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SOME PROPERTIES OF BARLEY STRIPE MOSAIC VIRUS*

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(With Plate 4 and 1 Text-figure)

Barley varieties differed greatly in their reactions to infection with barley stripe mosaic virus (BSMV) obtained from the variety Gloire du Velay growing in Cambridgeshire from seed imported from France. *Lolium multiflorum* Lam., previously considered immune, proved susceptible, as did *Beta vulgaris* L. and *Chenopodium amaranticolor* Coste & Reyn., which gave only local lesions, and *Spinacea oleracea* L. which became systemically infected.

Heating at 65° C. for 10 min. destroyed the infectivity and serological activity of BSMV. Sap was still infective after 32 days at 20° C., but lost its infectivity and half of its serological activity in 3 days at −20° C.; adding sugar to the sap prevented inactivation by freezing. The virus content of inoculated barley leaves increased most rapidly in plants kept at 24–28° C. At 24° C. increase was most rapid during the first 5 days, and the maximum virus concentration was reached about 10 days after inoculation. The highest infection end-point of sap was 1/2048, when the precipitation end-point with virus antisera was 1/256, and sap contained 10^{13} virus particles per ml.: the ratio of infectivity to serological activity and to number of virus particles is much smaller than with tobacco mosaic virus.

The rod-shaped particles are about 20 mμ wide and, in fresh sap, most are between 135 and 175 mμ long. Various treatments, especially heating to 50° C., cause the particles to aggregate linearly. Preparations partially purified by alternate cycles of low- and high-speed centrifugation and by precipitation with ammonium sulphate contain from 0·35 to 0·53 % P, 12·3 to 13·2 % N and 6·2 to 10·2 % carbohydrate. Such preparations are insoluble in water but dissolve in borate buffer at pH 7·5: they inactivate below pH 4·5.

INTRODUCTION

In 1956 symptoms of chlorotic and necrotic spotting and streaking were noticed on leaves of Gloire du Velay barley grown by the National Institute of Agricultural Botany at Cambridge, England, from seed obtained in France. A sample of the original seed was sown in the glasshouse at Rothamsted and about 10 % of the seedlings developed a chlorotic mottle. Sap from diseased plants was rubbed on the leaves of young wheat and barley plants, and in 4–5 days a chlorotic mottle began to appear. The type of symptoms and the seed-borne nature of the infection suggested that the disease might be barley stripe mosaic, originally called barley false stripe until McKinney (1951) showed it to be a seed-borne virus disease. Preliminary serological tests and electron-microscope observations showed that

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the virus from Gloire du Velay was similar to barley stripe mosaic virus (BSMV) isolated from infected Compana barley seed grown at Lethbridge, Alberta, Canada.

Many barley fields were examined in England, mostly in Cambridgeshire and Hertfordshire, without finding any further plants with the disease, but in the spring of 1957 it was found in experimental plots of barley at Versailles, France and Gatersleben, Germany (Slykhuis, 1958).

Our paper records the results we have obtained from studying some of the properties of this virus from Gloire du Velay and its rate of multiplication in barley plants kept in a range of constant temperatures.

Materials and methods

A selection of barley, HB 117/23/2 from the Plant Breeding Institute, Cambridge, proved very susceptible to infection and developed severe symptoms. It was used for propagating and testing the infectivity of BSMV. To inoculate plants, the index finger and thumb were wetted with inoculum, and the leaves were then pulled between them so that both surfaces of the leaves were rubbed for their entire length. The inoculum was sap extracted from barley plants about 2 weeks after inoculation, the time when the virus was at its highest concentration. The infected leaves were reduced to a pulp either with a pestle and mortar or with a household meat mincer, and the sap was extracted through muslin cloth. The sap could be stored at about 5° C. for several weeks with little loss of infectivity.

The virus contents of different preparations of sap were usually compared by infectivity and serological tests, but sometimes the electron microscope was used to count the virus particles, which are readily recognised (Gold, Suneson, Houston & Oswald, 1954). Infectivity was estimated by inoculating groups of about ten barley seedlings with a series of dilutions and finding what number became systemically infected.

Serological assays were made by finding the precipitation end-point with virus antiserum. Two antisera were prepared by injecting rabbits with virus that was partially purified as follows; infective sap was clarified by heating at 50° C. for 10 min. and centrifuging at 8000 *g* for 10 min.; the virus was precipitated by adding 2 vol. of saturated solution of ammonium sulphate to 3 vol. of clarified sap, and then centrifuged down. The precipitate was resuspended in a volume of distilled water equal to 1/3 the original volume of sap, and freed from insoluble material by centrifugation before it was injected into the rabbits. Each rabbit received six intravenous injections of 3–5 ml. of the virus preparation at intervals of 2–3 days. The rabbits were bled 10 days after the last injection and the antisera were stored at 5° C. with 0·5 % phenol. Both antisera had precipitation titres of 1/640. For most precipitation tests the antisera were diluted 1 to 50 in 0·85 % NaCl solution. The sap for serological tests was clarified by heating to 50° C. for 10 min. and centrifuging at 8000 *g* for 10 min. 1 ml. of the diluted antiserum to BSMV was added to 1 ml. of the heat-clarified sap diluted in 0·85 % NaCl, the dilutions varying by a factor of 2. The dilutions were made in tubes of 7 mm. diameter,

which were placed with their fluid columns half immersed in a thermostatically controlled water-bath held at 40° C. Floccules typical of a flagellar-type precipitate appeared in 5 min. to 2 hr. depending on the quantity of virus present. A final record was taken at the end of 3 hr.

The technique for counting the virus particles with the electron microscope was as described by Nixon & Fisher (1958). The sap was prepared for electron microscopy either by centrifuging and diluting with distilled water, or by centrifuging, dialysing against ammonium carbonate M/10 brought to pH 7·4 by adding HCl, then centrifuging and diluting.

RESULTS

Host range

Barley is the most common host of BSMV, but wheat is also susceptible (McKinney, 1951), and the yield of both cereals can be seriously decreased by infection (Hagborg, 1954), but different barley varieties differ considerably in the extent to which they are affected (Timian & Sisler, 1955). Some cultivated and wild grasses and the perennial grass *Bromus inermis* Leyss. have been reported susceptible to BSMV (Slykhuis, 1952; McKinney, 1953).

TABLE 1. *Reaction of twenty barley varieties to barley stripe mosaic virus*

Severity of chlorotic symptoms	Variety
Severe on 95–100 % of the plants	HB 117/23/2
	HB 169/5/22/6
Moderate on 40–75 % of the plants	Freja
	Ankara Tokak
	Domen
	Earl
	Kenia
	Provost
Mild to faint on 10–30 % of the plants	C 205
	B 2145
	Carlsberg
	Pioneer
	C 177
	Proctor
	B 2038
	C 174
Faint symptoms on less than 10 % of the plants	Rika
	Sprat Archer
	Maythorpe
	Plumage Archer

Twenty to twenty-five plants of each of twenty varieties of barley,* one variety of wheat and several other species of grass in the two- to three-leaf stage were inoculated in the Rothamsted glasshouse with the Gloire du Velay isolate of BSMV. In barley the percentage of plants that developed symptoms, and the severity of the chlorotic mottle, differed considerably with the different varieties (Table 1). Two unnamed varieties, HB 117/23/2 and HB 169/5/22/6, were very intolerant

* The barley varieties were obtained from Mr D. H. B. Sparrow, Plant Breeding Institute, Cambridge.

and nearly all the infected plants showed severe chlorotic symptoms (Pl. 4, fig. 1). Fewer plants in the other varieties developed symptoms, and the severity of the symptoms varied from moderate to very faint. Two varieties, Maythorpe and Plumage Archer, did not develop symptoms in these tests, but when later inoculated with isolates of BSMV from Canada a few plants developed faint symptoms and the virus was recovered from them. The wheat variety Cappelle des Prés was readily infected and showed moderate symptoms. Italian rye grass (*Lolium multiflorum* Lam.), not previously recorded as a host, became infected.

Among the dicotyledonous plants, McKinney (1953) has reported that tobacco plants, variety Samsum, developed local lesions after inoculation with BSMV. This variety did not become infected with the isolate of BSMV from Gloire du Velay barley, but sugar-beet plants (*Beta vulgaris* L. var. Kleinwanzleben E.) and *Chenopodium amaranticolor* Coste & Reyn. developed chlorotic local lesions 1 week after inoculation. Young *C. amaranticolor* plants produced numerous lesions when the inoculum contained 'Celite' and this species can be used for infectivity assays. In one test to find how infectivity is affected when sap from infected barley is diluted, the average numbers of lesions per *C. amaranticolor* leaf were 55, 82, 37, 12 and 2, respectively, with sap undiluted and at dilutions of 1/5, 1/25, 1/125 and 1/625. The most interesting new host for BSMV was summer spinach (*Spinacea oleracea* L. var. 'Round Summer'), because no other dicotyledonous plant is known to become systemically infected. In the summer, infected plants showed a bright yellow mottle, sometimes in the form of rings and oak-leaf patterns (Pl. 4, fig. 2). Because spinach sap contains a powerful inhibitor of infection, BSMV could be transmitted from spinach to barley only after the virus was separated from the inhibitor by ultracentrifugation. That sap from the spinach plants contained BSMV was also demonstrated directly by serological tests, in which specific precipitates were obtained up to a dilution of 1/16. Spinach plants did not become infected with the isolate of BSMV obtained from the Canadian barley variety Compana.

Size of the virus particles

Gold *et al.* (1954) reported that the BSMV particles had rods approximately 30 mμ wide and 130 mμ long. At Rothamsted, H. L. Nixon found that the Canadian and the Gloire du Velay isolate both had particles of the same size, about 20 mμ wide and, in freshly expressed sap, mostly from 135–175 mμ long. The lengths in partially purified virus preparations varied greatly, presumably depending on whether the treatments fractured or aggregated particles. Heating sap at 50° C. for 10 min. caused considerable aggregation, and one aggregate in a heated preparation consisted of approximately forty particles attached end to end (Pl. 4, fig. 3). This degree of heating usually increased the precipitate end-point with antisera.

Heat inactivation

The effect of heat on the infectivity and serological activity of BSMV from Gloire du Velay was tested in six experiments, in which sap from infected barley was held for 10 min. at temperatures between 50–70° C. Temperatures up to 55° C. did not

affect infectivity, and in four out of the six experiments the sap remained highly infective at 60° C. Only once was there any infectivity at 65° C., and inactivation was always complete at 70° C. These results agree with Hagborg's (1955) for a Canadian strain of BSMV. Changes in serological activity closely paralleled these in infectivity; temperatures of up to 60° C. had no effect on the precipitation end-point and at 65° C. or higher sap ceased to react with virus antisera.

Inactivation by freezing

Sap from diseased barley retained some infectivity after 32 days at 20° C. and lost infectivity still more slowly at 5° C. Sap frozen at −15 to −20° C., however, lost much infectivity in a day and most or all in 3–4 days. By contrast, freezing leaves had very little effect on the infectivity of the virus. For example, in one test the leaves from twenty diseased barley plants were cut into ½ in. pieces, the pieces were mixed and the mixture divided into three equal portions. One portion, and the sap expressed from another, were stored in sealed tubes at −20° C. The sap from the third portion was immediately tested for its serological activity and infectivity, and those kept at −20° C. were similarly tested 3 days later. Table 2 shows that the virus in the frozen sap was almost all inactivated, whereas sap from

TABLE 2. *Effects of freezing expressed sap and leaves on the serological activity and infectivity*

	Precipitation end point	No. of plants infected with sap diluted at				
		1/1	1/4	1/16	1/64	1/256
Fresh sap	1/128	8/8*	9/9	9/9	12/12	8/10
Sap stored 3 days at −20° C.	1/64	2/11	3/10	3/10	0/10	0/10
Leaves stored 3 days at −20° C. then sap expressed	1/64	11/11	9/9	12/12	11/11	3/11

*Numerator indicates number of plants infected and denominator plants inoculated.

the frozen leaves was as infective as the fresh sap, and that the loss of infectivity was not accompanied by a comparable decrease in serological activity. This behaviour contrasts with that described by Bawden & Pirie (1950) with Rothamsted tobacco necrosis virus, which was inactivated by freezing in leaves but not in expressed sap.

There seems to be no previous record of a virus becoming inactivated by freezing sap: indeed, keeping sap at −20° C. is the accepted method for storing many viruses. Bawden & Pirie (1943) found that purified salt-free preparations of tomato bushy stunt virus are denatured by freezing, but that this is prevented by the presence of 1·5 % glucose or pseudoglobulin. As Table 3 shows, adding sucrose or glycerol also preserved, partly or wholly, the infectivity of barley sap kept at −20° C. for 3 days.

Effects of environment on multiplication and virus content

McKinney (1953) reported the dilution end-point of BSMV in sap from infected wheat leaves to be more than 1/10,000. We found that the virus concentration in sap from barley varied with the age of the plants, the elapsed time after infection,

and the environmental conditions to which the plants were exposed after infection. Sap extracted 15 days after inoculation from young plants grown at 20° to 24° C. (Table 4) gave the highest infection end-point, 1/2048, but not all the plants inoculated with sap diluted as little as 1/256 became infected (Table 4). This sample of sap also gave a precipitation end-point with virus antiserum of 1/256, and counts made with the electron microscope showed that 1 ml. of sap contained 10^{13} virus particles. The ratio of the infection end-point to either the precipitation end-point or to the number of virus particles per volume of sap is very small compared with

TABLE 3. *The protective effect of different materials against inactivation by freezing*

	No. of plants infected by sap diluted at		
Preparation frozen	1/4	1/16	1/64
Undiluted sap	0/11*	0/11	0/12
1 ml. sap + 1 ml. distilled water	0/12	0/11	0/10
1 ml. sap + 1 ml. 12 % sucrose	10/11	11/11	5/10
1 ml. sap + 1 ml. 6 % sucrose	11/11	10/10	7/11
1 ml. sap + 1 ml. 3 % sucrose	8/8	7/11	7/12
1 ml. sap + 1 ml. 0·2 % gelatine	1/10	1/11	0/11
1 ml. sap + 1 ml. 2 % glycerol	5/11	4/9	6/10
Unfrozen control—sap at 5°C.	10/11	11/11	11/11

* Numerator indicates number of plants infected and denominator plants inoculated.

TABLE 4. *Virus concentration in barley plants 15 days after inoculation at* 24° C.

		No. of plants infected with sap diluted at						
Source of sap	Precipitation end-point	1/16	1/64	1/256	1/572	1/1024	1/2048	1/4096
Inoculated leaves	1/256	11/11*	11/11	4/9	4/11	0/11	1/12	0/9
Systemically infected leaves	1/128	11/11	10/10	6/11	4/9	0/9	1/10	0/8

* Numerator indicates number of plants infected and denominator plants inoculated.

that of tobacco mosaic virus, with which sap has an infection end-point of more than 10^{-6}, the precipitation end-point is over 1/1000 and the highest recorded number of virus particles per ml. of tobacco sap is about 10^{14} (Steere, 1952). As the ratios of serological activities and numbers of particles are similar for the two viruses, it seems that either a much smaller proportion of BSMV particles in sap is infective or that many more infective particles are needed to start an infection by BSMV than by tobacco mosaic virus. BSMV in this respect resembles broad-bean mottle virus, which has an infection end-point only a little higher than the precipitation end-point. (Bawden, Chaudhuri & Kassanis, 1951).

During periods of cloudy weather the symptoms on barley were faint and often indefinite. To see whether light intensity affects symptom development and virus concentration, barley plants were inoculated in the three-leaf stage and divided into three lots, one of which was covered with a muslin tent, a second with a heavy

brown paper tent and the third left unshaded in the same glasshouse. On a sunny day, when the light intensity in the glasshouse was about 4000 foot candles, the light intensity under the muslin was 400 foot candles and under the heavy brown paper it was about 40 foot candles. The tents were well ventilated so that the temperatures differed no more than 0·5° C. At 6 and 11 days after inoculation the concentration of the virus was estimated serologically, and Table 5 shows that both the concentration of virus and severity of symptoms decreased as the light intensity was lowered.

TABLE 5. *Effects of shading on symptoms and virus concentration*

	Precipitation end-point			
	6 days	11 days		
Shading	Inoculated leaves	Inoculated leaves	Systemically infected leaves	Symptoms 11 days
None	1/8	1/64	1/128	Moderate
Moderate	1/8	1/32	1/64	Mild to moderate
Heavy	0	1/8	1/32	Faint

Preliminary tests showed that the concentration of BSMV was a function of the air temperature, and experiments were made to determine the relation more precisely by keeping infected plants at constant temperatures between 16–36° C. The plants were kept in thermostatically controlled glass chambers in a cool glasshouse, and the temperature did not vary more than ±0·5° C. from the stated value. To avoid any possibility of the different temperatures affecting the number of the initial infections, the plants were not exposed to the constant temperature until 1 day after they were inoculated. The concentration of virus in the inoculated leaves was estimated serologically. In the first tests, leaf samples were taken at 2, 4, 8 and 16 days, but no virus could be detected serologically at any of the temperatures in the 2-day sample, and at the higher temperatures the older leaves became senescent after 10 days. In later experiments, therefore, samples were taken only between the 4th and 10th day after inoculation. Table 6 gives the results of two such experiments, which together cover the entire range of temperatures. No symptoms developed, and there was no serological evidence of virus multiplication in plants kept at 36° C., but such plants were infected, for when returned to ordinary glasshouse conditions after 2 weeks at 36° C. they later developed symptoms. Symptoms appeared sooner and virus concentration increased more rapidly in plants at 24° and 28° C. than at higher or lower temperatures. In plants kept at 20° C. the initial rate of virus increase was slow, but after 10 days the virus concentration was nearly as high as at 24° and 28° C.

For a more detailed study on the rate of increase of BSMV in the inoculated leaves of barley, the temperature of 24° C. was selected because at this temperature the plants appeared more vigorous and showed more pronounced symptoms than at 28° C. Samples were taken daily for the first 5 days, and on the 7th, 9th and 15th day after the inoculation. The virus concentration in the samples was estimated

serologically by infectivity tests and by counting particles with the electron microscope. The results (Table 7 and Text-fig. 1) show that most of the virus in the inoculated leaves was produced in the first 5 days. The sensitivity of the three methods for detecting the early stages of virus increase differed; electron microscopy showed the presence of 4×10^9 virus particles the first day after the inoculation (Text-fig. 1), whereas virus was not detected by infectivity and precipitation tests until the 2nd and 3rd days, respectively. The only other attempt to use these three methods to detect early stages of virus increase is with tobacco mosaic virus, with which the infectivity test was much more sensitive than the other two (Nixon, unpublished). Again, this difference may mean that many BSMV particles are not infective, or that more particles of BSMV than of tobacco mosaic virus are needed to infect.

TABLE 6. *Effects of temperature on symptoms and virus concentration in barley plants at different intervals after inoculation with barley stripe mosaic virus*

Temperatures (°C)	4 days		8 days		5 days		10 days	
	Virus	Symptoms	Virus	Symptoms	Virus	Symptoms	Virus	Symptoms
36	0	0	0	0	—	—	—	—
32	0	0	1/8	0	—	—	—	—
28	1/16*	Faint	1/128	Moderate	1/64	0	1/256	Moderate
24	1/64	Faint	1/256	Moderate	1/64	Faint	1/256	Severe
20	—	—	—	—	1/2	0	1/128	Moderate
16	—	—	—	—	0	0	1/4	Mild

* Virus content indicated by serological precipitation end-point of the sap.

Attempts at purification

Heating infective sap for 10 min. at 50° C. does not decrease the infectivity or serological activity of BSMV, and is useful in purifying the virus because it coagulates much other material that can then be removed by 10 min. centrifugation at 8000 *g*. The clear, light-brown, supernatant fluid obtained is 40% saturated with ammonium sulphate, the resultant precipitate sedimented by 10 min. centrifugation at 8000 *g*, when it is resuspended in a volume of pH 7·5 borate buffer (8·75 vol. of 0·2 M boric acid: 1·25 vol. of 0·05 M borax) equal to about 1/3 of the original volume of sap. A second precipitation with ammonium sulphate does not improve the virus preparation and increases the chances of the virus becoming insoluble. In its tendency to become insoluble during purification BSMV resembles potato virus *X*, but unlike virus *X* this does not seem to happen because particles aggregate (Pl. 4, fig. 4) (Kleczkowski & Nixon, 1950). Precipitates insoluble in water dissolve in borate buffer at pH 7·5, but to prevent the virus becoming insoluble the precipitates were always suspended in borate buffer.

Attempts to remove contaminating materials by acidifying heat-clarified sap were unsuccessful, because BSMV inactivated at pH values lower than 4·5.

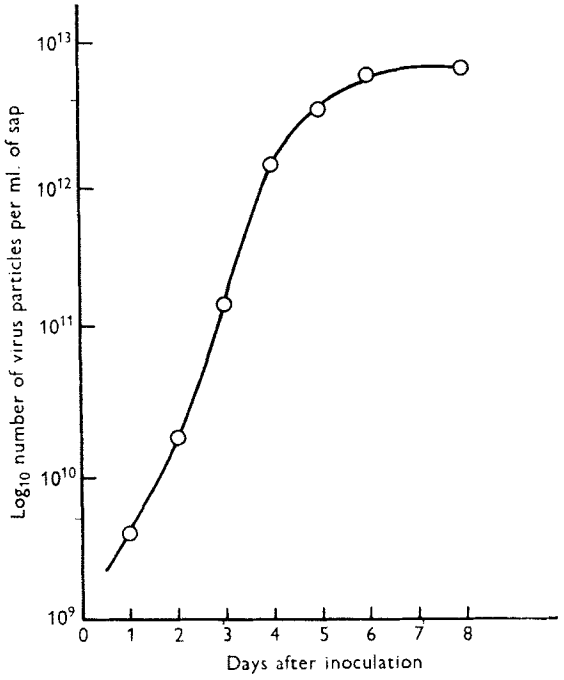
The best preparations of BSMV were made by alternate cycles of high- and low-speed centrifugation. The virus was sedimented from heat-clarified sap at 50,000 *g* and the pellet was suspended in a volume of borate buffer at pH 7·5 equal to 1/10

the original volume of sap. To extract the maximum amount of virus, the suspended pellet was left overnight at 5° C., before being centrifuged at 8000 *g*. The supernatant fluid was strongly opalescent but only faintly coloured and showed streaming birefringence. A second ultracentrifugation removed some more contaminating materials, but a third had little or no extra effect. Preparations at concentrations of 0·8% or over became gelatinous when dialysed against distilled water, but redissolved in borate buffer.

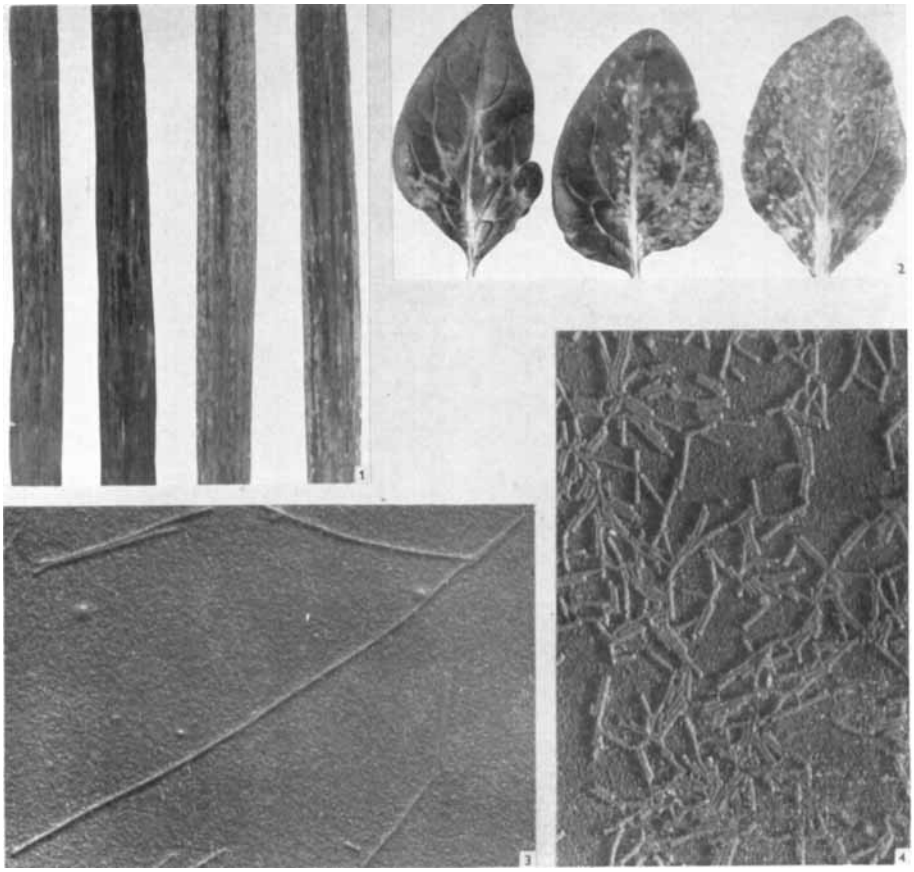
The contents of P, N and carbohydrate in different preparations varied from 0·35 to 0·53%, 12·3 to 13·2% and 6·2 to 10·2%, respectively. The variations suggest that the preparations contained varying amounts of a contaminant rich in carbo-

TABLE 7. *Serological activity and infectivity of sap from inoculated leaves* of *barley plants after various intervals at* 24° C.

Days after inoculation	Serological precipitation end point	Number of plants infected at each of a series of sap dilutions									
		1/1	1/2	1/8	1/32	1/64	1/256	1/512	1/1024	1/2048	1/4096
0	0	0/11	—	—	—	—	—	—	—	—	—
1	0	0/11	—	—	—	—	—	—	—	—	—
2	0	5/10	10/10	—	—	—	—	—	—	—	—
3	1/4	9/9	10/10	12/12	7/9	1/11	—	—	—	—	—
4	1/32	—	9/10	8/9	9/9	8/10	—	—	—	—	—
5	1/64	—	—	12/12	8/11	9/12	3/11	0/10	—	—	—
7	1/128	—	—	12/12	11/11	9/10	3/11	1/9	0/10	—	—
9	1/256	—	—	—	11/11	9/10	3/10	0/7	1/11	1/11	—
15	1/256	—	—	—	—	11/11	4/9	4/11	0/11	1/12	0/9



Text-fig. 1. Particle increment curve for BSMV in inoculated leaves of barley plants kept at 24° C.



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hydrate. Combining precipitation by ammonium sulphate with ultracentrifugation gave no extra fractionation; nor did it give preparations with a more constant composition. Purified preparations had little infectivity and it seems that much virus lost infectivity during separation. To quote one result with a preparation containing 0·5 % P, of ten barley plants inoculated with solutions containing 0·13, 0·026 and 0·005 mg/ml. of the preparation, the numbers infected were 5, 1 and 0, respectively.

We are indebted to H. L. Nixon and R. D. Woods for particle counts and electron-micrographs.

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EXPLANATION OF PLATE 4

Fig. 1. Leaves of barley plants infected with BSMV; the two on the left with a few chlorotic but mainly necrotic streaks are inoculated leaves, and the two on the right with mainly chlorotic streaks are systemically infected leaves.

Fig. 2. Spinach leaves infected with BSMV showing a bright yellow mottle and an oak-leaf pattern.

Fig. 3. Electron micrograph of heat-clarified sap from infected barley showing aggregated virus particles, × 23,000

Fig. 4. Electron micrograph of a BSMV preparation which became insoluble after dialysis, × 56,000.

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