Control of Acremonium Wilt of Shasta Daisy

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ABSTRACT

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Shasta daisies (*Chrysanthemum maximum*) were propagated in vitro using shoot tips (0.5 mm long) from field-grown 'Killian' daisies infected with *Acremonium strictum*. Viable explants were assayed, and those free from internal fungal and bacterial contaminants were multiplied in vitro. Commercial fumigation of naturally infested fields (sandy loam soil) at the rate of 340 kg per hectare of a 2:1 mixture of methyl bromide and chloropicrin delayed reinfection of pathogen-free plants for as long as 6 mo.

Acremonium wilt of Shasta daisy (*Chrysanthemum maximum* Ram.) was first reported in 1978 (2). Symptoms include vascular browning, chlorosis and necrosis of the lower leaves, wilting, stunting, and overall debilitation of the host. The causal agent, *Acremonium strictum* W. Gams (3), is a weak pathogen dependent on stress of the host for maximum development in the plant. Elimination of the fungus from the plant and reduction of fungal propagules in the soil are essential to control of the disease. Pyeatt et al (8) produced Shasta daisy plants through tissue culture methods, and plants were reported to be free from *Verticillium* sp. and other soilborne pathogenic fungi.

In this paper we report the feasibility of tissue culture methods and soil fumigation for control of Acremonium wilt.

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MATERIALS AND METHODS

The four steps in producing pathogen-free Killian by tissue culture techniques were: 1) production of viable explants from shoot tips, 2) assessment of explants for fungal and bacterial contaminants, 3) multiplication of explants, and 4) acclimatization and transfer of explants to soil. A fifth step, assessment of plant variation and health in commercial practice, was not completed at the time of this report.

Shoot tips approximately 1 cm long obtained in July from vigorously growing plants were washed in tap water, and all leaves longer than 5 mm were trimmed away. The tips were surface-disinfested by immersion for 10 min in a solution of 0.6% sodium hypochlorite and two drops of Tween 20 per 250 ml, then rinsed in sterile deionized water (SDW) three times. All remaining leaves were removed aseptically under magnification (×6). The final explant was no longer than 0.5 mm and consisted of the apical meristem and two or three leaf primordia. Each explant was placed in a 25 × 150 mm test tube containing modified Murashige and Skoog (6) medium (0.3 MS). The medium was modified by decreasing the amount of the six "macro" salts to 0.3 times their original concentration and adding a number of vitamins prepared in a stock solution to yield a final concentration per liter of 1 mg of thiamine HCl, 0.5 mg of nicotinic acid, 2 mg of glycine, 0.5 mg of pyridoxine HCl, and 100 mg of inositol. The pH of the medium was adjusted to 5.7 with 1N NaOH and 1N HCl before addition of the last ingredient (agar). The medium was melted in an autoclave for 7 min at 121 C, distributed into the tubes at 25 ml per tube, capped with Kaputs

(plastic test tube caps), sterilized for 15 min in an autoclave at 121 C, and cooled in a slanted position. Explants were grown under fluorescent lamps (1,000 lux) at 27 C using a 16-hr photoperiod and were transferred to fresh nutrient tubes every 4 wk. These procedures were done three times using 80 shoots each time.

The proximity of *A. strictum* to the apical meristem was tested by preparing tissue as just described. The shoot tip was aseptically sectioned into 1-mm pieces and placed in plates containing 1.5% agar and 60 μg/ml of streptomycin sulfate medium. After 6 days of incubation at 24 C, the presence of *A. strictum* or other organism was determined.

The chance that the explants were contaminated internally with an organism was explored using a technique similar to the one developed by Knauss (5) to assay *Dieffenbachia* spp. Three liquid media were used: potato-dextrose broth, Sabouraud liquid medium, and Tryptic soy broth without dextrose (Difco Laboratories, Detroit). The media were dispensed into 9 × 100 mm tubes, capped with foam rubber plugs, and sterilized in an autoclave at 121 C for 15 min. The base of each explant was aseptically sectioned into 1-mm pieces, and one piece was placed in a tube of each of the media; the tip of the explant was then placed in a fresh tube of 0.3 MS medium. The cultures that developed cloudiness were discarded along with the corresponding explant. Explants whose cultures remained clear were assayed a second time 4 wk later.

Pathogen-free explants, 12–16 wk old, were allowed to multiply in a second medium (MS+) identical to the first (0.3 MS) with the exception that the "macro" salts were used at full strength and the concentration of sucrose was increased by 50%. The plants were divided every 4 wk into sections consisting of an apical tip or three leaves attached to the stem, and these were planted in tubes containing MS+ medium.

Before being transplanted into soil, the explants were trimmed and transferred into 1-qt Mason jars containing 40 ml of 0.5 MS+ medium (MS+ modified by decreasing the concentration of sucrose to 15 g per liter). The 0.5 MS+ medium was allowed to harden with the Mason jar set on its side. Eight explants were placed in each jar and grown in increased light intensity (approximately 10,000 lux) for 3 wk. They were transplanted into steam-sterilized U.C. soil mix (1) in 5-cm peat pots and grown in a growth chamber at 24–27 C with daily watering and misting and reduced light for 1 wk. After growing 3 wk at the same temperature with normal water and light, they were placed in a greenhouse.

For isolation and estimation of the number of *A. strictum* propagules in sandy loam field soil, approximately 50 g of soil was collected from three places in the field, pooled, and thoroughly mixed by hand. Rhizosphere samples were collected, and 1 g of adhering soil was gently scraped from the roots. The soil (10 g of nonrhizosphere soil or 1 g of rhizosphere soil) was added to a 300-ml bottle containing 100 ml of SDW and shaken on a wrist action shaker for 10 min. At least three samples were taken from each soil tested, and three 1-ml samples from each bottle were further diluted in SDW to 10^{-4} g of soil (dry weight) per milliliter. Then, 1 ml was placed in each of 10 plastic petri dishes and mixed with 15 ml of rose bengal medium (4) previously cooled to approximately 47 C. Plates were wrapped in polyethylene bags and incubated in the dark for 1 wk at 24 C and examined for colonies of fungi. Colonies similar to *A. strictum* were transferred to fresh potato-dextrose agar medium in plates and incubated in continuous light to confirm their identity. This procedure was used to evaluate differences in soil populations of *A. strictum* between rows, in rows, and in the rhizosphere before and after fumigation of the field by commercial methods. Fields (sandy loam soil) previously planted to Shasta daisy were fumigated with a 2:1 mixture of methyl bromide and chloropicrin at the rate of 340 kg per hectare and covered for 7 days with a polyethylene tarp 0.025 mm thick. Soil moisture ranged from 10 to 14% and soil temperature was 28 C at the time of fumigation. A second series of experiments was performed to determine how long the fields were free from *A. strictum* after fumigation by sampling the soil of fields replanted with either infected Killian daisies or pathogen-free seedlings or left fallow. Assays were repeated once a month until the fungus was recovered from fallow soil on two consecutive months.

RESULTS

Approximately 10% of the shoot tips dissected and planted on the 0.3 MS medium were viable and free from fungal and bacterial contaminants. *A. strictum* was usually in the tissue within 2–3 mm of the tip but was occasionally isolated within 1 mm of the tip. For this reason, the size of the explant was not increased, although this would have increased chances of survival. When the plants were transferred to MS+ medium, shoot growth increased and root growth decreased, which was desirable because the roots were discarded each time the explants were divided. Explants multiplied at a mean rate of 2.5 shoots per month. Root growth increased after the explants were transferred to the Mason jars containing the 0.5 MS medium, and plants were successfully transplanted to soil. Subsequent growth in a growth chamber and greenhouse was vigorous and uniform.

A. strictum was isolated from the soil and from plant debris before fumigation (Table 1). The concentration of propagules depended on the source of the soil (proximity to the roots) and was highest in the region of the rhizosphere. Commercial fumigation reduced the population of *A. strictum* to undetectable levels. Most other fungi were affected in the same manner. *A. strictum* was reisolated from soil within 3 mo of fumigation when the fields were planted with infected stock but after 6 mo when pathogen-free stock was used (*unreported experiment*). When the soil was left fallow, the fungus was recovered after weeds began to grow, usually after 6 mo.

DISCUSSION

Perhaps the most desirable method of controlling a disease is through the use of resistant cultivars. Field observations indicated that Majestic was more resistant to Acremonium wilt than Killian, but Majestic is not as widely grown in California because the flowers do not survive shipping as well as Killian flowers do. If a breeding program were initiated to produce new selections possessing the resistance of Majestic and the shipping quality of Killian, the best possible control system could be used. Since this is not yet possible, alternate methods were investigated. In an unreported series of experiments, both benomyl and fumazone were used in in vitro growth studies of *A. strictum*. Benomyl at concentrations up to 40 mg(a.i.)/ml did not have any effect on the growth of the fungus. Fumazone did

Table 1. Recovery of *Acremonium strictum* from sandy loam field soil fumigated with methyl bromide and chloropicrin[a]

Sampling time	Test 1	2	3	4	5	Mean
Before fumigation						
Between rows	27[b]	20	2	···	···	17
In rows	5	25	20	10	···	15
In rhizosphere	130	120	···	···	···	125
After plowing	30	27	40	2	···	25
After fumigation	0	0	0	0	0	0

[a] A 2:1 mixture of methyl bromide and chloropicrin, respectively, was applied at the rate of 340 kg/ha and covered with a 0.025-ml polyethylene tarp.
[b] Values are mean number of propagules per gram of soil (dry weight × 10^{-2}).

affect growth and even killed the fungus but is not recommended because of its potential health hazards. Heat treatments (44 C for 4 hr) of infected root divisions of the plant did not eliminate *A. strictum* from the tissue and killed the plants. The host range of this fungus (7) is so wide that crop rotation could not be used effectively. Thus, the most feasible approach to control of this disease was through use of cultured cutting techniques.

Shoot tip propagation of the Killian daisy was first reported by Pyeatt et al (8). Unfortunately the method used to assay the explants for fungal and bacterial contamination was not included in the report. Our results were somewhat different with respect to size of the shoot tips used. Pyeatt and co-workers used tips 3–4 mm long; we consistently isolated *A. strictum* from this area, and only tips 0.5 mm long were free from contamination. The discrepancy in the results may be due to the time of the year in which the explants were made, since the infection is not always extensive. Secondly, *Verticillium* (the fungus Pyeatt et al were attempting to eliminate) may not have invaded the tip of the plant as extensively as *A. strictum* did. The explants we developed were successfully multiplied and transferred to soil. Their cultural characteristics will not be ascertained until they have flowered, but the vegetative characteristics appear stable at this time.

The final concern was reinfection of the plants through the soil. Our experiments showed that this was possible when pathogen-free plants were planted in proximity to infected field plants. Soil fumigation diminished the populations of the fungus in the soil to undetectable levels. When the soil was left fallow or planted with pathogen-free plants widely separated from the field stock, *A. strictum* was not recovered until 6 mo after fumigation. After that time, the plants were infected with the fungus and slight symptoms developed (unilateral necrosis and chlorosis of the lower leaves and very faint vascular browning). Because reinfection occurs, the use of pathogen-free plants each season is essential. In addition, soil fumigation at the beginning of each season would reduce the population of the pathogen in the soil and produce a period in which the plants could grow free from infection. It is difficult, at present, to assign a monetary gain to control of this disease by these measures. The growers do not have any figures on disease losses, and these control practices have not been commercially applied. In seasons in which plants are stressed severely by low temperatures or excessive rainfall, however, the practices should reduce disease losses significantly.

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