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Abiotic Transmission of Southern Bean Mosaic Virus in Soil

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Abstract

When soil in pots was infested with southern bean mosaic virus (SBMV), it became infective for highly susceptible *Phaseolus vulgaris* bait plants. Soil could be infested by adding either infected plant tops or roots or by growing infected plants in it. Release of SBMV by roots of *P. vulgaris* cv. Red Kidney reached a maximum at flowering and thereafter declined, but some virus was still recovered in drainage water when the plants were dead. When grown in infested soil *P. vulgaris* cv. Bountiful was more susceptible to infection at 26°C than at 21 or 32°C, temperatures which were less favourable for plant growth. The addition to virus-infested soil of tomato roots carrying nematodes (*Meloidogyne* sp.), fungi and other soil biota did not increase virus infection in bait plants. It is concluded that SBMV resembles tobacco mosaic virus in being able to infect plant roots abiotically in infested soil.

Introduction

When virus-infected plants are harvested or die, large amounts of root and top debris remain as a potential source of virus for release into the soil (Zeyen 1979). Furthermore, living infected plants may sometimes release large amounts of virus from their roots into the surrounding soil, and this virus can be conveniently assayed from soil leachate (Yarwood 1960; Smith *et al.* 1969; Hollings *et al.* 1977). Except in the case of tobacco mosaic virus strains (Broadbent 1965), such virus released from living roots and plant debris formerly was considered to play no part in plant infection, unless transmitted by a specific fungal or nematode vector (Cadman 1963; Grogan and Campbell 1966; Smith *et al.* 1969). This conclusion was restated recently by Zeyen (1979) as follows: 'The inability of viruses to enter root tissues, in the absence of wounds or root penetrating vectors has been demonstrated repeatedly ..... Undamaged susceptible roots may quite literally be bathed in solutions containing high concentrations of infectious virus and remain uninfected.'

The occasional transmission in soil of sugarcane mosaic virus to *Sorghum bicolor* (L.) Moench (Bond and Pirone 1970), and the frequent transmission in soil of cymbidium ringspot virus to *Nicotiana clevelandii* Gray (Hollings *et al.* 1977), of galinsoga mosaic virus to *Galinsoga parviflora* Cav. (Shukla *et al.* 1979), and of tomato bushy stunt virus to *Celosia argentea* L. (Kleinhempel and Kegler 1982) in the apparent absence of virus vectors indicated that abiotic transmission of plant viruses in soil might not be such a rare phenomenon.

Southern bean mosaic virus (SBMV) was selected as a candidate to test for abiotic transmission in soil. It is the type virus of the Sobemovirus group and is transmitted

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by infected seed (Zaumeyer and Harter 1943) and by beetles of the genus *Ceratoma* (Walters 1964). The virus was shown to be released in the drainage water from systemically infected bean (*Phaseolus vulgaris* L.) plants, but healthy bean seedlings were not infected when drainage water containing this virus was poured around their roots (Smith *et al.* 1969). However, when bean seed was allowed to imbibe moisture for 12 h near the roots of SBMV-infected bean roots, 50–85% of the resultant seedlings became infected (Teakle and Morris 1981). Therefore SBMV was tested for its ability to be transmitted from soil-borne debris under glasshouse conditions. Tests were also carried out to determine if this virus might be transmitted between the roots of infected and healthy plants growing together, as has been shown in the case of potato virus X (Roberts 1948).

Materials and Methods

The virus used was an isolate of the bean strain of SBMV originally obtained from R. J. Shepherd, University of Kentucky, Lexington. Before its use in transmission tests it was subjected to three single local lesion transfers in Pinto bean. The virus was propagated in Bountiful or Red Kidney bean and assayed in Pinto bean.

Unless otherwise stated all plants were grown in autoclaved U.C. mix IIc potting soil (Matkin and Chandler 1957) and were watered daily with a dilute nutrient solution.

Plants were usually grown at fluctuating temperatures in the range of 18–26°C, but in the winter months Pinto assay plants were grown at a constant temperature of 26°C.

To eliminate surface contamination with SBMV, the roots to be assayed were washed, dipped in 10% (w/v) trisodium phosphate (TSP) for 1 min and then rinsed in running tap water. Tests with roots contaminated by dipping in an extract of SBMV-infected leaves showed that the TSP treatment was 99–100% effective. Plants were accepted as being infected only when assay plants had five or more local lesions per leaf or half-leaf. Infected plants usually yielded 100 or more local lesions from assay of either roots or tops.

Cross-contamination between pots was prevented by careful watering, by separating pots on the bench, by avoiding handling the pot, and by placing each pot inside another empty pot of equal diameter so that any roots projecting through the drain hole were not damaged or allowed to contact the bench top.

Results

Effect of Growing Plants in Soil Containing Infected Leaf Material

Leaf inoculum was mixed with potting mix at the rate of 1% (w/v), the contaminated soil mix was then sown with bean or cowpea seed, and approximately

Table 1. Effect of growing plants in soil containing SBMV-infected leaf material

Chopped, infected French bean leaves were added to potting mix at the rate of 8 g per 12·5-cm pot

Test plant	Proportion of test plants with: Infected roots[A]	Infected foliage[A]
Bountiful bean	4/5	2/5
Red Kidney bean	2/5	1/5
Pinto bean	0/5	0/5
Blackeye cowpea	0/5	0/5

[A] The numerator is the number of plants with infected roots or foliage and the denominator is the total number of plants. No infection was detected in a similar number of plants grown in uninoculated potting mix.

3 weeks later the roots and leaves were indexed separately. The results (Table 1) showed that Bountiful and Red Kidney bean, which are systemic hosts of SBMV, were sometimes infected, but that the local lesion host, Pinto bean, and the insusceptible Blackeye cowpea were not infected. Control plants grown without soil infestation with SBMV were also not infected.

Effect of Inoculum Dosage and Temperature on Root Infection of Beans Growing in SBMV-contaminated Soil

The roots and soil from pots of systemically infected Bountiful bean plants were thoroughly mixed and then diluted with autoclaved U.C. mix to give 50, 10 and 4% of 'soil inoculum'. The soil was placed in 15-cm diameter clay pots, seeded with 12 Bountiful beans, and the pots placed in the glasshouse at either 21, 26 or 32°C. Uninoculated U.C. mix was similarly seeded and incubated.

One month after seeding, the roots of the plants were assayed for SBMV. Incidence of infection was highest with the highest inoculum dosage (50%), and also was highest at the intermediate temperature (26°C) where plant growth was best (Table 2).

Table 2. Effect of inoculum dosage and temperature on root infection of Bountiful bean plants growing in soil containing SBMV-infested roots

The inoculum was the roots and soil from pots of systemically infected Bountiful bean plants

Infested soil in potting mix (%)	Proportion of plants with infected roots when incubated at:		
	21°C	26°C	32°C
50	1/12	7/11	5/11
10	0/12	5/12	0/11
4	0/12	0/12	0/11
0	0/11	0/10	0/11

Root Infection of Transplants

Some of the infection reported above (Tables 1 and 2) could have resulted from infection of the germinating seed, as has been shown for SBMV and bean seed (Teakle and Morris 1981). To determine if SBMV could infect roots of older seedlings directly, SBMV-containing roots and soil were placed at the bottom of a pot and were covered by a $1\frac{1}{2}$-cm layer of autoclaved soil. Healthy Red Kidney bean seedlings at the primary leaf stage were then transplanted into further autoclaved soil above these soil layers.

When assayed about three weeks later, two out of 10 test plants were infected in both roots and tops with SBMV, whereas none out of four control plants growing in non-infested soil were infected. In a similar test in which a 3-cm layer of autoclaved soil was interposed between the infested soil and the transplants, two out of six Bountiful bean were infected systemically and an additional two bean plants were infected in the roots only, whereas six control plants remained healthy.

Release of Virus in Soil Leachate from Systemically Infected Plants

When soil leachate from pairs of Red Kidney bean plants infected with SBMV was assayed, the amount of virus detected increased rapidly until flowering time

and then decreased gradually until death of the plants. A small amount of virus was recovered even 30 days after the death of all plants (Fig. 1).

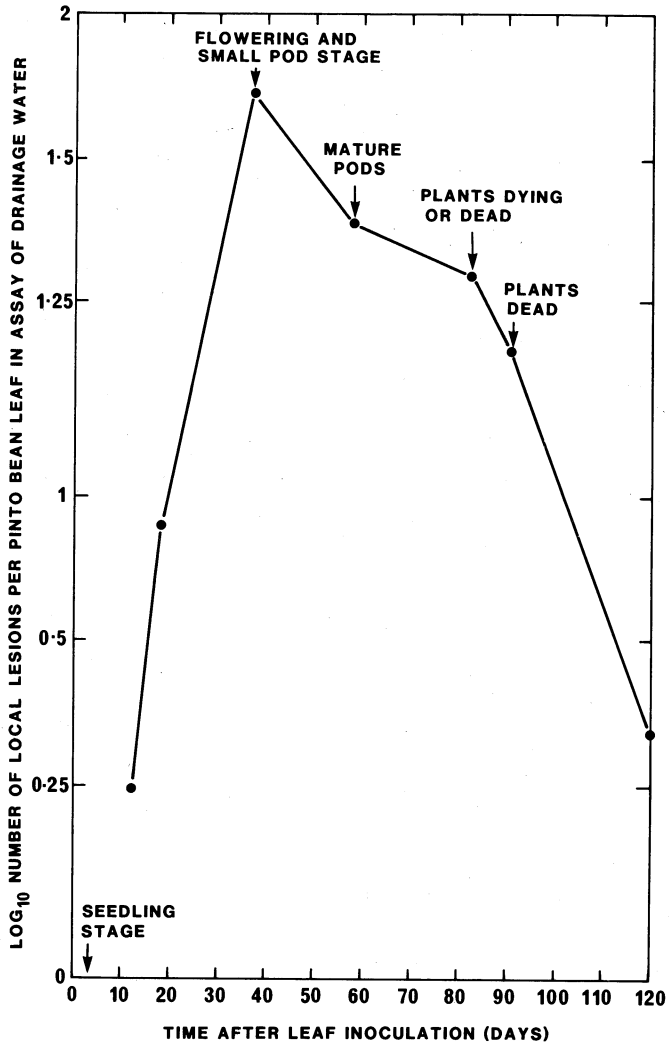

Fig. 1. Release of SBMV in drainage water from pairs of Red Kidney beans inoculated at the primary leaf stage.

Transmission by Growing Healthy and SBMV-infected Bean Plants in the Same Soil but with the Tops Separated

In one test pairs of Bountiful bean plants growing on one side of two 15-cm diameter pots were leaf-inoculated with SBMV when at the primary leaf stage. Sixteen days later the other side of the pots was seeded with two Red Kidney beans. The two sides of the pots were separated by erecting a plastic screen which penetrated 1–2 cm into the soil. When the Red Kidney bean plants were assayed 33 days after seeding, half of the plants had infected roots in each of the two similarly

treated pots. No root infection was detected in Red Kidney test plants growing in a third pot with uninoculated Bountiful bean plants.

In a second test, pairs of Bountiful bean plants in two pots were leaf-inoculated with SBMV and 5 weeks later the pots with growing, infected plants were baited by planting seeds of Bountiful bean. When the bait plants derived from the seed were indexed from the two pots 3 weeks later, two out of nine and one out of nine plants respectively had systemic infection, and an additional plant in the first pot had root infection only.

Effect of Soil Biota, Including Meloidogyne *sp., on SBMV Transmission in Soil Containing Infected Root Material*

Since nematodes and other organisms can wound roots, it was decided to determine if incorporation of heavily galled tomato roots in virus-infested soil would increase their infectivity. In one experiment the roots and associated soil from pots of SBMV-infected bean plants were mixed with autoclaved potting mix, and one pot of the virus-infested soil was supplemented with root knot nematode-infested roots from field-grown tomato plants. Another pot was similarly treated, except that the tomato roots containing soil biota were autoclaved before use. Other control pots had tomato roots or autoclaved tomato roots without virus-infested soil. Pots were seeded with bean and later the bean plants were assayed for virus. Shortly after assaying the pots were replanted with fresh bean seeds until a total of four crops had been grown in the same soil.

Table 3. Effect of soil biota on the soil transmission of SBMV to Red Kidney or Bountiful bean

Soil inoculum[A]	Proportion of plants[B] with systemic infection in:			
	Original planting	First replant	Second replant	Third replant
SBMV + biota	3/4[C]	0/4[D]	2/9	3/10
SBMV + autoclaved biota	2/5	6/10	5/8	5/7
Biota only	0/5	0/2	0/7	0/4
Autoclaved biota only	0/5	0/9	0/10	0/9

[A] The SBMV inoculum comprised roots and soil from pots of systemically infected bean plants mixed with autoclaved soil to a final concentration of 17% (v/v). The soil biota comprised roots of field-grown tomato plants heavily infected with root knot nematodes (*Meloidogyne* sp.) at a concentration of 1·7% (w/v).

[B] The numerator is the number of plants with systemic infection, and the denominator is the total number of plants which grew.

[C] The tops and a sample of the roots were harvested and assayed separately about 3 weeks after each seeding. The pots were kept moist and re-seeded with intervals between the four plantings of 1, 4 and 2 days, respectively.

[D] One of the four plants had root infection with SBMV.

The results (Table 3) showed that there was little effect of the soil biota on incidence of infection. Some infection was maintained in each crop as long as virus was present in the soil.

Discussion

In this work it is unlikely that one of the presently recognized soil-borne vectors was involved. Although *Olpidium brassicae* is a common glasshouse inhabitant,

precautions were taken to exclude this fungus from most of the experiments described, and when roots or root washings were examined, neither sporangia nor zoospores were observed. Further, *O. brassicae* has previously failed to transmit SBMV (Teakle and Gold 1963). It seems most likely that in the experiments described here, the virus infected the plants abiotically, perhaps through natural wounds.

In relation to this hypothesis it is pertinent to mention that Roberts and Price (1967) found that apparently uninjured leaves of French bean could be infected by dipping them in, or spraying them with, suspensions of SBMV. Apparently a similar situation exists with regard to infection of uninjured germinating seeds or seedling roots by SBMV (Teakle and Morris 1981). Further, the amount of infection was not increased by adding soil biota, including root knot nematodes associated with field-grown tomato roots (Table 3).

As a result of this work it is suggested that abiotic transmission of stable plant viruses in soil is probably a much more common phenomenon than previously realized. Presumably it has not often been recognized because it cannot easily be distinguished in the field from transmissions by other methods and because it has rarely been looked for. Also, it might be more important in natural situations, where healthy seedlings are growing near older, virus-infected plants, than in a cropping situation when growth of all plants is roughly synchronous.

A number of conditions might be expected to increase soil transmission by abiotic means. These include:

(1) *Release of a large amount of free virus in the soil.* This depends on the presence of heavily infected virus source plants, which release large amounts of virus from their roots, e.g. Red Kidney or Bountiful bean infected by SBMV (Table 2; Fig. 1). Amount of virus released is related to the vigour and size of the plant.

(2) *Highly susceptible bait plants.* Baiting with a highly susceptible systemic host provides a better chance for detecting a small amount of infection than using a local lesion host.

(3) *Suitable environment for infection.* In this work 26°C, which gave best growth of bean, was better than 21 or 32°C for infection of SBMV (Table 2). Moisture might have an effect in many ways, e.g. in bringing virus to a natural wound or taking it away, in affecting the susceptibility of the host plant through the number and type of infection sites, and in affecting the survival of the virus (Smith *et al.* 1969).

(4) *Stability of the virus.* Only highly stable viruses, such as SBMV, would be expected to survive well in soil. SBMV was shown to survive for 4 months when sap was air dried on cotton wool (D. S. Teakle, unpublished data). No doubt soil conditions, such as moisture, temperature, and microbial activity, affect soil survival; and

(5) *A continuing supply of susceptible plants.* A long delay between crops would be expected to decrease abiotic transmission. In this work soil infectivity was maintained for at least 2 months when the interval between three replant bean crops was 1–4 days only (Table 3).

These data support the concept that when stable viruses are released from living roots or debris in moderate to large amounts, they may cause plant infection abiotically. Therefore, care should be exercised when replanting soil which has grown a virus-infected crop that the soil has become virus free or is treated so that its infectivity is eliminated.

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References

Bond, W. P., and Pirone, T. P. (1970). Evidence for soil transmission of sugarcane mosaic virus. *Phytopathology* **60**, 437–40.

Broadbent, L. (1965). The epidemiology of tomato mosaic. 8. Virus infection through tomato roots. *Ann. Appl. Biol.* **55**, 57–66.

Cadman, C. H. (1963). Biology of soil-borne viruses. *Annu. Rev. Phytopathol.* **1**, 143–72.

Grogan, R. G., and Campbell, R. N. (1966). Fungi as vectors and hosts of viruses. *Annu. Rev. Phytopathol.* **4**, 29–52.

Hollings, M., Stone, O. M., and Barton, R. J. (1977). Pathology, soil transmission and characterization of cymbidium ringspot, a virus from cymbidium orchids and white clover (*Trifolium repens*). *Ann. Appl. Biol.* **85**, 233–48.

Kleinhempel, H., and Kegler, G. (1982). Transmission of tomato bushy stunt virus without vectors. *Acta Phytopathol. Acad. Sci. Hung.* **17**, 17–21.

Matkin, O. A., and Chandler, P. A. (1957). The U.C.-type soil mixes. In 'The U.C. System for Producing Healthy Container-grown Plants'. (Ed. K. F. Baker.) pp. 65–85. [Calif. Agric. Exp. Stn. Ext. Serv. Manual 23.]

Roberts, D. A., and Price, W. C. (1967). Infection of apparently uninjured leaves of bean by the viruses of tobacco necrosis and southern bean mosaic. *Virology* **33**, 542–5.

Roberts, F. M. (1948). Experiments on the spread of potato virus X between plants in contact. *Ann. Appl. Biol.* **35**, 266–78.

Shukla, D. D., Shanks, G. J., Teakle, D. S., and Behncken, G. M. (1979). Mechanical transmission of galinsoga mosaic virus in soil. *Aust. J. Biol. Sci.* **32**, 267–76.

Smith, P. R., Campbell, R. N., and Fry, P. R. (1969). Root discharge and soil survival of viruses. *Phytopathology* **59**, 1678–87.

Teakle, D. S., and Gold, A. H. (1963). Further studies of Olpidium as a vector of tobacco necrosis virus. *Virology* **19**, 310–15.

Teakle, D. S., and Morris, T. J. (1981). Transmission of southern bean mosaic virus from soil to bean seeds. *Plant Disease* **65**, 599–600.

Walters, H. J. (1964). Transmission of southern bean mosaic virus by the bean leaf beetle. *Plant Dis. Reptr.* **48**, 935.

Yarwood, C. E. (1960). Release and preservation of virus by roots. *Phytopathology* **50**, 111–14.

Zaumeyer, W. J., and Harter, L. L. (1943). Two new virus diseases of beans. *J. Agric. Res.* **67**, 305–28.

Zeyen, R. J. (1979). Viruses. In 'Ecology of Root Pathogens'. (Eds S. V. Krupa and Y. R. Dommergues.) pp. 179–205. (Elsevier: Amsterdam.)

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