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# BARLEY STRIPE MOSAIC VIRUS ON CAPE BARLEY IN QUEENSLAND

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SUMMARY

**Barley stripe mosaic virus was found to occur widely in Queensland, but to be confined to Cape, a relatively unimportant fodder variety. Most of the seed stocks of this long-established variety were found to be infected at the 1–5% level. Virus was purified using charcoal, an agarose column and sucrose rate zonal centrifugation in conjunction with differential centrifugation. The top virus band in rate zonal separations was composed of three distinct but closely associated minor bands. An antiserum was produced and used in infection testing of young seedlings.**

## I. INTRODUCTION

Barley stripe mosaic virus (BSMV) has previously been intercepted in quarantine plantings of barley (*Hordeum vulgare* L.) in Australia (Slykhuis 1962; Simmonds 1966). However, its widespread occurrence in the field was not previously known. During 1969, a sample of suspect Cape barley (a long-established 6-row variety) was forwarded by Mr. G. J. P. McCarthy (Queensland Department of Primary Industries, Kingaroy) for virus identification. About the same time a specimen was also received from the Darling Downs. Confirmation that these specimens were infected by BSMV led to the present study being initiated.

Considerable published work is available on the biological characteristics of this disease (McKinney and Greeley 1965) and on properties of the virus itself (Brakke 1959, 1962; Kassanis and Slykhuis 1959; Harrison, Nixon and Woods 1965, Timian and Savage 1966).

The virus particles are short thick rods (Gold *et al.* 1954; Kassanis and Slykhuis 1959; Gibbs *et al.* 1963) which are rather easily fractured and have a marked tendency for aggregation (Brakke 1959). It is seed and pollen borne (Gold *et al.* 1954; Crowley 1959; Inouye 1962; Hockett and Davis 1970; Timian 1970) and is also easily transmitted mechanically. The host range extends to many species in Gramineae and to some dicotyledonous species (McKinney 1951; Kassanis and Slykhuis 1959; Sing, Arny and Pound 1960; Inouye 1962).

The field importance of the disease seems to be variable, but plantings with a high level of infection have greatly reduced yields (Eslick 1953; Inouye 1962).

## II. MATERIALS AND METHODS

*Sources of isolates and seed samples.*—Isolations from barley and wheat specimens from the Darling Downs and South Burnett districts of Queensland were made during 1969 and 1970. Barley seed samples were obtained from farmers, seed merchants and Department of Primary Industries stocks during the same period. Because infection of the variety Cape was discovered early, approximately half of the total number of seed samples examined were of this variety, although it constitutes a fairly small proportion (approximately 8%) of barley grown in Queensland.

*Glasshouse methods.*—Vegetative samples were mechanically indexed by grinding with water or 0·1M, pH 7·0 phosphate buffer and then inoculating by finger onto carborundum-dusted seedlings of the barley varieties Clipper and Black Hulless. Seedlings for indexing were grown in steam-sterilised peat-sand-nutrient potting mixture, in an insect-screened glasshouse.

Samples from seed lots to be used for growing indexing test plants were sown and tested extensively to ensure virus freedom. Only one seed lot of each of the two varieties was used for glasshouse work.

Seed infection tests were carried out by planting samples, each of approximately 100 seeds, in wooden flats containing peat-sand potting mixture. Seedlings were inspected individually, without being handled, except to remove suspect specimens for sap inoculation tests. One pot of healthy seedlings was used for each plant tested.

*Virus purification and serology.*—Clipper barley planted in flats and manually inoculated at the 1-leaf and 2-leaf stages, provided the tissue source for virus purification. An isolate from Cape barley was used in the inoculations. Leaves were harvested approximately 1 week later, when inoculated leaves showed some necrosis and systemic symptoms were prominent.

The leaf tissue was macerated in a large cold mortar with 0.1M, pH 7 phosphate buffer at the rate of 1 ml per gram of tissue. Activated charcoal to 12% of the tissue weight was then incorporated and the mixture macerated further. All purification steps were assayed to pots of barley at 1:100 dilution. Species of *Chenopodium* were also used for this purpose.

A low-speed centrifugation at 6500 *g* for 10 min removed most of the charcoal and much of the pigment. The supernatant was then pelleted in the Spinco 30 rotor for 90 min at 30,000 r.p.m. These pellets were resuspended in 0·02M pH 7 phosphate buffer and given another low-speed centrifugation. Three millilitres of this low-speed supernatant were then injected into an upward flow, 2·5 x 40 cm agarose (Sepharose 2B) column and fractions collected for 24 hr. The residual green pigment came off the column early and virus was collected immediately after these green fractions until the main ribosome fractions began to come through. A Beckman DB spectrophotometer was used to check the fractions. After passage through the column the virus was found to be dispersed in 70 ml of buffer and was concentrated again by a further cycle of differential centrifugation.

One millilitre of the resulting virus suspension was then layered onto sucrose density gradient tubes containing 4, 7, 7 and 8 ml of 10, 20, 30 and 40% w/v sucrose solutions. The three major virus zones resulting from 2 hours' centrifuging at 25,000 r.p.m. in the Spinco 25·1 rotor were removed separately and combined again after infectivity assay and UV absorption tests. After a further

cycle of differential centrifugation the virus was used for intramuscular injection into a rabbit to prepare antiserum. Three injections of 1 ml + 1 ml Freund's adjuvant were given at 10-day intervals and ear bleedings begun 1 week later. After adding 0·01% sodium azide, serum was stored either frozen or 1 : 1 with glycerol at −20°C.

Serological tests were carried out in 9cm plastic petri dishes using either gel diffusion (Crowle 1961) or drop precipitin methods (van Slogteren 1954). The gel diffusion medium was prepared from buffered 0·85% saline with 0·75% Ionagar No 2 and 0·01% sodium azide. Wells were cut with a 5 mm cork borer.

Some of the virus seed-transmission tests were assessed by germination and crushing emerging seedlings in small mortars so that sap from these could be placed individually in eight peripheral wells around a central antiserum well. Three such patterns (24 plants) could be accommodated in one 9 cm plate.

*Electron microscopy.*—Cut-leaf dip preparations (Brandes 1964) were prepared with 1% potassium phosphotungstate on cellulose nitrate coated grids and examined in a Philips EM 300 electron microscope.

## III. RESULTS

*Early identifications and glasshouse testing.*—Examination and testing of specimens was begun in the spring of 1969. The first infected sample came from an experimental plot of Cape barley being grown by the Department of Primary Industries at Kingaroy. It showed typical symptoms of BSMV (Figure 1), which were reproduced by mechanical transmission to test plants in the glasshouse. Electron microscopy of leaf dip preparations confirmed the diagnosis by showing the characteristic short thick rods (Figure 2) with a tendency to fracture and aggregate.

It was soon evident from germination tests that commercial Cape barley samples carried the virus to the extent of a few seeds per 100 and some of the malting varieties were then tested. A line of Clipper barley was inspected in the field and seed collected and tested extensively by germination. When no virus was found by these procedures, the line was used for subsequent indexing and purification work.

As the 1969 season progressed, it became very difficult to locate likely virus specimens in the field because of the many other causes of leaf abnormalities in maturing plants. Consequently, work was diverted to seed infection tests, which could be conveniently carried out during the off-season.

Most Cape barley seed samples examined carried the virus. Only 3 of 28 bulk lines failed to produce some virus infected seedlings. Sometimes the symptoms on the young seedlings were very mild—a single short indistinct stripe—but indexing this to a pot of Clipper seedlings readily confirmed the diagnosis.

Seed samples of 18 other varieties of barley were tested, including 21 samples of the commonly grown malting varieties, Prior and Clipper. No infection was found in these lines.

*Field observations.*—During examination of barley plantings in parts of south-eastern Queensland in 1970, all the plantings of Cape barley that were inspected were found to contain some plants showing symptoms of BSMV, from

which the virus was isolated. Indeed it was possible to identify plantings of this variety at the early seedling stage by looking for BSMV infection. No diseased plants were found in other varieties.

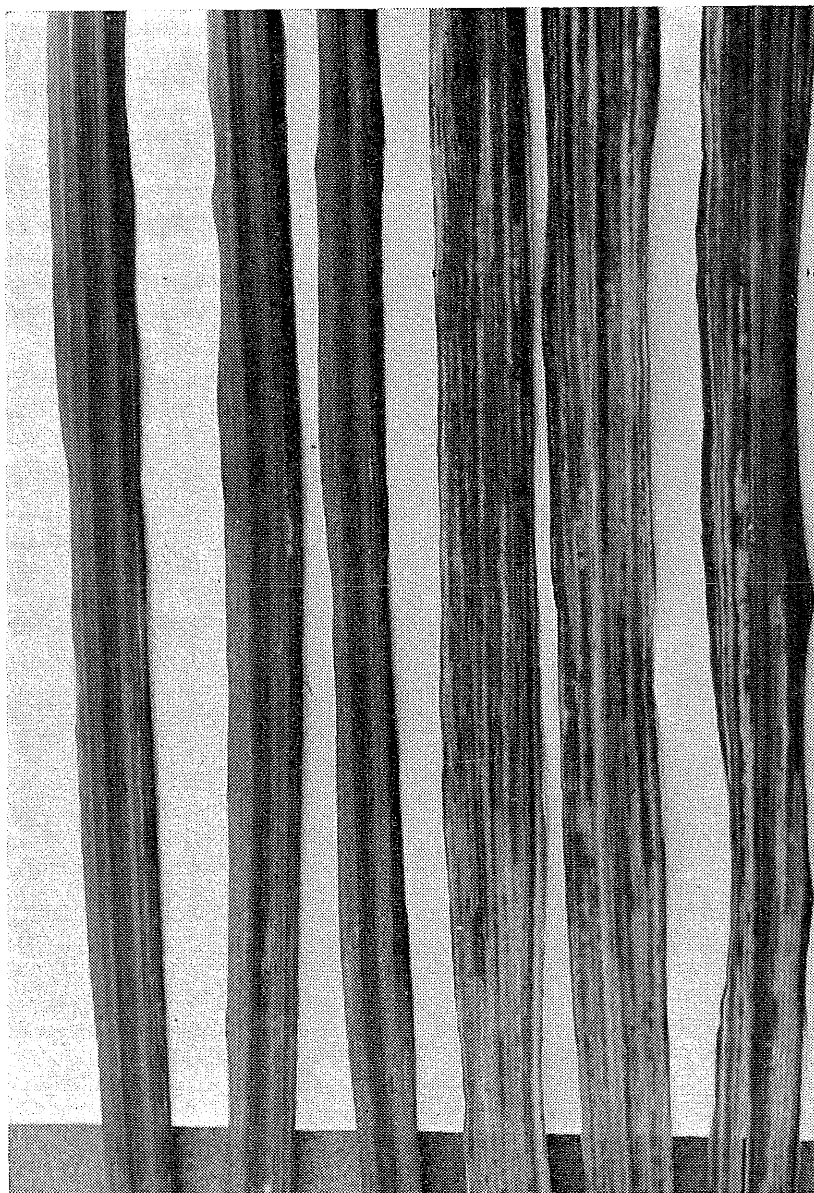

Fig. 1.—Barley leaves showing symptoms of mild and severe strains of barley stripe mosaic virus isolated from Cape barley.

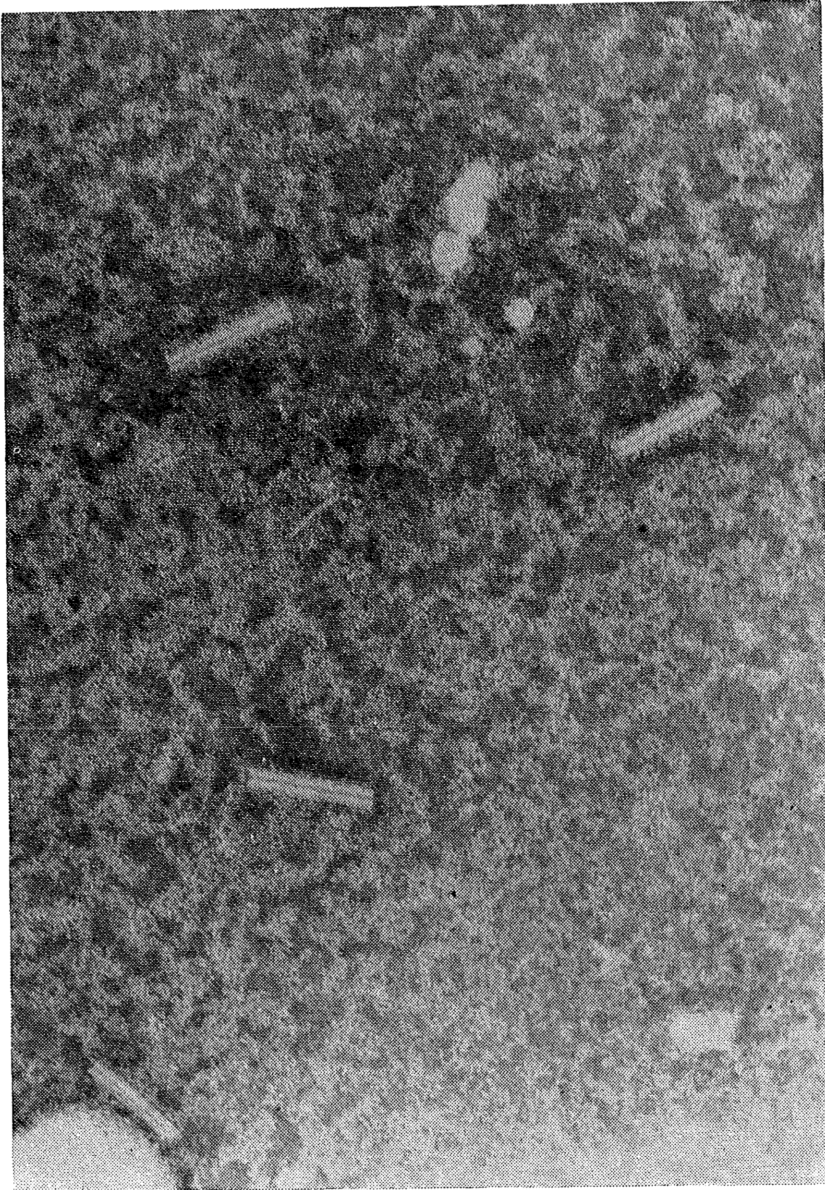

Fig. 2.—Electron micrograph of leaf dip preparation from Cape barley, showing short thick barley stripe mosaic virus rods.

In addition to the typical stripe symptoms, which became more difficult to detect in the field at maturity, it was found that infected plants usually had a short seed stalk carrying less grain in the head. Using the latter symptoms as a quick guide, the location of diseased plants in crops approaching maturity is simplified.

*Percentage infection.*—Neither in the field nor in seed infection tests was the rate of infection in Cape barley found to exceed 5%, except when non-commercial procedures (specially tended seed plots) had been followed. One such seed plot produced 42% of infected seed, as determined by germination followed by visual and serological assessment of 130 seedlings.

In most seed germination tests only 100 seeds of each line were planted and very low percentages could conceivably be missed. However, in the three instances where the first test of Cape barley seed produced no infected plants, repeated plantings totalling 300–400 seeds still showed no infection. Where infection was detected, the rate varied from 1 to 5% but was usually 1 to 2%.

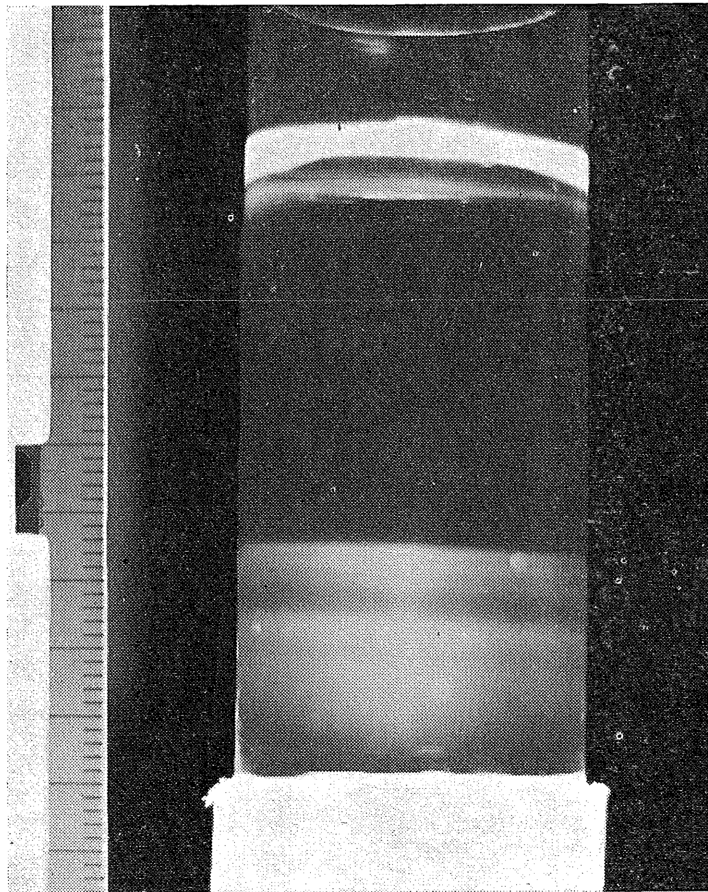

Fig. 3.—Rate zonal centrifugation of purified preparation of barley stripe mosaic virus, showing "triplet" top zone, dense narrow middle zone and bottom zone with diffuse lower edge.

*Seed transmission from inoculated plants.*—Seed of the Clipper variety harvested from glasshouse-grown plants which had been inoculated as seedlings was tested by the germination and serological indexing method. Of a total of 80 seeds 25 were found to be infected.

*Purification and serology.*—BSMV was purified by the method described. Charcoal clarification did not appear to reduce infectivity, as assessed from the assay test plants. However, some virus was evidently lost in each step involving a low-speed centrifugation.

The agarose column effectively separated the pigmented fraction from the virus and from the ribosome fraction. The green pigment had a high UV absorption but no observable A260–A240 "dip". The ribosome fraction also had high UV absorption, but with a conspicuous nucleoprotein "dip".

After rate zonal centrifugation in sucrose solution columns, the virus appeared as three major bands in the lower half of the tube (Figure 3). All were infective and had similar UV absorption profiles. They were grouped together for high-speed pelleting. The lower band had a diffuse lower edge and presumably represented a non-uniform aggregated particle mixture. No ribosome material was visible in the upper part of the tube. The peculiar triplet appearance of the upper virus band was not explained and the components were too close together to be effectively separated by the means available at the time.

The antiserum prepared by the method described proved to be very satisfactory for identification of infected crude sap by the gel diffusion method. Only faint host lines were produced and the virus line was dense and well defined, although rather close to the antigen well, as expected from the virus morphology.

## IV. DISCUSSION

The field occurrence of BSMV in Queensland appears to be largely or entirely confined to the variety Cape. This variety has been established here for over 40 years. It is not grown extensively and is used for fodder and grazing purposes. Because this is an old variety and the infection is so widely associated with it, a stable infection ecology seems likely.

Seed transmission experiments confirmed that the rate of seed transmission in Cape is higher than in the most popular malting variety grown in Queensland. The rate of spread in the field due to leaf contact and pollen transmission was not determined. Yield reduction in infected plants is inhibitory to infection increase because healthy plants will tend to provide more of the next season's seed. In any stabilized infection situation there must be a balance of contributory factors. In this case these factors include the positive contributions of seed, pollen and contact transmission and the negative effect of yield reduction.

Since the disease in Queensland appears to be varietally restricted, it should not be difficult to provide unfected seed of this variety and promote its use. Similar schemes have proved effective elsewhere (McKinney and Greeley 1965).

The purification method used here was rather long, but since the antiserum produced was mainly for use with crude sap preparations, an antigen preparation free of host components was desirable. The agarose column and density gradient steps probably resulted in less aggregation loss than many alternative methods. Heat clarification of infected sap at 40°C resulted in very poor yields from the

isolate used here. Considerable losses are inherent in a multi-step procedure such as the one employed, but crude infected material is relatively easy to produce for BSMV.

The sedimentation pattern produced by rate zonal centrifugation was not entirely explained, although all bands contained infective virus. The pattern resembled that obtained by Brakke (1960) with some of the procedures he employed. However, the complex upper band shown in the present study could be further investigated.

## V. ACKNOWLEDGEMENTS

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