Graft, Pollen, and Seed Transmission of an Agent Associated with Top Spotting in *Kalanchoë blossfeldiana*

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ABSTRACT

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Top spotting was shown to be caused by a graft-transmissible agent(s), which was also seedborne and transmitted through pollen from diseased plants. Seed transmission occurred in about 30–63% of the progeny if the pollen parent had top spotting. Bacilliform particles measuring 20–25 × 50–100 nm were observed in ultrathin sections of three of four diseased plants used as a source of scions in graft-transmission tests and in three of five diseased but none of five healthy progeny examined from seed-transmission tests.

Many cultivars of *Kalanchoë blossfeldiana* Poelln. commercially marketed as flowering pot plants showed chlorotic spots and ring spots on emerging leaves during periods of vigorous vegetative growth (Fig. 1). Spots usually faded as the leaves expanded, but sunken zones often developed on the upper leaf surface at sites that formerly showed chlorotic spots. The intensity and duration of the spotting varied among cultivars. Preliminary experiments showed that a graft-transmissible agent was associated with the disease (C. Mellinger, *personal communication*). Attempts to mechanically transmit a pathogen failed. The following research was conducted to identify the causal agent of the disease, herein referred to as top spotting, and to determine its means of transmission. A preliminary report has been made (7).

MATERIALS AND METHODS

Seed transmission in selfed plants. Three cultivars of *K. blossfeldiana* (Cactus Candy, Louise, and Osage Orange) were selected because they produced new leaves with obvious spotting during several months of every year. Cactus Candy showed symptoms almost year-round. No verified healthy plants of either cultivar were available to serve as controls at this time in the experiment. One naturally infected plant of each cultivar was allowed to self-pollinate (Fig. 2). Seeds were collected from each plant and stored at 4 C. Seeds were germinated under mist in seed pots 6 and 15 mo after harvest. Fifty seedlings in the first test and 100 seedlings in the second test were transplanted to 5-cm plastic pots and maintained in the greenhouse. Seedlings were observed for top spotting symptoms over a 9-mo period.

Graft transmission. Four Cactus Candy seedlings with top spotting and 11 sister seedlings that showed no symptoms were selected from the second germination test in the previously described experiment (Fig. 2). Five cuttings were rooted from each of the 11 symptomless (healthy) seedlings (Fig. 2). When the rooted cuttings were 12–15 cm tall, about 4–5 cm of the shoot tips of three of the five rooted cuttings were removed. A tip from one of the four seedlings with top spotting was grafted onto each of the 33 healthy stocks. Grafted plants were placed under a mist system for 7–10 days, then maintained in a shaded greenhouse at 18–28 C. The grafted stock plants were observed for symptoms of top spotting in the lateral shoots that emerged after the grafts were made.

The 33 tips removed from the healthy stock plants were rooted and maintained as controls (Fig. 2). Twenty-two healthy sister plants were also maintained as controls.

Pollen transmission. Cuttings of symptomatic lateral shoots from the grafted stock plants were rooted. Cuttings also were rooted from the plants derived from the apical tips of the unspotted control stock plants. Both sets of rooted plants were allowed to flower under natural short-day fall conditions. Four to 12 florets in each of one to three cymes were emasculated on each plant by slipping off the corolla tube and attached stamens with forceps. The remaining florets and those that developed later were removed from the cymes with forceps. Pollen was touched to the sticky, swollen stigmas as each ovary became receptive to fertilization. Glassine bags were placed over the cymes for several days. Cymes were uncovered after all stigmas were pollinated and past the receptive stage.

Duplicate crosses were made between a healthy seed parent and a pollen parent with top spotting, a seed and a pollen parent with top spotting, and a healthy seed and pollen parent. Plants pollinated in December and January produced seeds that were harvested in April. Seeds were dried for 2–3 wk at room temperature, then germinated and transplanted as described previously. Seedlings were maintained in the greenhouse and observed for top spotting symptoms through October. Healthy seed parents crossed with pollen from infected plants were observed for disease symptoms.

Electron microscopy. Leaf tissue from spotted areas of infected seedlings and comparable tissue from healthy sibling seedlings used in the graft-transmission tests were fixed and embedded for ultrastructural observation (6).

RESULTS

Seed transmission in selfed plants. Results of two germination trials of

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Cactus Candy, Louise, and Osage Orange confirmed the seed-transmissible nature of the agent associated with top spotting (Table 1). Seedlings of Cactus Candy and Osage Orange were phenotypically uniform and similar to the parent plants except for the occurrence of top spotting symptoms in some plants and not others. Louise seedlings, on the other hand, varied in size, branching, and leaf-shape characteristics. Symptoms on Louise seedlings also varied from chlorotic spotting to mottle and vein yellowing. Only the Louise seedlings with distinct chlorotic spots were rated positive for top spotting. This may account for the lower percentage of seed transmission recorded for Louise compared with Cactus Candy and Osage Orange. The reason for the variable symptoms in Louise seedlings is not presently understood.

Graft transmission. Twenty-nine of the 33 Cactus Candy grafts produced viable, growing scions with top spotting symptoms. Two grafted scions remained alive for 2 mo, but no union was formed with the stock plant and the latter failed to develop top spotting. Two grafts died and no symptoms were observed in the stock plants. Symptoms were observed on the lateral breaks of some stock plants about 6 wk after the grafts were made. Twenty-seven of 29 successfully grafted stock plants developed top spotting symptoms in one or more shoots within 3 mo.

Cuttings from symptomatic shoots of grafted stock plants were rooted and repropagated twice more. Symptoms were observed in the derived plantlets from each propagation; however, not all shoots of a diseased plant showed spotting during periods of symptom expression. Symptomless plants could be obtained occasionally by rooting cuttings from a symptomless shoot of an infected plant. This probably resulted from nonuniform distribution of the pathogen in the mother plant. The general persistence of the disease symptoms in the propagations demonstrated, however,

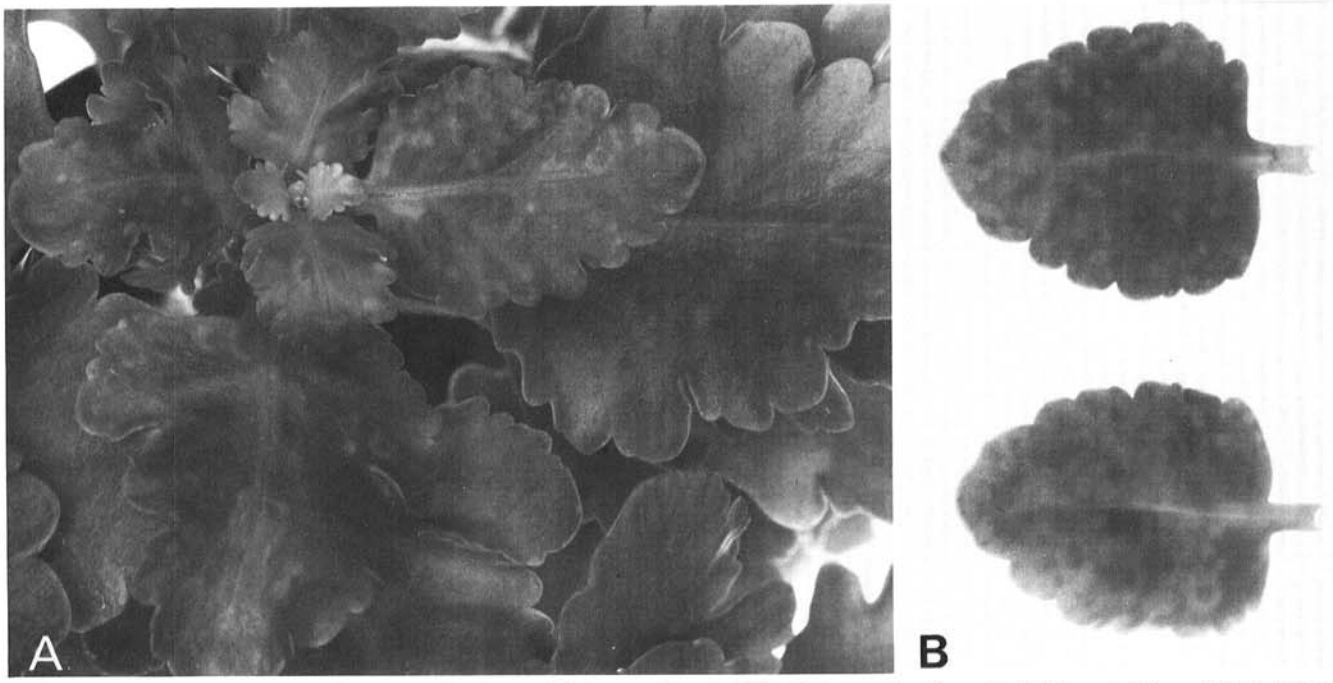

Fig. 1. *Kalanchoë blossfeldiana* showing chlorotic spots and ring spots characteristic of top spotting disease in **(A)** the apical tip and **(B)** individual leaves of a Cactus Candy seedling derived from a cross between infected seed and pollen parents.

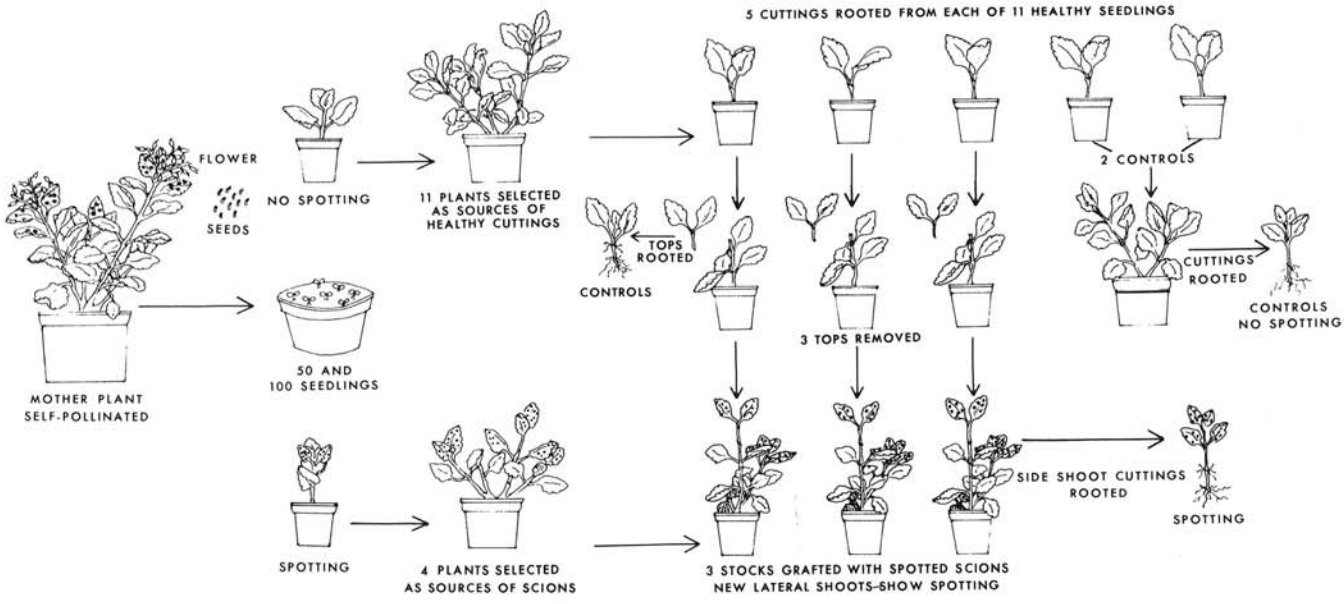

Fig. 2. Diagrammatic illustration showing the origin of plant materials and the experimental procedures used to test for graft, pollen, and seed transmission of the causal agent(s) of top spotting.

that a toxin produced in the original scions did not cause symptoms in the stock plants.

None of the 33 tip cuttings from stock plants (removed to make the scion insertions) developed top spotting when rooted. The 22 untreated sister plants of the grafted plants also remained symptomless.

Pollen transmission. Cactus Candy seedlings with top spotting were obtained from crosses of diseased pollen parents with healthy and diseased seed parents (Table 2). None of the healthy seed parent plants used in crosses with diseased pollen parent plants developed top spotting symptoms after the crosses were made.

Electron microscopy. Ultrathin sections of leaf tissue from diseased plants occasionally contained small bacilliform particles in a few cells. Particles in ultrathin sections measured about 20–25 × 40–100 nm. They were most prevalent in the vascular tissue, where they occurred in small clusters in the lumens of the mature sieve cells (Fig. 3A) or in small aggregates in the cytoplasm of the vascular parenchyma cells. Small aggregates of bacilliform particles also occurred in the cytoplasm of mesophyll cells (Fig. 3B), though they were less obvious than those in the cells of the vascular tissue that were devoid of cytoplasm and organelles such as ribosomes.

Bacilliform particles were found in three of the four diseased plants used as sources of diseased scions in the grafting experiments. Bacilliform particles were also found in three of five symptomatic progeny but in none of five symptomless progeny from the pollen-transmission tests. Less than 30% of the examined tissue pieces from the three positive progeny contained bacilliform particles. In addition, the particles were only found in spotted areas of the tissue, when tissue pieces were excised to include some of the surrounding green tissue. No additional viruses, mycoplasmas, or bacteria were detected in the sections of the diseased or healthy seedlings.

DISCUSSION

Top spotting in *K. blossfeldiana* was previously thought to be a genetic or nutritional disorder because symptoms appeared in many of the progeny in young seedling populations and symptom expression was erratic. Our tests demonstrated that a graft- and seed-transmissible agent(s) caused top spotting. The agent was shown to be transmitted in the pollen from infected plants. Mechanical transmission of the causal agent has not been accomplished using standard buffers, additives, and herbaceous indicator plants (S. S. Hearon, *unpublished*).

The causal agent of top spotting was not identified. Small, bacilliform particles were observed in ultrathin sections in a few cells of some diseased seedlings. These particles were not found in healthy seedlings. The bacilliform particles were smaller than those of the rhabdovirus potato yellow dwarf virus and larger than those of alfalfa mosaic virus. Both of these viruses infected kalanchoës upon mechanical inoculation (S. S. Hearon, *unpublished*). Leaf tissues infected with both these viruses as well as leaf tissue with top spotting were compared to verify that the small bacilliform particle associated with top spotting differed in ultrastructural appearance from the other two bacilliform viruses under the fixation and embedding conditions we used.

The particles associated with top spotting resembled virus particles of cacao swollen shoot (3), and particles associated with rubus yellow net (13), rice tungro (8,12), "Alomae" disease of *Colocasia esculenta* (9), internal brown spot of *Dioscorea alata* (5), viruslike particles in Mexican bean beetle (2), and a virus causing a lethal disease in house crickets (1). Particles with a similar morphology have been observed also in *Commelina diffusa* that was infected with a potyvirus (11). The particles we observed differed slightly from the particles of orchid fleck virus (4) and

Table 1. Seed transmission of top spotting in self-pollinated plants of three cultivars of *Kalanchoë blossfeldiana*

Cultivar	Seedlings with top spotting (%)		
	Trial 1[a]	Trial 2[b]	Avg. of two trials
Louise	18	42	34
Osage Orange	44	60	55
Cactus Candy	63	66	65

[a] Based on about 50 seedlings. Group size varied slightly due to losses from other causes during the experiment.
[b] Based on about 100 seedlings.

Table 2. Transmission of top spotting in Cactus Candy progeny from crosses of healthy and diseased parents

Parental cross	Progeny with top spotting	Total progeny	Transmission (%)
Healthy female × diseased male			
1[a]	63	145	43
2	40	130	30
Diseased female × diseased male			
1	23	48	48
2	22	58	38
Healthy female × healthy male			
1	0	77	0
2	0	37	0

[a] Cross number.

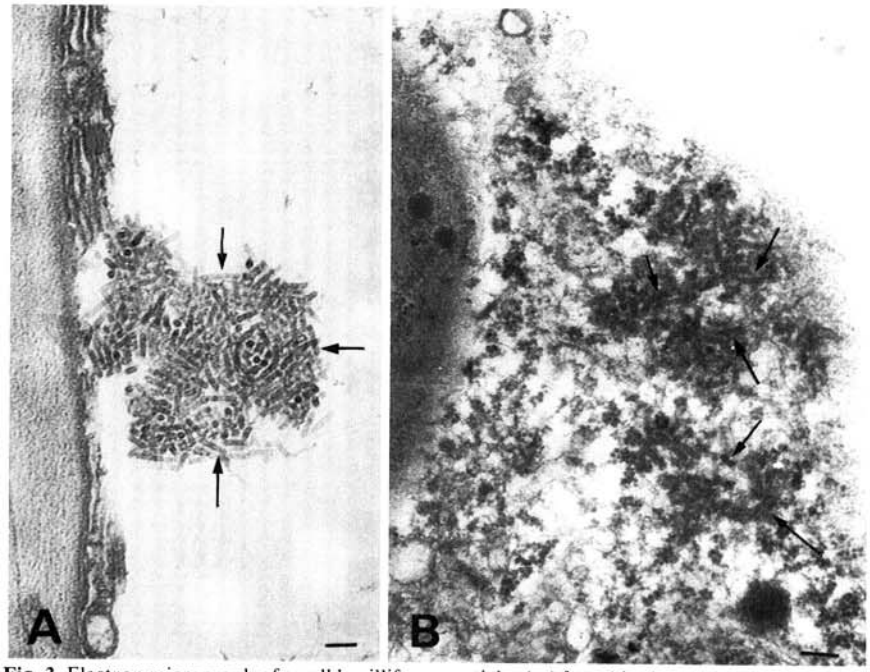

Fig. 3. Electron micrograph of small bacilliform particles (←) found in the **(A)** lumen of the sieve cells and **(B)** cytoplasm of mesophyll cells in seedlings of Cactus Candy with top spotting. Bar = 100 nm.

citrus leprosis virus (10) in that the latter are reported to be slightly wider (35–40 nm) and occur in the nucleus, as well as in the cytoplasm, of infected cells. With the exception of cacao swollen shoot and the rice tungro bacilliform viruses, none of the above-mentioned small, bacilliform, viruslike particles have been isolated and characterized.

Further work is needed to identify the role of the bacilliform particle, if any, in causing top spotting in kalanchoës. In the interim, growers and breeders of kalanchoës should be aware that pollen and seed transmission of the pathogen cause top spotting and should eliminate diseased seedlings from their breeding selections. Seedlings should be observed over several months to make the diagnosis. The observation period should include a time during which known diseased plants of the same cultivar are expressing top spotting symptoms.

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