Line Pattern of Birch Caused by Apple Mosaic Virus

A. R. Gotlieb and J. G. Berbee

Research Assistant, Department of Plant Pathology; and Professor of Plant Pathology and of Forestry, University of Wisconsin, Madison 53706.

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ABSTRACT

Fifteen white birch and 12 yellow birch trees were graft-inoculated with budwood from a white birch tree displaying line pattern and ringspot symptoms on scattered leaves. Within 2 years, one-third of the white birch and two-thirds of the yellow birch trees exhibited line pattern symptoms. Remission of all leaf symptoms in the continued presence of the virus usually occurred thereafter. A virus from the white birch tree that supplied the budwood was mechanically transmitted to cucumber, squash, cowpea, and bean plants. The birch virus was purified from infected cucumber cotyledons by differential centrifugation. It was identified as apple mosaic virus (ApMV) on the bases of symptoms in selected herbaceous hosts, serological relationships, virus components, and virus morphology. Cucumber proved to be a highly efficient and reliable host for indexing both white and yellow birch trees for ApMV. The virus also was detected consistently in the crude leaf sap of infected white and yellow birch trees by Ouchterlony gel double-diffusion tests.

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Both white and yellow birch (*Betula papyrifera* Marsh. and *B. alleghaniensis* Britton) trees are subject to an unexplained decline and dieback that may result in tree mortality. The most dramatic example of unexplained birch dieback occurred between 1935 and 1955 in the northeastern United States and in eastern Canada. Most of the merchantable birch trees in the severely affected areas were killed. A continuing, but less spectacular, decline problem of white birch in urban settings, and of yellow birch in the type of northern hardwoods forest found in Wisconsin, has emphasized a continuing need for investigation of every detectable pathogen of birch.

During the course of intensive etiological investigations of birch dieback in northeastern North America, certain symptoms suggestive of a virus disease were reported (3, 11, 12). Hansbrough (11) described gold ringspot of white birch and reported graft-transmission of its causal agent to white birch saplings. He discounted its importance in the etiology of birch dieback because the symptom was not associated consistently with the disorder. In eastern Canada, graft-transmission of the agent responsible for line pattern of yellow birch was reported by Berbee (4).

In Wisconsin, line pattern and ringspot symptoms on white and on yellow birch trees occasionally have been observed as a conspicuous curiosity in scattered locations for decades. On affected trees, leaf symptoms usually appeared only sporadically. Thus, although not conspicuously apparent, the agent responsible for birch line pattern may be widespread in nature and contribute to the ecological sequence of events leading to decline problems in birch.

The purposes of this study were: (i) to confirm graft-transmissibility of the birch line pattern agent in Wisconsin; (ii) to describe foliage symptoms; (iii) to isolate, characterize, and identify the causal virus; and (iv) to develop simple indexing methods for detection of the virus.

MATERIALS AND METHODS.—Initially, an individual white birch tree displaying line pattern and ringspot symptoms located on the Madison campus of the University of Wisconsin was selected for study. Branches displaying conspicuous foliage symptoms during the growing season were labeled and utilized the following spring as a source of budwood for graft-transmission trials and a source of newly emerging foliage for virus transmissions to herbaceous hosts.

Herbaceous plants were grown from seed in the greenhouse (22-26 C) in 10.3-cm (4-inch) pots or flats containing steamed soil. Cotyledons and primary leaves were most susceptible to virus infection when fully expanded, but prior to the onset of secondary growth. Herbaceous hosts routinely used, included cowpea [*Vigna sinensis* (Torner) Savi] 'Blackeye'; bean (*Phaseolus vulgaris* L.) 'Bountiful'; cucumber (*Cucumis sativus* L.) 'Chicago Pickling'; squash (*Cucurbita maxima* Duchesne) 'Buttercup'; and *Chenopodium quinoa* Willd. Healthy 2-year-old white and yellow birch trees were obtained from Boscobel State Forest Nursery, Wisconsin.

Mechanical transmission.—Infected leaf tissue (0.2 g) ground in 1 ml of .03 M phosphate buffer (pH 8.0) containing .02 M 2-mercaptoethanol (2-ME) was applied with a cheesecloth pad to leaves of herbaceous host plants previously dusted with Carborundum. For transmissions directly from birch to herbaceous hosts, sap expressed from newly emerging leaves was used as inoculum. Such material was made available throughout the spring

and early summer months by storing excised dormant birch branches in a cold room (4 C) with their cut ends either sealed with wax or immersed in water.

Purification.—Cucumber plants, grown in flats, served as a purification host. The cotyledons were inoculated just prior to primary leaf expansion with inoculum prepared from infected cucumber cotyledons. Cotyledons were harvested for virus purification 4-5 days after inoculation when confluent yellow rings appeared, or at first sign of wilting of the growing tips.

The birch virus was purified by a modification of the procedure described for apple mosaic virus (ApMV) by Fulton (8). Infected cucumber cotyledons were homogenized in .03 M phosphate buffer containing .02 M 2-ME (1.5 ml buffer/g tissue) in a precooled Omni-mixer immersed in an ice bath. The rate of homogenization was adjusted to keep the temperature below 16 C. The homogenate was centrifuged at 3,000 rpm for 20 min in a Sorvall GSA rotor. Hydrated calcium phosphate (HCP) was added to the supernatant (0.9 ml/g of original tissue) and the mixture was clarified by repeating the above centrifugation. The supernatant then was centrifuged at 30,000 rpm for 4 hr in a No. 30 Spinco rotor. Pellets resulting from high-speed centrifugations were suspended in phosphate buffer (0.03 M, pH 8.0). All further low-speed (10,000 rpm for 20 min) and high-speed (40,000 rpm for 90 min) centrifugations were done in a No. 40 Spinco rotor. Following a low-speed centrifugation, one additional alternating high- and low-speed cycle yielded virus preparations used in initial serological tests and for density-gradient centrifugations.

The virus was further purified for antiserum production and for cross-absorption tests by two additional cycles of alternating high- and low-speed centrifugations.

Serology.—Antiserum against the birch isolate was prepared by intramuscularly injecting a rabbit every 3-4 days for 6 weeks with 1 ml of a partially purified virus suspension ($A_{260/280}$ ratio of 1.43 - 1.51; A_{260} of 5) emulsified with 1 ml of Freund's incomplete adjuvant.

The rose strain of apple mosaic virus (ApMV), the G strain of Prunus necrotic ringspot virus (PNRV), and their homologous antisera were provided by R. W. Fulton, Department of Plant Pathology, University of Wisconsin.

Ouchterlony gel double-diffusion tests were used for virus identification and detection. These were conducted in plastic petri dishes (90-mm diam) each of which received 20 ml of Ionagar (0.6%) containing sodium chloride (0.85%) and sodium azide (0.2%). Three to six peripheral wells, 5 mm in diam, were punched in the agar 5 mm from a central well. To eliminate possible host protein precipitation lines, undiluted expressed sap from healthy cucumber cotyledons was placed in antiserum wells 6 hr prior to tests. Plates were read within 48 hr but, in cases of apparent negative results, were kept under observation for 10 days.

RESULTS.—*Symptoms.*—In both white and yellow birch, line pattern symptoms consisted of chlorotic lines forming oak-leaf designs, irregular rings or linear flecks, sometimes accompanied by a mild mosaic (Fig. 1-8). Figures 1-3 illustrate leaf symptoms on the white birch tree, from which a virus was first graft-transmitted and isolated. Symptoms appearing on white and on yellow birch leaves following graft-transmission from white birch are shown in Fig. 5 and Fig. 6-7, respectively. Line pattern and ringspot symptoms appeared on both species. Line pattern and ringspot symptoms that appeared in 1954 on a white birch tree and in 1956 on a yellow birch tree in Nova Scotia are illustrated in Fig. 4 and Fig. 8, respectively. The presumed virus responsible for the symptoms observed on birch in Nova Scotia was not investigated.

Naturally infected white birch trees were observed for symptom development throughout the growing season. Emerging leaves on infected trees generally remained symptomless until after they became fully expanded. On some of the infected trees leaf symptoms never developed, but on others a few leaves sometimes exhibited symptoms. Rarely did leaf symptoms appear throughout the crown. Most commonly, they were restricted to very few leaves on a few branches. During mid-summer, chlorotic leaf tissue turned almost white. Leaf symptoms persisted throughout the growing season. Leaves displaying symptoms and leaves without symptoms on infected trees and leaves on healthy trees all abscised at about the same time in the fall.

Graft-transmission.—During February 1969, 15 three-year-old white birch and 12 three-year-old yellow birch trees were forced in the greenhouse (16 C) and each was inoculated with two buds from a white birch tree exhibiting line pattern symptoms. Three white birch trees and five yellow birch trees served as noninoculated controls.

Line pattern symptoms appeared on about one-third of the bud-inoculated trees as soon as leaves became fully expanded (Table 1). During the next growing season, one-third of the inoculated white birch and two-thirds of the inoculated yellow birch trees showed symptoms on from one to six leaves (Fig. 5-7). All of the trees that had foliage symptoms during the first growing season also exhibited them during the second growing season. The eight noninoculated check trees remained symptomless. In this experiment, total remission of all line pattern symptoms occurred during the third and fourth growing seasons following inoculations (Table 1). However, the diseased white birch tree from which budwood was taken for graft-inoculations displayed conspicuous line pattern symptoms during every growing season from 1968 through 1972. In a separate graft-transmission trial, one of four infected white birch trees, and one of seven infected yellow birch trees, showed line pattern symptoms 3 years after bud-inoculation. Thus, although remission of leaf symptoms in birch trees usually occurs, line patterns on leaves may appear each year in certain individuals.

Transmission from birch into herbaceous

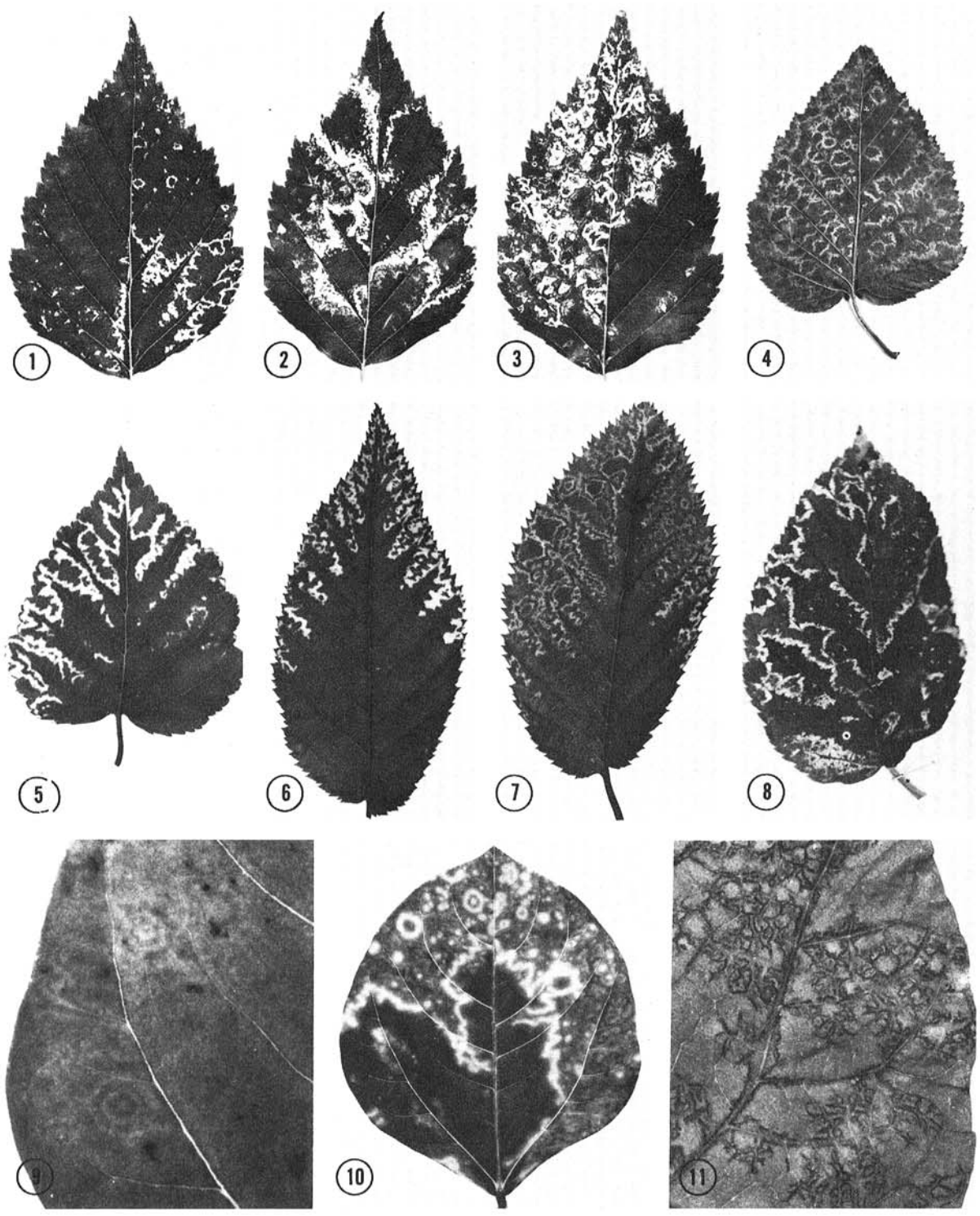

Fig. 1-11. Leaf symptoms. **1)** Line pattern and chlorotic rings, **2)** diffuse line pattern, and **3)** angular chlorotic rings on white birch leaves infected with ApMV in Wisconsin. **4)** Line patterns and rings on white birch in Nova Scotia. **5)** Line pattern and angular chlorotic rings on white birch, **6)** line pattern alone, and **7)** with chlorotic rings on yellow birch, all following graft-transmissions from a diseased white birch. **8)** Line pattern and angular chlorotic rings on a yellow birch in Nova Scotia. **9)** Symptoms of the birch isolate of ApMV on a cowpea primary leaf, **10)** on a cowpea trifoliate leaflet, and **11)** on a bean primary leaf, all following inoculations with the birch isolate of ApMV.

hosts.—In June, inoculum prepared by triturating newly expanded white birch leaves from the same tree that provided budwood for graft-transmission trials, was rubbed on the leaves of a series of herbaceous hosts. Initially, a single necrotic lesion was obtained on a squash cotyledon as a result of attempted inoculations of nine pairs of cotyledons. Serial transmission of virus from the necrotic lesion was made to squash and then to cucumber, cowpea, and *C. quinoa*. The following year, transmissions from newly emerging birch leaves into herbaceous hosts were routinely made from late December through July. Primary isolations of virus from birch were made on cowpea, cucumber, squash, and bean. With the exception of *C. quinoa*, symptoms on all of the herbaceous hosts were reproduced, both by serial transfers to the same host species, and by transfers between herbaceous host species.

TABLE 1. Numbers of white and yellow birch trees exhibiting line pattern symptoms and subsequent remission of symptoms following bud inoculation with apple mosaic virus (ApMV) in 1969

Species	Trees bud-inoculated	Trees showing line pattern during[a]		
		1969	1970	1971 and 1972
White birch	15	4	5	0
Yellow birch	12	4	8	0

[a] Trees that showed line pattern symptoms in 1969 also showed them in 1970. Eight noninoculated control trees remained healthy.

Symptoms of the birch virus and of ApMV on herbaceous hosts.—Since symptoms of the birch virus isolate on herbaceous hosts resembled those reported for the rose isolate of ApMV (7), the two isolates were compared in a limited host-range study. The birch virus and ApMV produced identical symptoms on cucumber, squash, bean, *C. quinoa*, and *Cyamopsis tetragonaloba* (L.) Taub. Lesions on cucumber cotyledons, and on *C. tetragonaloba* leaves, were identical to those illustrated for ApMV by Fulton (10). On cucumber cotyledons, confluent yellow rings appeared or the cotyledons simply collapsed. These symptoms usually were followed within 24 hr by necrosis of the growing tip, and ultimately by death of the plant. Both isolates produced necrotic lesions on squash cotyledons. A conspicuous necrotic veinbanding appeared, both on the inoculated primary leaves, and on trifoliate leaves of bean (Fig. 11). Both isolates induced chlorotic flecks on *C. quinoa*, but reisolation from this host was never achieved.

The birch virus could be distinguished from the rose isolate of ApMV only on cowpea. Both isolates produced necrotic rings with green centers and concentric chlorotic rings on the inoculated primary leaves of cowpea (Fig. 9). Systemic symptoms of both isolates included vein-clearing, mottling, ringspot, and malformations of trifoliate leaves as illustrated by Fulton (7). But only the birch isolate sometimes produced line patterns on the trifoliate leaves of cowpea during warm (above 26 C) conditions (Fig. 10). The known ApMV isolate caused a much greater reduction in height growth of cowpea plants than did the birch isolate.

Purification.—Clarification with HCP, followed by two cycles of alternating high- and low-speed centrifugation, yielded virus suspensions with $A_{260/280}$ ratios which ranged from 1.15 to 1.32. With two additional high- and low-speed cycles, 200-g lots of infected cucumber cotyledons each yielded 1 ml of virus suspension with A_{260} readings of up to 72, and with $A_{260/280}$ ratios ranging from 1.43 to 1.61.

The infectivities of these preparations, adjusted to an A_{260} of 0.1 or higher, were established by consistently successful inoculations of cucumber cotyledons.

Serology.—Twelve serial injections of a rabbit with 1 ml of purified birch virus suspension adjusted to an A_{260} of 5 ($A_{260/280}$ ratio 1.43-1.51) yielded a whole serum with an antibody titer of 1:1,024. This antiserum was used throughout the study.

Since the symptoms of the birch virus isolate on herbaceous hosts were similar to those of ApMV, serological tests were conducted to confirm the tentative identification of the birch virus as ApMV. For initial serological tests, both virus isolates were concentrated 20-fold from infected cucumber cotyledons by two cycles of differential centrifugation. However, an optimum concentration of antigen for reaction with antiserum in gel double-diffusion plates occurred in the crude sap of infected cucumber cotyledons. Thus, crude cucumber sap performed equally as well as partially purified virus suspensions in double-diffusion tests.

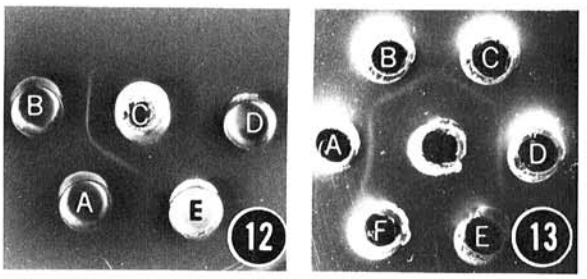

Fig. 12-13. Serology. **13)** Serological identification of the birch virus as apple mosaic virus (ApMV). Well C contained a 1:20 dilution of birch virus antiserum. Wells A, B, and E contained crude sap of cucumber; A) infected with the birch virus, B) infected with the rose isolate of ApMV, and E) infected with the G strain of PNRV. Well D contained crude sap of healthy cucumber. **14)** Serological indexing of birch leaves infected with ApMV. The center well contained birch virus antiserum diluted 1:80. Wells A and B and wells C and D contained crude leaf sap from two different white birch trees infected with ApMV. Wells E and F contained crude sap from healthy birch and from healthy cucumber leaves, respectively. The precipitation lines appeared within 24 hr and were photographed after 48 hr.

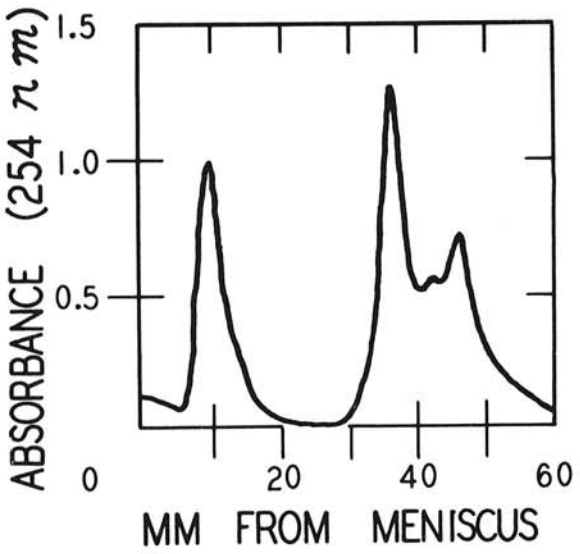

Fig. 14. Ultraviolet scanning pattern of a partially purified preparation of the birch isolate of apple mosaic virus (ApMV) in a 7-30% sucrose density-gradient after centrifugation for 4.5 hr at 25,000 rpm in a Spinco SW 25.1 rotor.

In Ouchterlony gel double-diffusion tests, both the known ApMV antiserum and the birch virus antiserum were tested against the birch virus, ApMV, and PNRV (Fig. 12). Birch virus antiserum (diluted 1:20) placed in the central well, reacted with birch virus antigen placed in well A, and with ApMV antigen placed in well B, both contained in crude cucumber sap, to produce a continuous precipitation line without any indication of spur formation. Conversely, PNRV antigen, contained in crude cucumber sap in well E, failed to react with the birch virus antiserum. The reciprocal tests, with ApMV antiserum in the central well, gave identical results. The PNRV antiserum, diluted for optimum line precipitation with PNRV contained in undiluted cucumber sap, failed to react with birch virus contained in undiluted cucumber sap. In this test and its reciprocal test, the concentrations of the virus antigens, and possibly of the heterologous antisera, were not high enough to detect the serological cross-reactions between the ApMV and PNRV serotypes that were demonstrated by Fulton (9) and confirmed by Casper (5).

Intragel cross-absorption tests.—The method described by Van Regenmortel was employed (14). Purified preparations of the birch virus and of ApMV and their homologous antisera were used in the tests.

Serial dilutions of ApMV, and of birch virus antigens, were tested in gel double-diffusion plates against serial dilutions of both of the antisera, to determine the optimum combinations of concentrations for line precipitation reactions. Both antigens initially were adjusted to an A_{260} of 32. A 1:64 dilution of the ApMV antiserum gave optimum precipitation lines with 1:32 dilutions both of birch virus antigen and of ApMV antigen. A 1:32 dilution of the birch virus antiserum gave optimum reactions with 1:32 dilutions of both virus antigens. Thus, a 1:64 dilution of the ApMV antiserum, a 1:32 dilution of the birch virus antiserum, and a 1:32 dilution of both antigens, were judged optimum for cross-absorption tests.

In separate reciprocal tests, concentrated birch virus antigen or ApMV antigen were placed in central wells, each surrounded by six outer wells. After 6 hr, a 1:64 dilution of ApMV antiserum was placed in the well that previously had received concentrated birch virus antigen and a 1:32 dilution of birch virus antiserum was placed in the central well that previously had received concentrated ApMV antigen. In each of these reciprocal tests, the six outer wells received 1:16, 1:32, and 1:64 dilutions of birch virus antigen and of ApMV antigen. The three concentrations were used to bracket the optimum dilution of the antigens.

In repeated reciprocal trials, no precipitation lines formed. The birch virus antiserum was completely absorbed by the ApMV antigen and the ApMV antiserum was completely absorbed by the birch virus antigen. In these tests, the birch virus proved serologically identical to Fulton's rose isolate of ApMV.

Virus components.—The birch virus was separated into components by density-gradient centrifugation. One ml of partially purified virus (A_{260} of 9.5, $A_{260/280}$ of 1.27) was layered on a 7-30% linear sucrose gradient in .03 M phosphate buffer (pH 8.0) which then was centrifuged at 25,000 rpm for 4.5 hr in a Spinco SW 25.1 swinging bucket rotor. Contents of the tube were scanned and fractionated with an ultraviolet scanner (254 nm) and recorder attached to an ISCO density-gradient fractionator.

In five separate trials, four peaks with high absorbency at 254 nm were recorded after density-gradient centrifugation (Fig. 14). The peak closest to the meniscus showed a very slight reaction with birch virus antiserum. When antiserum not absorbed with host protein was used, a diffuse host protein precipitation line appeared. Peaks two, three, and four reacted serologically with birch virus antiserum, but showed no host protein reaction. When the fraction containing the first peak was applied to 10 cucumber plants, no infection occurred. All 10 of the cucumber plants inoculated with the fraction containing a mixture of the second, third, and fourth peaks became infected.

Virus morphology.—Electron micrographs were taken of virus purified by density-gradient centrifugation. Fractions containing peaks two, three, and four were combined and centrifuged for 90 min in a No. 40 Spinco rotor at 40,000 rpm. The pellet was resuspended in 2 ml of phosphate buffer (.03 M, pH 8.0) and was fixed by dialysis for 5 days against 1% gluteraldehyde, followed by dialysis against distilled water for 24 hr, both at 4 C. A droplet of fixed virus mixed with 1% phosphotungstic acid (2:1) was placed on a grid covered with a carbon-coated collodion membrane. The droplet was dried down within 30 seconds by absorbing it with filter paper.

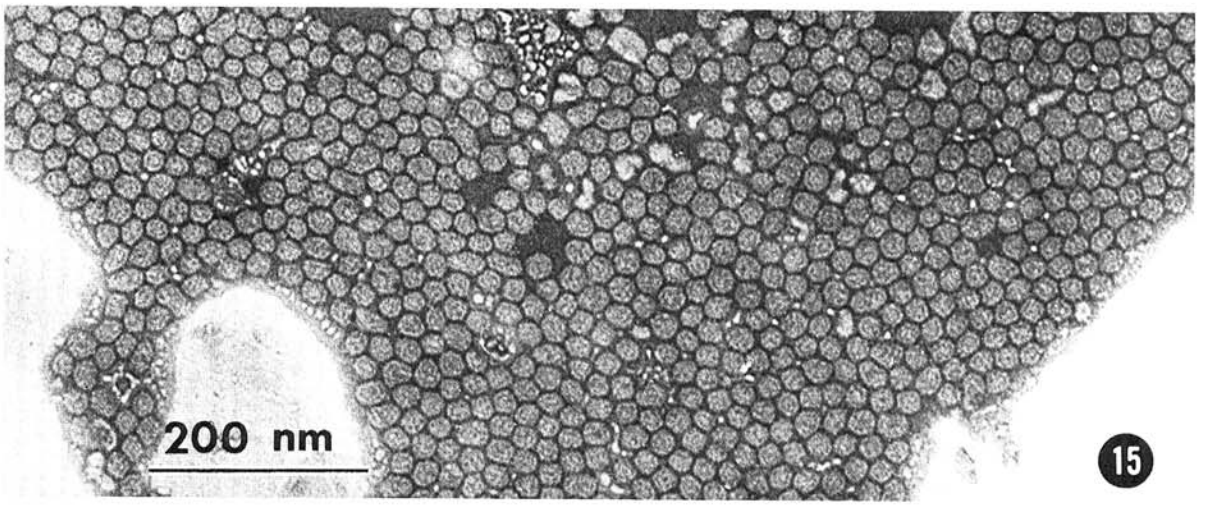

Fig. 15. Electron micrograph of the birch isolate of apple mosaic virus (ApMV) stained with phosphotungstic acid.

Chains of four birch virus particles were measured to determine particle size. The spherical particles ranged in diameter from 23.4 to 25.1 nm (Fig. 15). The average diameter of 400 particles was 23.9 nm, a size in general agreement with that reported for ApMV (8).

Indexing birch trees for ApMV.—Experiments were undertaken to determine the efficiency of primary virus transmissions from birch trees to cucumber. Cucumber was chosen because symptoms developed in as little as 3 days and the plants were highly susceptible to the virus. In each of the three separate trials, at least eight (average nine) of the ten cucumber plants per test inoculated with birch sap from infected leaves, showed diagnostic chlorotic ring symptoms within 5 days (Table 2). This result was confirmed in subsequent routine indexing of birch trees for ApMV. For example, of 12 infected white birch and 14 infected yellow birch trees, 22 trees each indexed positive on all five of the inoculated cucumbers per test; the remaining four trees each indexed positive on four of the five inoculated cucumber plants. Eighty percent or more of the inoculated cucumber plants always became infected. Thus, with inoculation of three cucumber plants, the probability of detecting existing virus in birch leaves would exceed 99%.

TABLE 2. Percentages of cucumber plants exhibiting symptoms, and rate of symptom development following mechanical inoculations of cotyledons with crude leaf sap of birch infected with apple mosaic virus (ApMV)[a]

Symptom	Days after inoculation		
	3	4	5
None	60	20	10
Chlorotic rings	40	60	90
Tip necrosis	0	20	70

[a] Based on averages from three separate trials using 10 cucumber plants per trial.

White and yellow birch trees that were bud-inoculated in 1969 were indexed for ApMV during the spring of 1972 (Table 3). The five inoculated white birch and eight inoculated yellow birch trees that subsequently showed line pattern symptoms all indexed positive for ApMV on cucumber. In addition, two inoculated white birch trees that never showed line pattern symptoms indexed positive on cucumber. All of the white birch and yellow birch trees that indexed positive on cucumber, also were positive in serological gel double-diffusion tests. These indexing results were confirmed in a separate graft-transmission trial involving four infected white birch and seven infected yellow birch trees.

The practicality of serologically indexing birch trees for ApMV is illustrated in Fig. 13. Success in the serological indexing of birch trees for ApMV requires that the virus contained in birch sap be placed in the antigen wells 6 hr before ApMV antiserum is deposited in the central well.

Although no effort was made to index large numbers of birch trees in the field, ApMV has been identified by serology in white birch trees in northwestern, northeastern, and southern Wisconsin in the vicinities of Hayward, Sturgeon Bay, and Madison, respectively.

DISCUSSION.—The virus transmitted from white birch into cucumber and other herbaceous hosts proved serologically identical to R. W. Fulton's rose isolate of ApMV. The serological identification was confirmed by particle morphology, component analysis, and a limited host-range study.

The birch virus could be distinguished from Fulton's rose isolate of ApMV only on cowpea. However, the differences in height growth of cowpea plants infected with the two isolates, had limited diagnostic value. The line pattern symptoms on

cowpea leaves induced by the birch isolate, but not the rose isolate, appeared only under warm conditions during the summer months. Symptoms of the two isolates were identical on all of the other herbaceous hosts tested. Thus, symptoms produced by the birch virus in herbaceous host plants were consistent with its identification as ApMV.

Serological and infectivity tests demonstrated that the first peak obtained by density-gradient centrifugation contained host protein mixed with a small quantity of noninfectious virus antigen. The remaining three components each contained birch virus antigen but no host protein and were deemed identical to the three components reported by Fulton for the rose isolate of ApMV (8). The three components combined contained birch virus particles and were infectious on cucumber cotyledons. The infectivities of the individual components were not determined.

The identification of ApMV in white birch extends the natural host range of the virus. The similarity of naturally occurring line pattern symptoms in yellow birch to those obtained by bud-inoculations, suggests that ApMV also occurs naturally in yellow birch. Further serological tests will be required to confirm this suspicion as well as to determine the identity of the presumed virus responsible for birch line pattern symptoms commonly observed in northeastern North America and elsewhere.

We have concluded that ApMV causes line pattern of white birch in Wisconsin. Final proof of this assertion will require the reproduction of line pattern symptoms by mechanical inoculations of birch with purified virus preparations. This has not been accomplished. However, since each of the 30 birch trees that showed line pattern symptoms indexed positive for ApMV, it is highly probable that ApMV is the cause of the disorder.

Since remission of line pattern symptoms commonly occurred in birch, an indexing method was needed to detect the virus. Provided that sap from newly emerging birch leaves was used as inoculum, cucumber cotyledons proved a highly efficient indexing host. Every birch tree in which symptoms had appeared was indexed positive by this method. However, serological indexing, either of the birch trees themselves or of cucumber cotyledons successfully inoculated with birch sap, is required for positive identification of ApMV. In other woody plant species, direct serological indexing of crude sap for ApMV has been relatively unsuccessful or dependent upon the availability of flower petals (6). Direct serological indexing of birch trees was enhanced considerably by placing crude leaf sap in antigen wells 6 hr before the antiserum was placed in its well.

The usual remission of all foliage symptoms within 3 years in the continued presence of the virus suggests that undetected ApMV infections of birch may be common. This possibility is supported by the widespread geographic range of apple mosaic and rose mosaic (10), and of birch line pattern, all caused by ApMV. In any case, no longer tenable is the contention that viruses are not involved in birch decline problems because conspicuous foliage symptoms are not consistently associated with them.

TABLE 3. Numbers of bud-inoculated and noninoculated white and yellow birch trees, with and without a history of line pattern symptoms, that indexed positive for apple mosaic virus (ApMV) on cucumbers and by serology

Material	Trees indexed for ApMV	Indexed positive	
		On cucumber	By serology
White birch			
Inoculated trees that -			
Showed line pattern[a]	5	5	5
Remained symptomless	10	2	2
Noninoculated trees[b]	3	0	0
Yellow birch			
Inoculated trees that -			
Showed line pattern[a]	8	8	8
Remained symptomless	4	0	0
Noninoculated trees[b]	5	0	0

[a] Remission of all foliage symptoms occurred prior to indexing.

[b] All of the noninoculated trees remained symptomless.

Infections of birch trees with ApMV may have both direct and indirect effects. Since this virus reduces the yields of apple trees by up to 40% (13), it might also reduce the growth rates of birch trees. More important is the possibility that virus-infected birch trees may be predisposed to severe damage by secondary agents, particularly the bronze birch borer (*Agrilus anxius*). Although the primary predisposing or inciting factor remains a mystery (2), this insect was the most important known biotic factor in the near-total destruction of the commercial birch forests of northeastern North America that occurred primarily during the 1940's. Weakening of yellow birch trees also would expose them to increased damage by shoot and branch cankers developing from latent bark and vascular infections caused by *Diaporthe alleghaniensis* (1). Any widespread infectious agent that weakens birch trees enough to predispose them to successful attack by the bronze birch borer, or to increased canker damage, would have major significance. Inconspicuous infections by viruses, such as ApMV, may be in this category.

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