Dark Treatment of Wheat Inoculated With Soil-Borne Wheat Mosaic and Barley Stripe Mosaic Viruses

A. S. Rao and M. K. Brakke

Postdoctoral Fellow, Department of Plant Pathology, University of Nebraska, Lincoln 68503; and Research Chemist, Crops Research Division, ARS, USDA, Department of Plant Pathology, University of Nebraska, respectively. Present address of senior author: Department of Botany, S. V. University, Tirupati, A. P., India.

Cooperative investigations, Crops Research Division, ARS, USDA, and Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln. Supported in part by Crops Research Division, ARS Contract No. 12-14-100-7790(34).

Journal Series Paper No. 2680. Published with approval of the Director, Nebraska Agricultural Experiment Station.

Accepted for publication 25 November 1969.

ABSTRACT

The percentage of Michigan Amber wheat (*Triticum aestivum*) plants with systemic soil-borne wheat mosaic symptoms was increased by a postinoculation dark treatment, or by removing the leaves and observing new growth. The increase was found in plants grown at 13 C and inoculated in the two-leaf stage by leaf-rubbing or through the roots by the vector, *Polymyxa graminis*. A 6-day dark treatment, starting 6 days after inoculation, was the best of those tried. A similar increase in systemic symptoms was observed after a postinoculation dark treatment of plants inoculated with barley stripe mosaic virus. Phytopathology 60:714-716.

Wheat inoculated through the roots by the vector with soil-borne wheat mosaic virus (SBWMV) develops symptoms in the leaves in a few weeks only if inoculated when very young (3). Older plants become infected and can serve as a source of infectious root washings, but usually do not have leaf symptoms when grown in the greenhouse or growth chamber. Winter wheat in the field normally develops leaf symptoms of SBWMV in the spring, but not on foliage in the fall (9). SBWMV apparently moves slowly out of the roots. There is a tendency for soil-borne viruses to remain localized in the roots (2, 7, 8, 10). This tendency probably is not a unique property of soil-borne viruses, since Fulton (6) showed that many plant viruses move more slowly upward than downward in roots. Schneider (11) and Bennett (1) have discussed this problem and its possible relation to food transport in reviews on virus translocation.

A more rapid development of leaf symptoms of some plant viruses occurs after defoliation or dark treatment of the host (1). We report here an increase in the percentage of wheat plants showing mosaic after inoculation with SBWMV or barley stripe mosaic virus and subsequent defoliation or dark treatment.

Materials and Methods.—The source of SBWMV was described earlier (3). Michigan Amber winter wheat (*Triticum aestivum* L.) was used in most experiments. It was grown at a constant 13 to 17 C with 9 hr/day of fluorescent light at about 1,200 ft-c. For one set of experiments, Michigan Amber wheat and Moore barley (*Hordeum vulgare* L.) were grown in the greenhouse. Plants were grown in a soil: sand mixture in 10-cm pots, usually 15-20 plants/pot (4).

Wheat was inoculated with SBWMV via the vector, *Polymyxa graminis* Led. (5), by pouring root washings into pots with young seedlings or into petri dishes containing seedlings (4). Plants were manually inoculated by rubbing leaves with extracts prepared by grinding leaves infected with SBWMV in a mortar and pestle with a few ml of 0.1 M K_2HPO_4/g of leaves. Celite (Johns Mansville diatomaceous earth) was added as an abrasive.

Type cultures of wheat streak mosaic virus (WSMV, AC 29), brome mosaic virus (BMV, AC66), and barley stripe mosaic virus (BSMV, AC 69) were used in some experiments. Plants were manually inoculated by rubbing leaves with an extract prepared by grinding infected leaves in a mortar and pestle with about 5 ml of water/g of leaves. Celite was added as an abrasive.

Results.—*Plants inoculated with SBWMV through the roots via P. graminis.*—The percentage of plants with leaf symptoms was increased by cutting off the leaves and observing new growth if the seedlings were several days old when inoculated (Table 1). A similar increase was observed in plants given a postinoculation dark treatment. In two experiments, 2-week-old seedlings were pot-inoculated with root washings and kept at 17 C. Plants in half the pots were placed in the dark for 4 days, starting 1 week after inoculation. Six weeks later, there were 38/37 and 69/86 mosaic plants/inoculated plants in the two experiments. None of the plants not placed in the dark (62 and 50 in the two experiments, respectively) showed mosaic. None of 32 and 28 plants, respectively, showed mosaic when inoculated with root washings from healthy plants.

A dark treatment before inoculation with root washings resulted in less infection. In two experiments, no mosaic developed in 44 and 123 plants kept in the dark for 4 days before inoculation with root washings at 2 weeks of age. Among comparable plants not kept in the dark, 7 out of 40 and 7 out of 77, respectively, developed mosaic.

TABLE 1. Number of Michigan Amber wheat plants that developed leaf symptoms in new growth after the first leaves had been cut off. The plants had been inoculated with soil-borne wheat mosaic virus through the roots by the vector in root washings[a]

	Plants with leaf symptoms after inoculation of seedlings at an age of				
	2 days	4 days	6 days	8 days	10 days
Pot inoculation					
30 Days, first leaves	84/118[b]	99/117	57/98	14/113	3/61
60 Days, new leaves	65/94	74/89	42/56	68/113	43/61
Petri dish inoculation					
30 Days, first leaves	30/47	26/56	20/55	8/45	3/47
60 Days, new leaves	30/44	32/56	20/39	19/44	22/38

[a] Seeds were planted or placed in petri dishes every other day to give seedlings of different ages that were inoculated on one time with one inoculum. Plants were kept at 17 C and symptoms were read at 30 days after inoculation. The leaves were then cut off. After an additional 30 days at 17 C, the symptoms were again recorded. Some plants died between 30 and 60 days.

[b] Numerator is number of plants with leaf symptoms; denominator is number inoculated. Combined results of two experiments.

Manually inoculated plants.—The difficulty of obtaining systemic invasion and mosaic symptoms in plants inoculated with root washings can be lessened by inoculating them when they are very young. However, plants to be manually inoculated must be large enough to have leaves that can be rubbed. With manually inoculated plants, dark treatment had little effect on the number of plants showing systemic symptoms of BMV and WSMV, but markedly increased the number of plants showing systemic symptoms of BSMV and SBWMV (Table 2).

Plants were placed in the dark starting at 1 week after inoculation for the experiments reported in Table 2, and were kept in the dark for 2, 4, or 6 days. In a second series of experiments, plants were inoculated with SBWMV and kept in the dark for 4 days, but the time when the dark treatment started was varied. Placing manually inoculated plants in the dark for 4 days beginning at 0, 2, 3, 6, 8, 10, and 12 days after inoculation resulted in 17, 30, 40, 55, 46, 29, and 28%, respectively, of the plants developing mosaic. Inoculated plants not dark-treated had 9% with mosaic, and noninoculated plants remained free of virus symptoms.

The symptoms of BMV, WSMV, and BSMV at 13 C differed from those at higher temperatures. Wheat streak mosaic at 13 C was expressed as pronounced yellow streaks on systemically infected leaves. Symptoms on many of the inoculated leaves were severe, and many of these leaves died. BSMV gave necrotic local lesions and a dark green-yellow systemic mosaic with some yellow eyespots with green centers. Brome mosaic virus (BMV) was initially severe, causing death of leaves and of some plants. Some leaves and stems turned purple and necrotic. Most leaves did not have a typical mosaic. Later, in the chronic phase, plants had an odd growth pattern with short nodes, stiff, short, and upturned leaves, occasionally trapped and curled leaves, and some leaves with wrinkled and serrated edges.

TABLE 2. Number of Michigan Amber wheat plants with systemic symptoms at 13 C when given a dark treatment after manual inoculation of the leaves with a virus

	Plants with systemic leaf symptoms (days in dark[a])			
Virus	0	2	4	6
Brome mosaic	56/81[b]	82/85	85/86	78/78
Wheat streak mosaic	68/77	58/73	92/94	76/78
Barley stripe mosaic	8/87	47/102	48/81	84/103
Soil-borne wheat mosaic	3/61	28/87	31/86	74/93
None	0/90			

[a] Plants were manually inoculated in the two-leaf stage. One week later they were placed in the dark and kept there from 0-6 days. Symptoms were read 6 weeks after inoculation. Plants were at 13 C. Data of two experiments combined.

[b] Numerator is number of plants with systemic symptoms; denominator is number inoculated.

Local symptoms of BMV infection appeared in 8-10 days in manually inoculated plants at 13 C, and those of WSMV and BSMV in about 2 weeks. First appearance of systemic symptoms was variable, but was usually 2-3 weeks for BMV and BSMV, and 3-4 weeks for WSMV and SBWMV.

Michigan Amber wheat and Moore barley were inoculated with BSMV and kept in a greenhouse at about 25 C to find if a postinoculation dark effect occurred at this temperature. In each experiment, plants were inoculated with several 10-fold dilutions. A variety of postinoculation dark treatments was tried. The dark treatments delayed the appearance of symptoms, but the percentage of plants showing symptoms was not significantly changed.

DISCUSSION.—The viruses we studied moved slowly in plants at low temperatures. Tu & Ford (12) reported similar results with maize dwarf and soybean mosaic viruses. Barley stripe mosaic virus and SBWMV moved very slowly, and a postinoculation dark treatment speeded movement of these two viruses. The dark treatment was less effective with WSMV and BMV, possibly because these viruses became systemic in most plants without special treatment.

Previous theories of virus movement would suggest that the dark treatment and removal of leaves made food move from roots to shoots, and that virus was carried along (1). However, the dark treatment might also have had other effects on physiology of the plants that speeded virus movement (11).

The utility of a dark treatment or removal of leaves, to increase systemic invasion of cereal viruses in experiments, is obvious. The slow movement of these viruses at low temperatures must also influence the occurrence and pathogenesis of the diseases they incite in the field.

LITERATURE CITED

1. Bennett, C. W. 1956. Biological relations of plant viruses. Annu. Rev. Plant Physiol. 7:143-170.
2. Bergeson, G. V., K. L. Athow, F. A. Laviolette, & Sister M. Thomasine. 1964. Transmission, movement, and vector relationships of tobacco ringspot virus in soybeans. Phytopathology 54:723-728.
3. Brakke, M. K., A. P. Estes, & M. L. Schuster. 1965. Transmission of soil-borne wheat mosaic virus. Phytopathology 55:79-86.
4. Brakke, M. K., & A. S. Rao. 1967. Maintenance of soil-borne wheat mosaic virus cultures by transfer through root washings. Pl. Dis. Reptr. 51:1005-1008.
5. Estes, A. P., & M. K. Brakke. 1966. Correlation of *Polymyxa graminis* with transmission of soil-borne wheat mosaic virus. Virology 28:772-774.
6. Fulton, R. W. 1941. The behavior of certain viruses in plant roots. Phytopathology 31:575-598.
7. Harrison, B. D. 1957. Studies on the host range, properties, and mode of transmission of beet ringspot virus. Ann. Appl. Biol. 45:462-472.
8. Hewitt, W. B., D. J. Raski, & A. C. Goheen. 1958. Nematode vector of soil-borne fanleaf virus of grapevines. Phytopathology 48:586-595.
9. McKinney, H. H. 1923. Investigations on the rosette disease of wheat and its control. J. Agr. Res. 23:771-800.
10. Price, W. C. 1938. Studies on the virus of tobacco necrosis. Amer. J. Bot. 25:603-612.
11. Schneider, I. R. 1965. Introduction, translocation and distribution of viruses in plants. Advances Virus Res. 11:163-221.
12. Tu, J. C., & R. E. Ford. 1969. Translocation of maize dwarf mosaic and soybean mosaic viruses from inoculated leaves. Phytopathology 59:1158-1163.