Factors Affecting Epidemiology of Bacterial Fasciation of Chrysanthemum maximum

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Accepted for publication 18 February 1975.

ABSTRACT

The severity of bacterial fasciation of *Chrysanthemum maximum* (which is caused by *Corynebacterium fascians*) appeared to increase with the length of time plants are diseased, and with the removal of the apical buds. Garden pea seedlings responded rapidly to the disease in greenhouse studies. Since daisy root divisions used for propagation were the main sources of inoculum in the field, the use of clean planting materials for control is reemphasized.

Phytopathology 65:719-721

Additional key words: Shasta daisy, *Pisum sativum*, cytokinin/auxin ratio.

Bacterial fasciation of Shasta daisy (*Chrysanthemum maximum* Ram.) caused by *Corynebacterium fascians* (Tilford) Dowson (5) was first observed and described in California in 1945 by Baker (1). He considered the disease to be economically important because it caused reduction in flower production and in root divisions for propagation purposes. The disease is still prevalent, and in some fields virtually all plants may be diseased.

The purpose of this study was to determine factors affecting the epidemiology of the disease in the field.

MATERIALS AND METHODS.—Monthly visits were made to a farm in San Diego County to observe cultural practices, and to record the incidence and severity of the disease. Diseased plants were collected and isolations of the pathogen were attempted from the hypertrophied shoots. The abnormal shoots were immersed in 0.6% sodium hypochlorite solution for 10 minutes, and rinsed three times in sterile distilled water (SDW). Pieces of the shoots were squeezed with sterile forceps and left in 10 ml SDW in test tubes for 1 hour. The resulting suspension was used to streak potato-dextrose agar (PDA) plates which were incubated at room temperature (25 C). Fast-growing fungi and bacteria appeared as contaminants within 7 days of culturing. After 7 days, the bacterium appeared as punctiform colonies, orange in color, with entire colony margins (9).

When rapid confirmation of pathogenicity was required, garden pea (*Pisum sativum* L. 'Alaska') was used as the bioassay plant (7,8). Seeds were immersed in 95% ethyl alcohol for 3 minutes, in 1% mercuric chloride solution for 10 minutes, rinsed three times in SDW, and transferred to a petri dish lined with moist sterile filter paper. The seeds germinated in 1-2 days. Two-day-old seedlings were inoculated by placing them in a suspension of the isolated bacterium (1.7×10^8 cells/ml). Controls were placed in SDW. After 1 hour the seedlings were removed and either placed in a petri dish lined with moist, sterile filter paper and placed at room temperature, or planted in 12.5-cm diameter clay pots (four plants per pot) in a greenhouse, of which the temperature varied from 23-28 C.

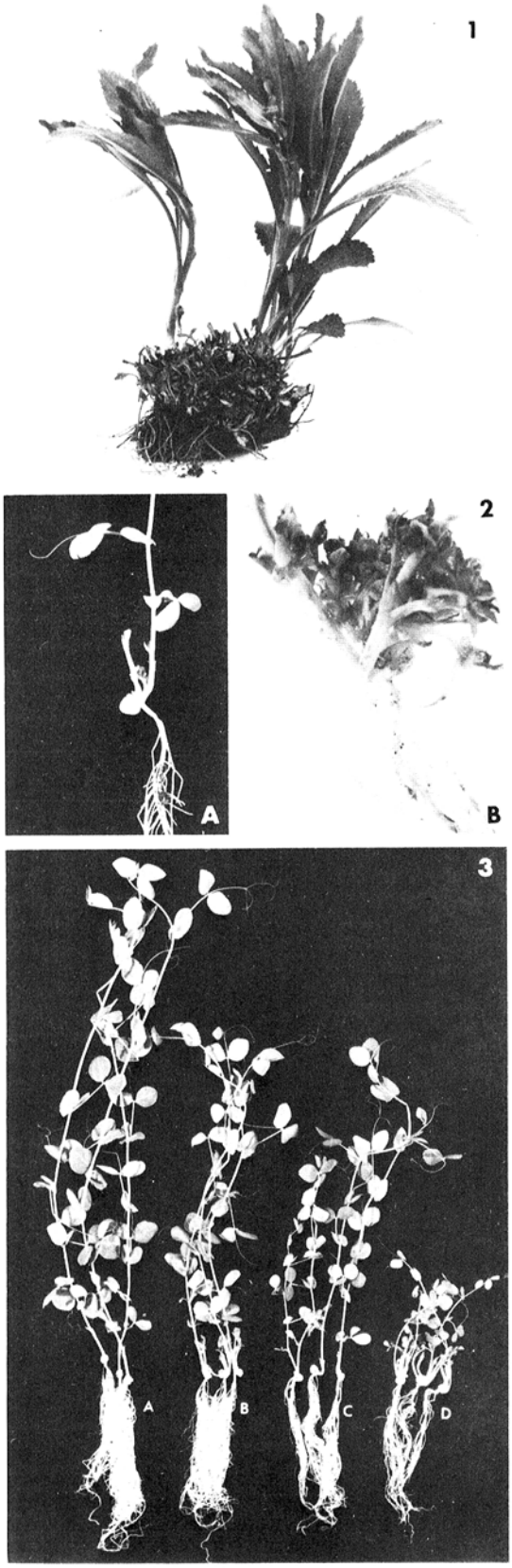
RESULTS.—*Pathogen and field symptoms.*—The bacterium isolated from the diseased plants was confirmed to be *C. fascians* on the basis of cultural characteristics, pathogenicity, and identity with the two reference cultures (ATCC 12975, ICPB 103R) supplied by M. P. Starr, University of California, Davis. It caused emergence of hypertrophied lateral shoots in seedlings of Shasta daisy (cultivar Killian) grown from bacteria-free seeds, and it could be reisolated; thus Koch's postulates were fulfilled.

The primary symptoms of the bacterial fasciation disease of Shasta daisy were numerous hypertrophied shoots which emerged from the basal buds and spread horizontally at the base of the main stems to produce what Lacey (4) called leafy gall (Fig. 1). Stunted growth and reduction of flower size appeared to be secondary effects resulting in loss of yield.

Factors affecting disease severity.—The incidence of severe fasciation symptoms increased with progressively longer periods of infection after plants first became diseased, and with removal of the apical buds. During the initial visit to the field in March 1972 when most of the 1,000 plants were in the vegetative stage, only 0.5% of the plants had leafy gall symptoms. After the first flower harvest in April and the second in May, the incidence had increased to 4.9% and 25.9%, respectively. At the end of the third and final harvest in July, 100% of the observed plants were leafy-galled.

The field observations were verified in greenhouse studies with garden pea inoculated in the seedling stage (Fig. 2,3). During the first 3 weeks after inoculation, only a few hypertrophied shoots emerged from the cotyledonary node (Fig. 2-A). Thereafter, more abnormal shoots emerged continuously from the nodes of the initial hypertrophied shoots, and after the sixth week many hypertrophied shoots emerged from the second and third nodes of the main stem to produce the leafy gall symptoms (Fig. 2-B). Thus, a long period of infection following symptom expression increased disease severity.

The greenhouse studies also showed that the removal of apical buds increased the severity of fasciation (Fig. 3). When the apical buds of 25 pea seedlings were left intact and not inoculated, there were no abnormal shoots (Fig. 3-A). When the apical buds were left intact and the plants inoculated with *C. fascians,* there were five abnormal shoots per plant (Fig. 3-B). When the apical buds were excised but plants were not inoculated, there were no abnormal shoots (Fig. 3-C), but when the apical buds were excised and plants inoculated, there were 15



abnormal shoots per plant (Fig. 3-D). Thus, the proliferation of abnormal lateral shoots was greatest in inoculated plants on which the apical buds had been excised.

Source of inoculum.—Since the main symptoms of fasciation occurred at the base of stems, contamination of the soil was suspected. An attempt was made therefore to isolate the pathogen from soil around the diseased plants by a baiting technique and by using a selective medium. To bait the pathogen, 50 germinated pea seeds were planted in 12.5-cm diameter clay pots containing a mixture of 10 soil samples collected from the field, and maintained in the greenhouse for 2 weeks. To isolate the pathogen on a selective medium, the composite soil sample was mixed with SDW 1:1 and 10^{-4} dilutions made from the suspension. Portions of the dilution were plated (0.2 ml per plate) on Kado's (3) D2 medium (which is selective for *Corynebacterium*) and incubated at room temperature. Both experiments were repeated.

The bacterium isolated on Kado's medium was confirmed to be *C. fascians* by testing pathogenicity of cultures from ten typical colonies on garden peas. All ten isolates caused the primary fasciation symptoms in peas. No disease symptoms appeared on plants treated with water only.

Although *C. fascians* was isolated from the soil on Kado's selective medium, none of the 50 pea plants used for baiting the pathogen from the composite soil sample became fasciated 2 weeks after treatment. The results indicated that the concentration of bacterium in the soil was presumably too low to cause disease.

To determine whether field soils were important sources of inoculum, it was necessary to follow the fate of the pathogen when diseased daisy plants were incorporated in soil. Four 25-cm diameter clay pots were filled with unsterilized top soil, and soil in two of the pots was artificially infested by burying in each, 800 gm pieces of daisy stems with leafy gall symptoms. The other two pots served as noninfested controls. All the pots were put in saucers and placed 1 meter apart on a bench in a greenhouse. One set of infested and noninfested soils was watered with fertilized water, once from the top and subsequently only from the bottom. The other set was left completely dry during the entire period of the experiment. After 3 months, samples of each soil were taken from different places and depths of the pot, mixed thoroughly, and suspended in SDW. Isolation of *C. fascians* from

Fig. 1-3. 1) Field symptoms of fasciation disease of Shasta daisy caused by *Corynebacterium fascians*. Note uprooted Shasta daisy plant exhibiting abnormal growth composed of short, hypertrophied shoots which spread horizontally at the base. **2-(A, B)** Symptoms expressed by garden pea infected with *C. fascians* from Shasta daisy. **2-A)** Three weeks after inoculation. Hypertrophied shoots emerged from buds at the cotyledonary or first node. **2-B)** Over 6 weeks after inoculation. Stimulation of lateral buds of the hypertrophied shoots and emergence of abnormal shoots from second and third nodes to produce the leafy gall symptom. **3-(A to D)** Effects of removal of apical buds on proliferation of shoots of garden pea inoculated with *C. fascians*. **3-A)** Apical buds intact, noninoculated. **3-B)** Apical buds intact, inoculated. **3-C)** Apical buds excised, noninoculated. **3-D)** Apical buds excised, inoculated.

each suspension was attempted as described above. At the time of isolation, plant parts that were buried in the wet soil were completely decomposed, while those buried in the dry soil were not. When portions of the soil suspension were plated on Kado's selective or PDA media, *C. fascians* was isolated on the two media only from the inoculated dry soil. The concentration of the bacterium, determined on Kado's medium 7 days after plating, was about 1.6×10^7 cells per gram of soil. The bacterium was not isolated from either the infested wet soil or the two noninfested control soils. Thus, when infected Shasta daisy plant remains were completely decomposed, the bacterium was no longer isolatable. In the field, decomposition of diseased plant parts is usually achieved before replanting because, as a part of the cultural practices, old plants are plowed under. Also, the soil is treated with fumigants such as methyl bromide and chloropicrin in certain farms. It is safe to assume that under such conditions, most field soils will be free from the pathogen at the beginning of the season. Planting materials must therefore be the main source of inoculum.

Root divisions were investigated as the main source of inoculum, since Shasta daisy is asexually propagated and root divisions for the following season are prepared by the growers from existing plants at the end of the season when almost all plants are fasciated. To determine whether these plants were infected, 50 of the root divisions planted in flats and maintained in the grower's greenhouse were randomly collected and grown in sterilized soil in the greenhouse at Riverside. After 2 months, over 50% of the plants were fasciated and *C. fascians* was isolated from the leafy galls of the diseased plants. The experiment was repeated with similar results.

DISCUSSION.—The increase in disease severity resulting from the removal of apical buds can best be explained in terms of hormonal imbalance. Hildebrandt (2) reported that a high ratio of cytokinin to auxin stimulated the growth of lateral buds. The removal of apical buds during flower harvest could reduce auxins in plants while the presence of *C. fascians* could increase the cytokinin content in the diseased plants (8). This condition appears to create the high cytokinin/auxin ratio which produces the leaf gall symptoms.

As infection by the bacterium progressed, multiplication, spread, and reinfection by the pathogen would be possible. This could explain the increasing severity of disease resulting from long period of infection after plants first became diseased.

Root divisions were the main source of inoculum in the daisy field. A similar conclusion was made by Lacey (5), who studied the disease on chrysanthemums. Therefore the use of clean planting materials to control the disease must be reemphasized. Root divisions must be selected from nondiseased plants and the divisions dipped in disinfectant; e.g., sodium hypochlorite solution, to preclude contaminants. Propagators could also provide growers with clean planting materials by adopting the technique of Pyeatt et al. (6): prepare the planting materials from tip cultures and then screen them against *C. fascians*.

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