Filiform Enations in Virus-Infected Soybeans

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ABSTRACT

Filiform enations were produced in 4 of 14 soybean cultivars by double infections of soybean mosaic virus and bean pod mottle virus. The enations developed 3 to 5 weeks after inoculation from the midrib of the trifoliolate leaves and were 0.5-2.5 cm long, 1 mm wide at the base, and tapered at tips. Enations produced synergistically by two unrelated viruses have not been reported previously. Both viruses occur together in naturally infected soybeans in Iowa. Phytopatbology 61:763-766.

Additional key word: synergism.

Filiform (threadlike) enations were produced in some soybean (*Glycine max* [L.] Merr.) cultivars inoculated with the combination of soybean mosaic virus (SMV) and bean pod mottle virus (BPMV) during a host range and symptomatology study. The enations were produced on the midrib on the upper surface of the trifoliolate leaves.

Enations due to virus infection occur on such diverse plant types as clover (wound tumor virus), peas (pea enation mosaic virus), tomato (tobacco mosaic virus), and *Tetragonia expansa* (chrysanthemum aspermy virus) (8). Virus-infected plants belonging to the genus *Nicotiana* are particularly prone to produce enations. Among these are *N. paniculata, N. tomentosa,* and *N. tabacum angustifolia* infected by the tobacco mosaic virus (TMV) (5); *N. rustica* infected by the chrysanthemum aspermy virus (7); and *N. tabacum* 'Samsun' infected by the alfalfa mosaic virus (1, 9). However, the filiform enations in soybeans were different from those induced by TMV and other viruses on tobacco.

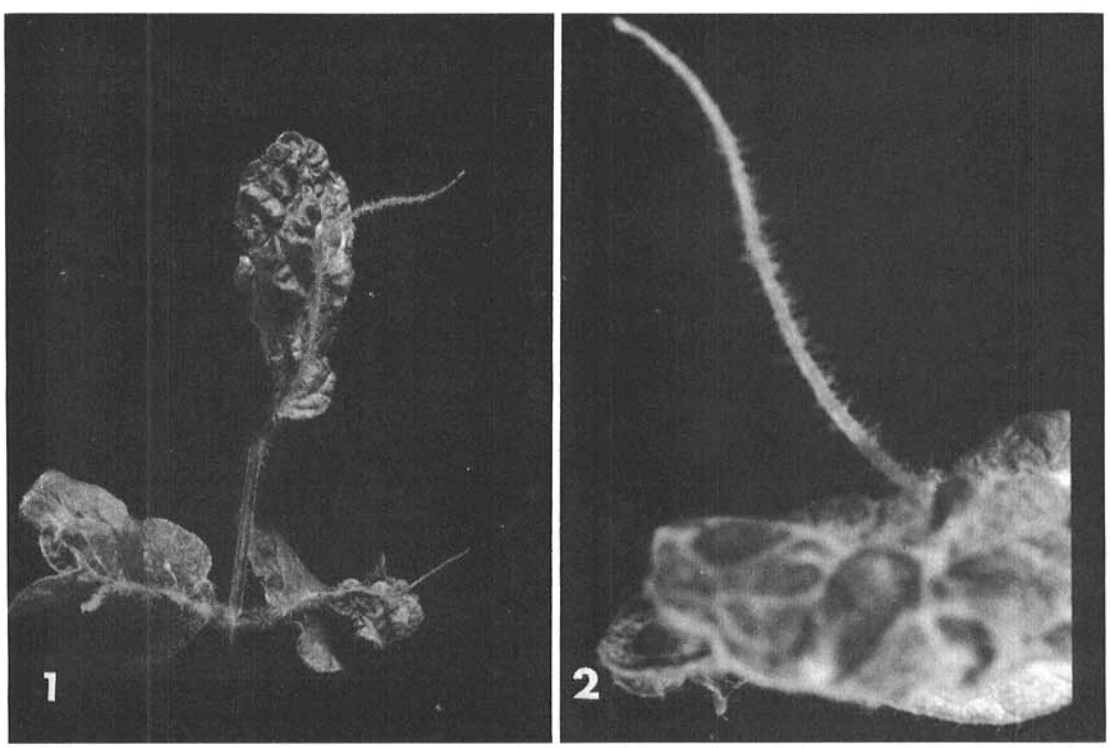

Fig. 1-2. **1)** Filiform enations produced on Lindarin soybean leaves following inoculations with a mixture of soybean mosaic virus (SMV-O) and bean pod mottle virus (BPMV) (×0.5). **2)** Filiform enations produced on Mandell soybean leaves following inoculations with a mixture of SMV-O and BPMV (×3).

Several viruses infect soybeans singly or in combination, but none produces enations. Symptoms produced by virus infection of soybeans include vein-clearing, vesicle formation on leaves, chlorotic mosaic, rugose leaves, curved shoot tips, bud blight, pod streaking, and stunting (2, 3, 6, 10). This paper describes histologically the development of the filiform enations in soybeans infected with SMV and BPMV.

MATERIALS AND METHODS.—SMV-O (soybean mosaic virus, Ottumwa strain) and BPMV used in this study were obtained earlier from naturally infected Clark soybeans in Ottumwa, Iowa (10). They were maintained in Bansei soybeans, and all inoculations were made from sap extracted in neutral 0.01 M phosphate buffer. Seedlings were inoculated by rubbing sap on the Carborundum-dusted unifoliolate leaves with the forefinger. Twenty-five plants of each of 14 soybean cultivars (Table 1) were inoculated with either

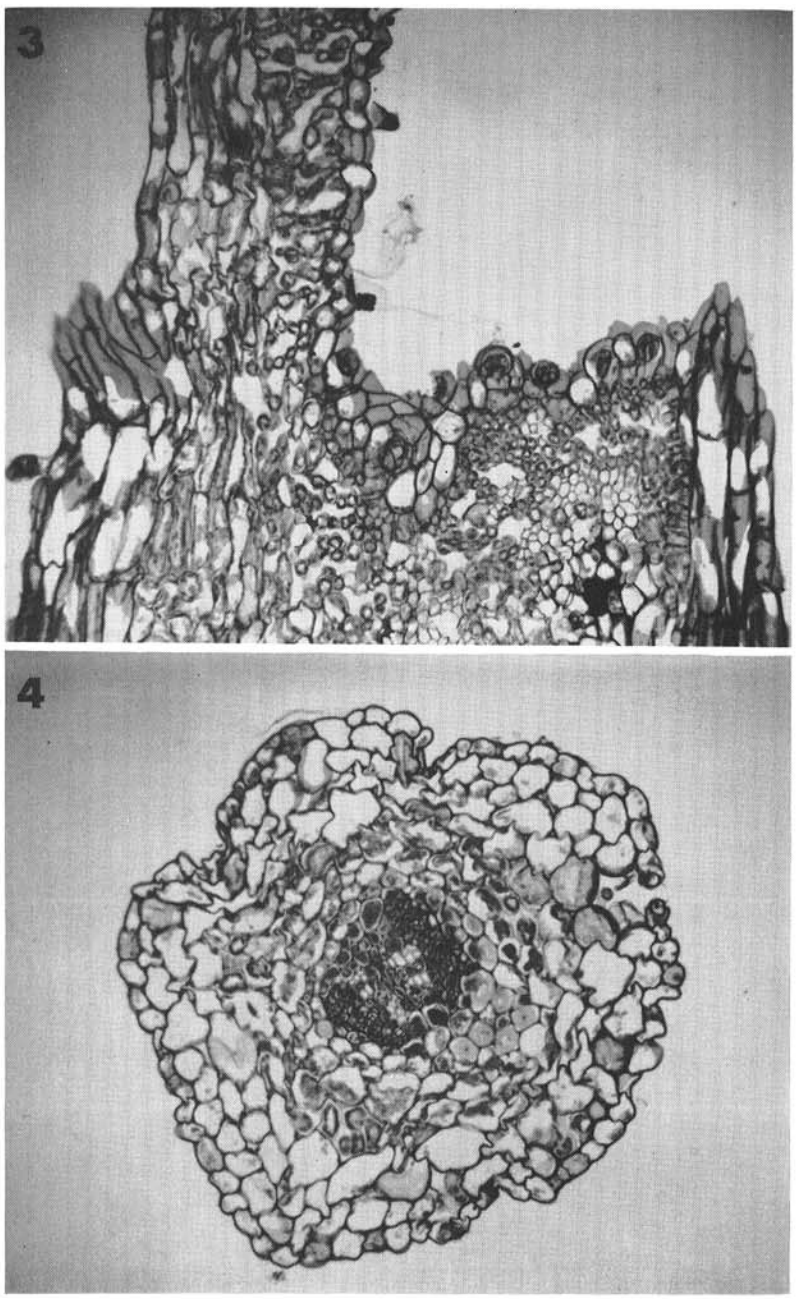

Fig. 3-4. 3) Longitudinal section (10 μ thick) through a midvein with enation transected. The concentric cells are meristematic and initiated the enation (×1,027). 4) Cross section (10 μ thick) of enation near the midvein showing vascular bundles (×1,095).

TABLE 1. Response of soybean cultivars inoculated singly or doubly with soybean mosaic and bean pod mottle viruses

Cultivars	Viruses and type of symptoms induced[a]		
	SMV-O & BPMV	SMV-O	BPMV
Adams	M, C	M, R	M
Amsoy	M, C	M, R	M
Bansei	M, C, E	M, R	M
Chippewa	M, C	M, R	M
Clark	M, C	M, R	M
Ford	M, C	M, R	M
Hark	M, C	M, R	M
Hawkeye	M, C	M, R	M
Hill	M, C	M, R	M
Lee	M, C	M, R	M
Lindarin	M, C, E	M, R	M
Mandarin (Ottawa)	M, C	M, R	M
Mandell	M, C, E	M, R	M
Wayne	M, C, E	M, R	M

[a] SMV-O = severe strain of soybean mosaic virus from Clark soybeans collected from Ottumwa, Iowa; BPMV = bean pod mottle virus isolated from same Clark soybeans; M = mosaic; R = rugose; C = chlorotic; E = enation.

one or both viruses. After inoculation, the plants were grown in a greenhouse maintained at 25 ± 3 C.

For the histological study, enations were taken at different stages of development, fixed with FAA (11) overnight, dehydrated through an ethanol-xylene series, and embedded with paraplast (55 C mp). Microtome sections were cut 8 μ and 10 μ thick, and stained with safranin-fast green.

RESULTS.—Filiform enations (Fig. 1, 2) were produced in 4 of 14 soybean cultivars (Table 1) inoculated with SMV-O and BPMV. Those cultivars which consistently produced enations were Bansei, Lindarin, Mandell, and Wayne.

Enations were formed on the midrib on the upper surface of the trifoliolate leaves 3 to 5 weeks after inoculation. The laminae were rugose, as in ordinary virus-infected soybean leaves. The enations were filiform, hirsute, 0.5-2.5 cm long, 1 mm wide at the base, and tapered at the tips.

Histological studies revealed that the enations were leaflike in structure, with an upper epidermis, sometimes with a palisade layer, spongy parenchyma cells, and a lower epidermis (Fig. 3). They originated from the chlorotic area at the end of the midrib. Cross sections of an enation near the attachment to the midrib showed a cortex of parenchymatous cells with a single vascular bundle at the center (Fig. 4). The bundle was not found throughout the enation.

DISCUSSION.—Few descriptions of leaf development in soybeans have been published. The first detailed study on shoot apex and leaf histogenesis was reported by Sun (12). He observed that when a leaf primordium developed to a height of about 80-90 μ, the next leaf primordium was initiated. Leaf initiation was indicated by anticlinal divisions in the tunical layer and periclinal divisions in the outer corpus.

The development of filiform enations in soybeans differs from those reported by Tepfer & Chessin (13) in tobacco. They reported two principal effects of tobacco mosaic virus on the early development of shoestring leaves in tobacco: (i) lack of complete dorsiventrality in the primordium; and (ii) an inhibition of meristematic activity. Furthermore, plants that produced shoestring enations almost invariably also produced narrow-bladed leaves. In our study, soybean leaflets which produced enations had normal laminae, and the enations developed concurrently with the development of the leaflets.

The mechanism of enation formation is not understood. However, soybean cultivars producing the enations showed "acute" symptoms in unifoliate leaves 10-14 days after inoculation with the combined viruses, and developed enations in the next three to four trifoliolate leaves. In our opinion, these teratological outgrowths were produced in response to the physiological "shock" of the hosts. According to the observations of Kunkel (8), enations are produced in chlorotic areas that have lost their normal morphogenetic control of the tissues.

Ford (1) reported an inconsistency in enation production in *N. tabacum* 'Samsun NN' plants inoculated with alfalfa mosaic virus, where 1 out of 17 plants produced enations. In Italy, Graniti et al. (4) also reported inconsistency in the number of enations produced within cultivars in the enation disease of grapes.

Went (14) proposed that two growth factors are involved in leaf development, one concerned with vein growth and the other with mesophyll development. The former factor is probably involved in this study, since it is possible that a secondary meristem forms directly on the chlorotic area on the upper surface of the midrib.

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