Persistence of Pea Cotyledons Induced by Corynebacterium fascians

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ABSTRACT

Corynebacterium fascians caused fasciation in garden pea and a permanent retention of the morphological integrity of its cotyledons. Dry weight of the cotyledons decreased to 14% of the original weight 4 weeks after inoculation and planting. In contrast, cotyledons of noninoculated pea plants shriveled in 2 weeks and decomposed shortly thereafter. Nutrients were utilized much more slowly in the diseased plants than in the control. The effects on the cotyledons may serve as a new bioassay for determining cytokinins, and a tool for studying infection by *C. fascians*.

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When garden pea (*Pisum sativum* L.) was inoculated with *Corynebacterium fascians* (Tilford) Dowson, primary symptoms of bacterial fasciation developed. Besides these symptoms, secondary effects involving the persistence of pea cotyledons were constantly observed in numerous experiments: the morphology of cotyledons of infected plants remained unchanged even after flowering, whereas cotyledons of noninfected plants shriveled and some decomposed three weeks after germination and planting.

The phenomenon had not been reported by Samuels (3) or Thimann and Sachs (5) who had used peas in their studies of the relationship of kinetin to disease response following infection with *C. fascians*. One of the reasons that they did not observe the effect on the cotyledons may have been because their experiments rarely lasted more than two weeks. Three experiments were conducted to assess accurately the effect of infection by *C. fascians* on pea cotyledons. The results of these and associated experiments are reported herein.

MATERIALS AND METHODS.—Seeds of garden pea (*Pisum sativum* L.) were immersed for 5 minutes in 95% ethyl alcohol followed by 10 minutes in 0.1% mercuric chloride solution, rinsed three times in sterile distilled water (SDW), and transferred to a petri dish lined with moist sterile filter paper. Two-day-old seedlings were placed in SDW (control) or were inoculated with *C. fascians* by placing them in a suspension of the bacterium (1.7×10^8 cells per ml). After one hour the seedlings were removed and planted in 12.5-cm clay pots (four plants per pot) in a greenhouse at 23-28 C.

RESULTS.—Symptoms resulting from infection by *C. fascians* were stunting; stimulation of growth of lateral buds (initially at the cotyledonary node and later spreading to second and third nodes); retention of morphological integrity of cotyledons; and reduction in yield.

The differences in morphology of infected plants as compared to noninfected plants are shown in Fig. 1. In the first 2 weeks following inoculation there were no apparent changes in morphology of cotyledons (Fig. 1-A). Between the second and third week pronounced differences were observed: the cotyledons of noninfected plants shriveled and decomposed, whereas those of noninfected plants remained unchanged (Fig. 1-B). Cotyledons of noninfected plants were completely decomposed by 6 weeks (Fig. 1-C), whereas those of fasciated plants were intact and they persisted, even after the plants had flowered and had formed seed pods (Fig. 1-D).

Although the gross morphology of cotyledons was

unaffected by infection, significant changes occurred in the dry weights of cotyledons of infected plants (Table 1). After 1 week, the dry weight was 69% of the initial weight; this loss was comparable to that of cotyledons of noninfected plants. After 2 weeks considerable weight loss also occurred, but it was 10% less than that of noninfected plants. After 3 weeks, the differences between infected and noninfected plants were most obvious, with the loss being least in infected plants. Finally, a steady-state of 14% dry weight was maintained in cotyledons of infected plants, but noninfected plants were completely decomposed. Nutrients were utilized in the cotyledons of both the treated and control plants as indicated by the decrease in dry weights following germination and growth, but utilization was slower in the inoculated plants.

Analysis of the cotyledons of inoculated plants (six weeks after inoculation and planting) by Sudan dye, Molisch, and biuret tests showed traces of lipids, carbohydrates, and protein, respectively, as compared to healthy, nongerminated pea cotyledons.

Efforts were made to isolate *C. fascians* from cotyledons of fasciated plants without success. Bacteria were readily isolated from the swollen shoots arising from the cotyledonary, the second, and the third nodes, but not from the roots of inoculated plants. Thus, the effect on morphological integrity of cotyledons is presumably due to bacteria-induced growth factors, and not to direct infection of cotyledons by *C. fascians*.

Cytokinins were suspected to be involved in the symptoms caused by *C. fascians,* so an attempt was made to mimic the phenomena using pure cytokins. Kinetin [6-furfurylaminopurine] or zeatin [trans-6-(4-hydroxy-3-methylbut-2-enyl)-aminopurine] manufactured by Calbiochem, San Diego, California, were used. Two-day-old pea seedlings, prepared as earlier described, were soaked in 25 μg/ml SDW-cytokinin solutions for 24 hours. The seedlings were transferred to petri dishes lined with sterile filter papers, and moistened with cytokinin solutions. Control plants were treated with only SDW. After 5 days, 24 seedlings per treatment were planted in sterilized soil in a greenhouse. The experiment was repeated once.

The effects of zeatin on the cotyledons of peas are shown in Fig. 2. Similar responses were obtained from the kinetin treatments. After continuous contact with zeatin for 5 days, swollen shoots emerged from the treated seedlings (Fig. 2-A). About 3 weeks later, cotyledons of treated plants were morphologically intact, whereas those

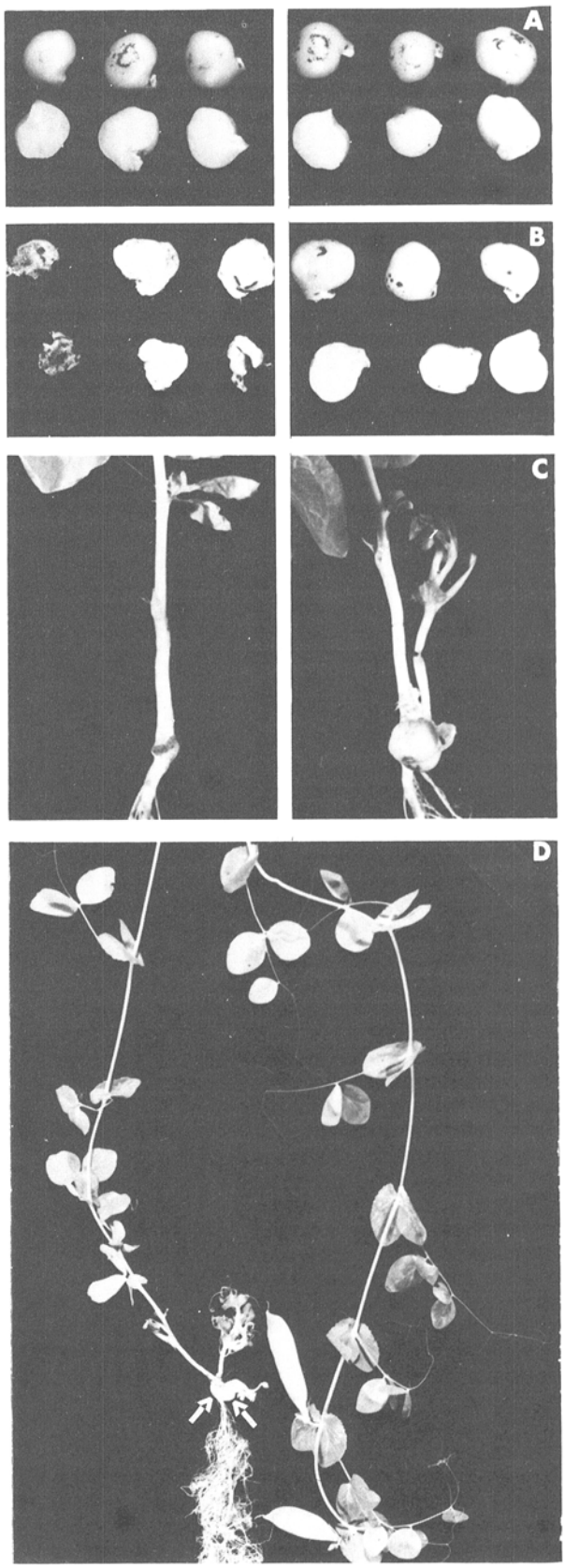

Fig. 1-(A to D). Effect of infection by *Corynebacterium fascians* on cotyldeon morphology of garden pea. **A)** Two weeks after inoculation. Right, cotyledons removed from inoculated plants; Left, cotyledons from noninoculated plants. Note that no differences in morphology were apparent. **B)** Three weeks after inoculation. Right, intact cotyledons from inoculated plants; left, shriveled and decomposed cotyledons from noninoculated plants. **C)** Six weeks after inoculation. Right, intact cotyledon on infected mature plant showing symptoms of fasciation; Left, noninoculated plant, showing that cotyledons had completely decomposed. **D)** Six weeks after inoculation. Entire plant showing intact cotyledon even though plant had formed seed pods.

of nontreated plants were shriveled (Fig. 2-B). After about 4 weeks over 60% of the cotyledons of treated plants were still intact, and the remainder were only slightly shriveled. In contrast, cotyledons of nontreated plants were extensively shriveled and some completely decomposed. The abnormal development of lateral shoots, usually observed in peas infected with *C. fascians,* was not as advanced as in zeatin-treated plants.

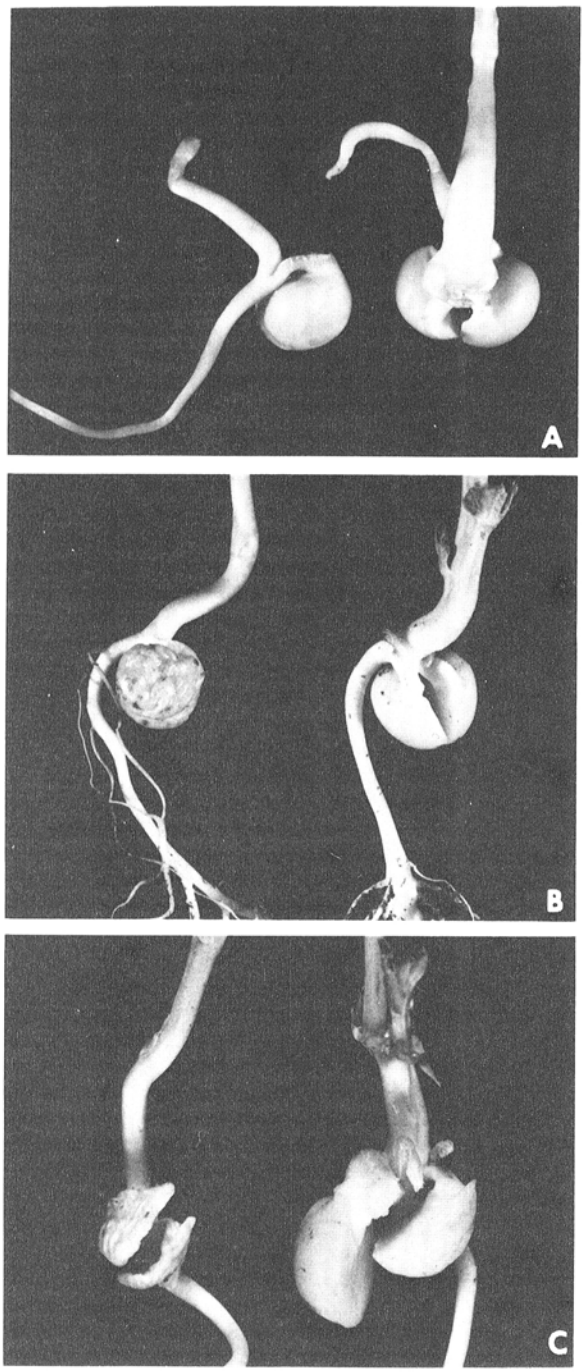

Fig. 2-(A to C). Effect of cytokinin on cotyledon morphology of garden pea. **A)** Five days after continuous treatment with zeatin in petri dishes. Right, treated seedlings showing primary symptoms of fasciation. Left, nontreated seedling. Note that no differences in morphology of cotyledons were apparent. **B)** Five days continuous treatment with zeatin in petri dishes, plus 12 days in soil in the greenhouse. **C)** Five days continuous treatment with zeatin, plus 23 days in soil in the greenhouse.

TABLE 1. Effect of infection by *Corynebacterium fascians* on the dry weight of cotyledons of garden pea during growth[a]

Time (weeks)	Cotyledon dry weight (% of 0-time control)	
	Not inoculated	Inoculated
0	100	100
1	71	69
2	28	38
3	8	27
4	4	14
5	0	14
6	0	14

[a]Per plant, mean of 12 plants.

DISCUSSION.—No attempt was made to extract cytokinins from the cotyledons; however their presence in both *C. fascians* and bacterial-fasciated plants is well documented (1,2,5). Thimann and Sachs (5) mimicked bacterial fasciation by application of pure cytokinins. Similar work done in these studies produced both the primary and secondary symptoms of the disease, namely emergence of hypertrophied shoots and persistence of cotyledons. The lack of extensive proliferation of abnormal lateral shoots to produce leafy gall symptoms and the shriveling of cotyledons of some of the treated plants could be due to a lack of continuous irritation as might be the case of tissues infected with the bacteria. *C. fascians* probably stimulates a continuous supply of cytokinins as the plant grows, resulting in formation of prolific shoots. In contrast, with a single application of pure cytokinin, as supplied here, the effects might be partially overcome as the plant ages.

Cytokinin (kinetin) is reported to maintain the integrity of the cell membrane (4). Apparently, another effect of these compounds is the retention of morphological integrity of pea cotyledons. These secondary effects of bacterial fasciation of garden pea may serve as a new bioassay system for both infection by *C. fascians* and for cytokinin studies.

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