicator over calcium chloride until ready for use.
9. The determinations are made by attaching these strips to strawberry leaves by means of a paper clip. The time from attachment until a full pink color develops on the disk should be recorded in seconds. This time is designated as "T" in the water vapor loss formula.

c. Calculations.

Reference Standard - Equivalent to the water vapor per 100 square centimeters of paper surface.

$$\frac{GM}{A} = \frac{X}{100}$$

Where GM - Gain in weight in grams of 20 disks in a saturated atmosphere.

A - The area of these disks in square centimeters.

X - Reference standard.

Water Vapor Loss

$$G = \frac{(\text{Reference Standard} \times 3600)}{T}$$

Where G - The grams of water vapor lost per hour.

T - The time of color change from blue to pink in seconds.

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