Surface Contamination of Pollen by Plant Viruses

R. I. Hamilton, E. Leung, and C. Nichols

Research Scientist and Technicians, respectively, Research Station, Agriculture Canada, 6660 N. W. Marine Drive, Vancouver, B. C., Canada V6T 1X2.

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ABSTRACT

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Electron microscopic examination of anthers from plants infected with bromegrass mosaic virus (BMV), southern bean mosaic virus (SBMV), or tobacco moasic virus (TMV) showed that the exine of mature pollen was infested with virions. No evidence was obtained for infection of pollen by these viruses. Homogenates of pollen (SBMV-infested) or dry pollen (TMV-infested) were infectious when assayed on appropriate hosts. The implication of these findings in the epidemiology of certain mechanically transmissible viruses is discussed.

Additional key words: pollen transmission of viruses.

Several virus diseases of plants have been reported to be transmissible via pollen to the egg cells of healthy female plants (8) and in some cases to the female plant itself (2, 7). The underlying assumption is that the male gametes are infected with the pollen-transmissible virus and, indeed, this has been shown in pollen from barley infected with barley stripe mosaic virus (1) and in pollen from soybean infected with tobacco ringspot virus (9). While examining anthers of common bean infected with southern bean mosaic virus and tobacco mosaic virus, we noted that the pollen exine was often coated with masses of virions. The results of these observations and their possible significance in the epidemiology of plant viruses are communicated here. A preliminary report was published earlier (5).

MATERIALS AND METHODS

Viruses.—Southern bean mosaic virus (SBMV) originally was derived from AC 17 (American Type Culture Collection) and tobacco mosaic virus from cowpea (TMV-L) was obtained from T. J. Morris, Department of Plant Pathology, University of California, Berkeley. An isolate of common tobacco mosaic virus (TMV-C) and bromegrass mosaic virus (BMV) were obtained from stocks maintained at the Vancouver Research Station.

Host plants.—Bean (*Phaseolus vulgaris* 'Bountiful') and cowpea (*Vigna unguiculata* 'Ramshorn') were inoculated with SBMV or TMV-L at the primary leaf stage. Isolate TMV-C was inoculated to tobacco *(Nicotiana tabacum* 'Haranova') at the five-leaf stage. Barley (*Hordeum vulgare* 'Atlas') was inoculated with BMV at the first-leaf stage (10 days after seeding). All experimental plants were maintained in a greenhouse at 25 C under a photoperiod of 16 hr. Infectivity assays to determine the presence of these viruses in plant parts was by manual inoculation to Pinto bean (SBMV), *Chenopodium amaranticolor* (TMV-C and TMV-L), and Black Hulless barley (BMV).

Examination of anthers.—Anthers at or near dehiscence were removed from healthy and infected plants and processed for electron microscopy. They were fixed for 1 hr in 5% glutaraldehyde in 0.1 M potassium phosphate, pH 7.2, then washed for 15 min, twice in phosphate buffer, and finally postfixed in 1% OsO_4 in Palade's buffer. Dehydration was for 15 min in 70%, 95%, and 100% ethanol, and in propylene oxide; three incubations in 100% ethanol and in propylene oxide were done to effect complete dehydration. The tissues were infiltrated with Epon 812 overnight in a mixture of 50% Epon and 50% propylene oxide, embedded in pure Epon, and cured at 60 C. Sections were cut with a diamond knife on a Reichert OMU-2 ultramicrotome, stained with 5% uranyl acetate (in 50% methanol) for 20 min, and stained with Reynold's lead citrate for 10 min. They were examined with a Philips EM 200 or EM 300 electron microscope.

RESULTS

A typical section from an anther of healthy bean showing a mature pollen grain lying near the anther wall is shown in Fig. 1. A higher magnification of an area of the same pollen grain (Fig. 2) shows the prominent bacula in the exine. A similar section from an anther of SBMV-infected bean shows deposits of SBMV in the space between the exine of mature pollen and the cells of the anther wall (Fig. 3). An infected cell in the anther wall near the thecal cavity is shown in Fig. 4; it contains

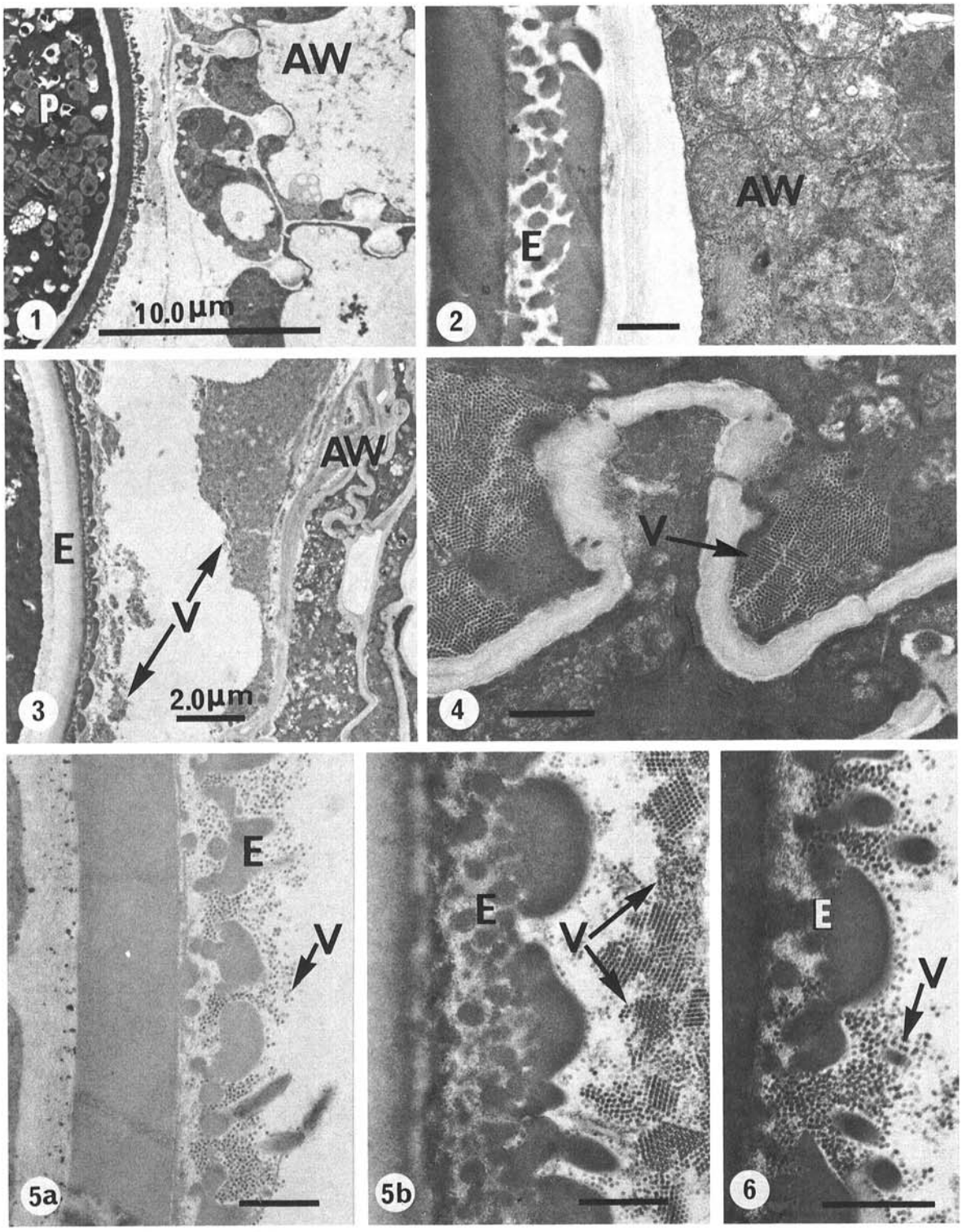

Fig. 1-6. Electron micrographs of anthers of *Phaseolus vulgaris* 'Bountiful'. **1)** Mature pollen (P) in noninfected anther lying adjacent to cells of the anther wall (AW); **2)** Higher magnification of a noninfected anther showing exine (E) of mature pollen; **3)** Mature pollen in anther infected with SBMV showing large masses of virions (V) lying between exine and cells of the anther wall; **4)** Crystalline masses of SBMV (V) in an infected anther cell near the thecal cavity; **5-A, B)** Virions of SBMV (V) in the bacula of the exine (5-A) and coating the surface of the exine (5-B); and **6)** Virions of SBMV (V) on the exine of pollen which had been deposited on a glass slide and air-dried. Bars represent 0.5 μm unless otherwise indicated.

crystalline masses of SBMV. Virons can be seen within the bacula of the exine (Fig. 5-A) or near the surface of the exine in large numbers (Fig. 5-B). A similar association of SBMV virions with the exine of mature pollen which had been collected from anthers by dusting onto a glass slide is shown in Fig. 6. Virus-infested pollen was observed in 18 of 30 anthers (60%) from 10 flowers.

Cells of the anther wall in TMV-infected bean also contained TMV aggregates (Fig. 7-A, 7-B) and the exine of some pollen grains was infested with TMV virions (Fig. 8). Similar masses of virions were seen on the exine of mature pollen from TMV-infected cowpea (Fig. 9, 10). In both hosts, about 40% of the anthers contained infested pollen. Although virions of TMV-C could be detected in anther cells of TMV-infected tobacco (Fig. 11-A, 11-B) it was difficult to detect them on the exine. A group of such virions can be seen between the pollen coating and the exine (Fig. 11-C).

Pollen from BMV-infected barley also showed extensive infestation of the exine although only one of eight anthers contained infested pollen.

No evidence was obtained by electron microscopy for the infection of pollen grains by any of the viruses studied.

Infectivity was associated with extracts of pollen from

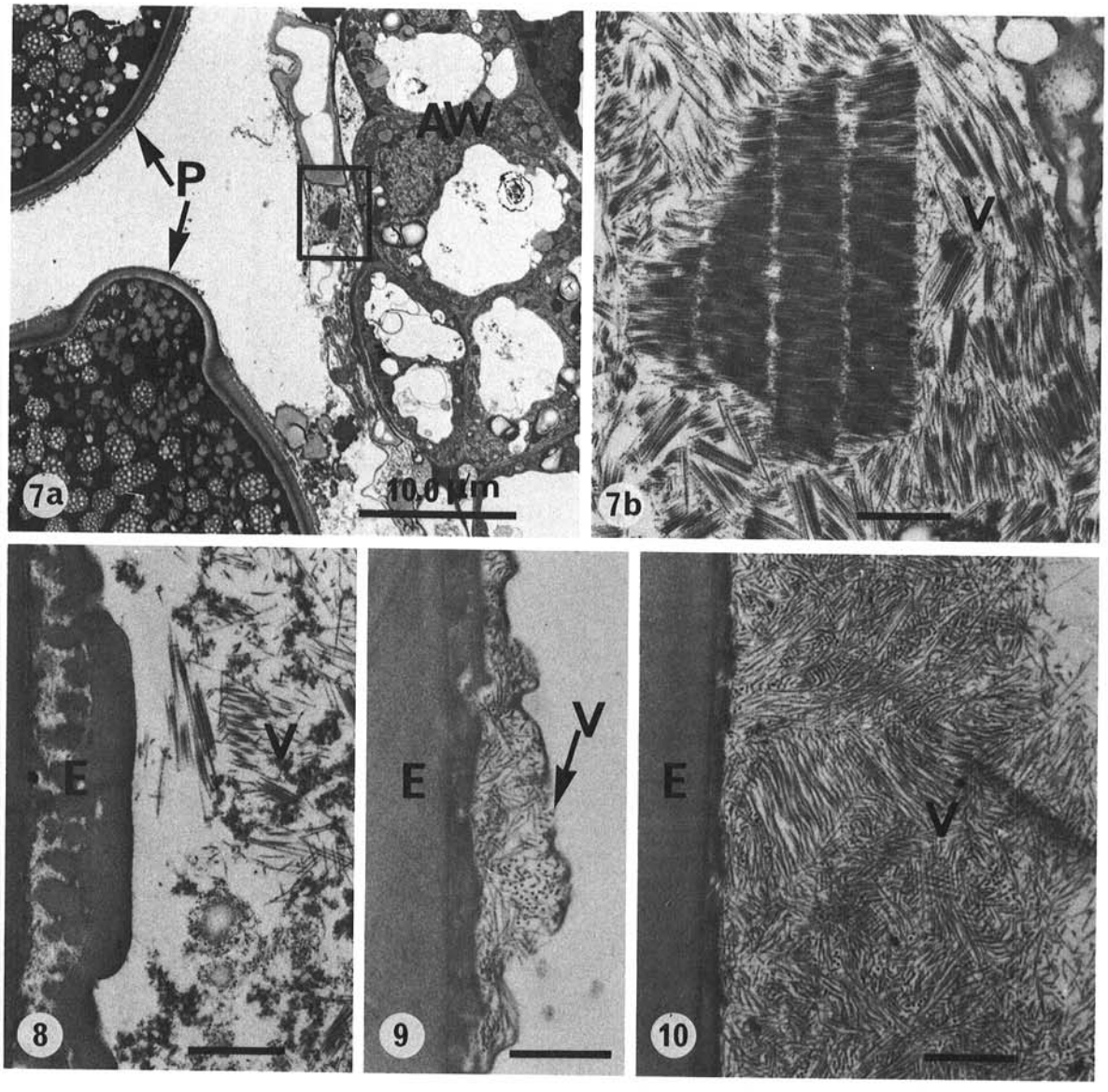

Fig. 7-10. Electron micrographs of anthers of *Phaseolus vulgaris* 'Bountiful' infected with TMV-L; **7-A)** Section of anther showing two mature pollen grains lying adjacent to infected anther wall cell; **7-B)** Higher magnification of anther wall cell in 7-A showing masses of TMV (V); **8)** Virions of TMV (V) adjacent to pollen exine. Bars represent 0.5 µm unless otherwise indicated. **9-10)** Electron micrographs of virions of TMV (V) infesting surface of exine in anthers of infected *Vigna unguiculata* 'Ramshorn'. Bars represent 0.5 µm unless otherwise indicated.

SBMV-infected bean. Pollen from the anthers of individual flowers was dusted onto a glass microscope slide and homogenized with another slide, using 0.01 M potassium phosphate, pH 7.5 and 1% Celite as the homogenizing medium; such extracts induced about 20 lesions per leaf when assayed on Pinto bean in several experiments. Infective TMV was also associated with pollen from TMV-infected tobacco, but inoculation results were not always positive. An average of 10 lesions per leaf was induced on Xanthi-nc tobacco when pollen grains were dusted onto the leaf surface and the leaf then rubbed with a Q-tip previously soaked in 0.01 M potassium phosphate, pH 7.5; no lesions were induced with the same inoculum in two replicates applied with a dry Q-tip. Appropriate controls for all inoculations (pollen from healthy bean and tobacco, and buffer) always were negative.

DISCUSSION

The results of these experiments show that contamination of the pollen exine with virions is a consequence of the development of the pollen grain in some virus-infected plants. The fact that pollen dusted from SBMV-infected anthers which had dehisced could

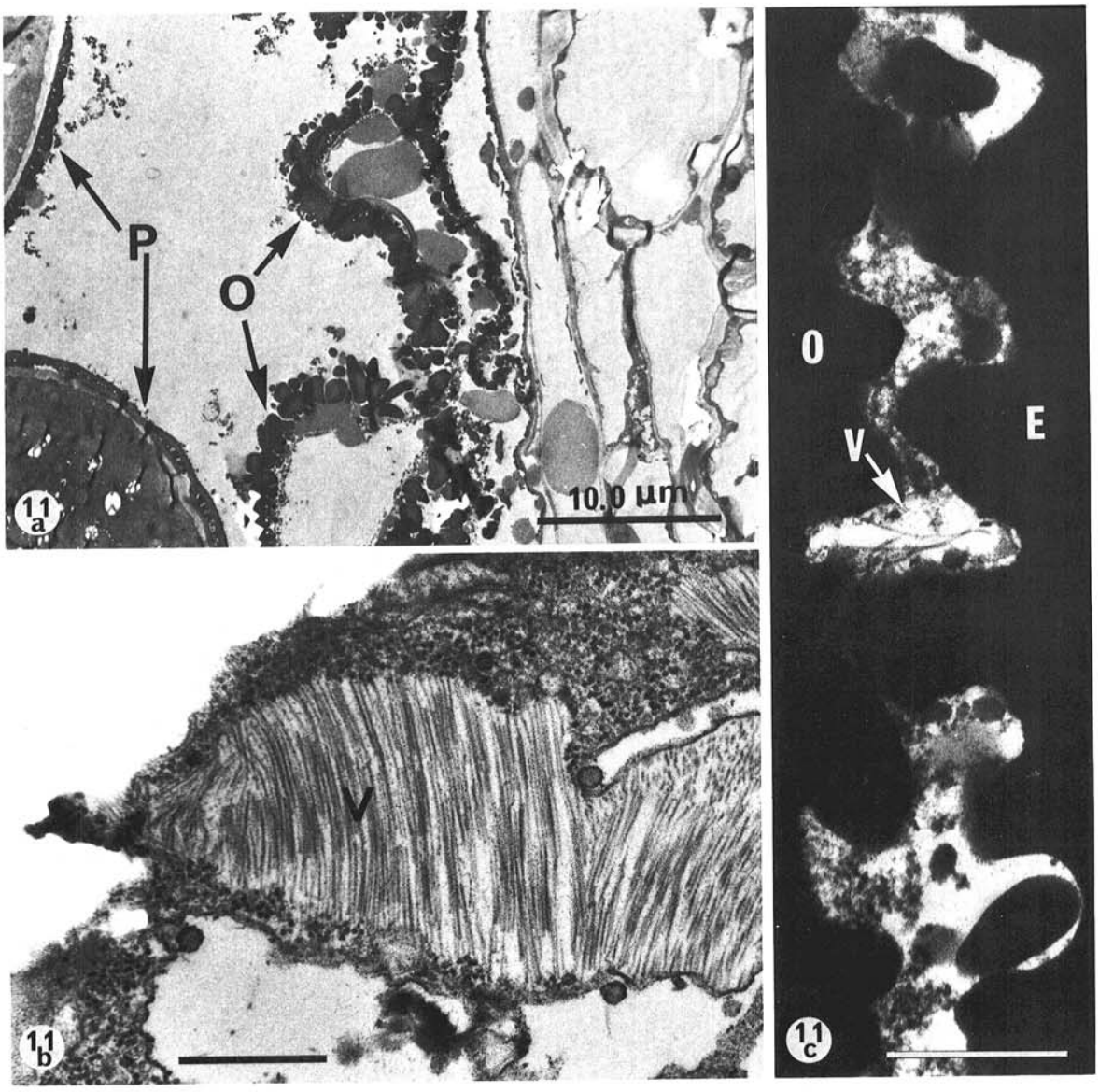

Fig. 11-(a to c). Electron micrographs of an anther of TMV-infected *Nicotiana tabacum* 'Haranova'. **a)** Section of anther showing two pollen grains (P) and osmiophilic globules (O); **b)** Section of anther wall cell showing aggregate of TMV (V); **c)** Area between osmiophilic globule (O) and the pollen exine (E) showing entrapped TMV virions (V). Bars represent 0.5 μm unless otherwise indicated.

be shown to contain SBMV virions in the bacula of the exine suggests that the association of virions with the exine is not an artifact of the procedures used to prepare the anthers for electron microscopy. It is likely that the virions which coat the exine are derived from the tapetum, a layer of specialized cells in the anther which have been shown to undergo lysis with the consequent release of substances which provide essential compounds to the surface of the pollen grain (4).

The observation that the pollen surface can be contaminated with infectious plant viruses is of interest because it suggests a new route by which certain mechanically transmissible viruses may be spread in the natural state. It is perhaps fortuitous that the observations were made on plants infected with BMV, SBMV, and TMV, three highly infectious viruses which occur in very high concentrations in their hosts, thus enhancing the probability of their detection on the exine. It may be that the pollen exine of many plants is contaminated with viruses, but the probability of detecting such surface infestation may be low if the concentration of virus in the tapetal cells is correspondingly low.

If surface-contaminated pollen is to function as a vehicle for the spread of a mechanically transmissible plant virus, several factors will undoubtedly affect the efficiency of the transmission process. Firstly, since the release of pollen to the atmosphere is much more prevalent in open-pollinated plants, the process of transmission by contaminated pollen is likely to be more efficient in open-pollinated plants than it is in self-pollinated plants. This does not negate the possibility that contaminated pollen could transfer viruses from self-pollinated plants because out-crossing of 5-10% does commonly occur in such plants. Secondly, the concentration and properties of the virus which is adsorbed to the surface of the pollen grain also will affect the efficiency of transmission. The results of the assays of pollen from SBMV-infected bean and from TMV-infected tobacco indicate that the number of local lesions induced is very low, despite the fact that large numbers of virions can be shown attached to the pollen surface. It may be that pollen homogenates are inimical to the infectivity of some plant viruses and experiments are in progress to determine the effect of homogenates of pollen from healthy plants on the infectivity of viruses. Thirdly, the mode of transport of contaminated pollen may influence the efficiency of the transmission process. Windblown contaminated pollen, after deposition on leaf surfaces, probably would have to await introduction into wounds as a consequence of leaf abrasion or by the locomotion of insects (6). On the other hand, flower-visiting insects could transport such pollen to other plants and in the process of walking on leaf surfaces directly introduce contaminated pollen into wounds caused by their locomotion.

The possibility that surface-contaminated pollen may play a role in the seed transmission of plant viruses also is raised by these observations. The demonstrated presence of virions in pollen from some virus-infected plants (1, 9) would indicate that in most, if not all, cases of pollen transmission of viruses leading to seed transmission the virus is introduced to the egg from infected pollen. What is intriguing is the possibility that germ tubes emanating from contaminated pollen may become infested, or infected by mechanical inoculation, during the germination process, leading to transmission of virus to the ovule. It is also possible that the stigma could become infected as a consequence of the deposition of contaminated pollen, and this may lead to infection of the integuments of the ovule. These possibilities are under investigation with SBMV- infested pollen in an attempt to determine the route by which a relatively high proportion (20%) of seeds of Early Red Valentine bean were reported to be infected with SBMV when healthy mother plants were pollinated with pollen from SBMV-infected plants (3). In the absence of direct proof that SBMV infects either the generative or vegetative cells of pollen, the results of Crowley (3) may be more likely explained on the basis of virus infection of the floral apparatus as a consequence of pollination with contaminated pollen.

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