ASEPTIC PROPAGATION OF SHASTA DAISY
Progress Report

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Shasta daisy (*Chrysanthemum maximum* Ramond) is a herbaceous perennial with many cultivars that are asexually propagated. The plant has a spreading, free-rooting growth habit, which permits rapid propagation by division of the stolon-like basal stems. However, Shasta daisy is subject to infection by several species of pathogenic fungi and bacteria that affect root and vascular systems. *Verticillium* wilt is particularly troublesome to commercial growers. Such diseases are perpetuated from one planting to the next as a result of the propagation method.

In recent years, techniques have been developed for aseptic culture of many plant species. Proper aseptic procedures yield plantlets free of fungi and bacteria and thus free of fungal and bacterial diseases. The work reported here represents the search for a suitable aseptic culture procedure for Shasta daisy propagation.

PROCEDURES

Tissue used in this study was obtained from two Shasta daisy cultivars 'Thomas Killin'[1] and 'Majestic.' Two propagation methods were attempted.

Stem Node Sections With Axillary Buds

Leaves were removed from immature vegetative stem pieces 10 to 20 cm. long. The stem pieces were scrubbed with a detergent solution and divided into individual test batches for agitation in various concentrations of chemical disinfectants at several time intervals (table 1). After treatment, they were rinsed three times in sterile once-distilled water and six times in sterile

[1] Also known in the horticultural trade under such names as: 'Killian,' 'T. E. Killian,' 'T. E. Killen,' 'Thomas Killen.'

twice-distilled water to remove residual disinfectant chemical. Using a sterile transfer chamber, one-node stem sections approximately 3 mm. long were cut from the central portion of the chemically treated stem pieces and placed individually in screw-cap vials containing 10 ml. of modified White's medium (1).

Excised Shoot Tips

After hand removal of as many leaves as possible, a terminal section of stem was held under a binocular dissecting scope during shoot tip preparation. Using aseptic techniques, all remaining leaves were removed except the first four to six readily discernible leaves immediately below the apical meristem. Tissue was sliced away to produce a nearly cube-shaped cutting 3 to 4 mm. in size. No stem surface cleansing treatments were used in this propagation method. The excised shoot tip was implanted in 10 ml. of modified White's medium (1) in a screw-cap vial.

During development on modified White's medium, the plant material was exposed to a constant 70° F. air temperature and 1,500 foot-candles of light for 16 hours a day. This photoperiod did not initiate flower bud formation in the shoot tips even though flowers can be initiated in more mature plants under similar light conditions.

RESULTS

Stem Node Sections With Axillary Buds

None of the disinfectant treatments (table 1) yielded a significant percentage of plantlets that were free of contamination, without causing lethal damage.

Excised Shoot Tips

Those shoot tips that showed symptoms of fungal or bacterial contamination while on the sterile growth medium were discarded. Losses due to contamination did not exceed 10 percent on six batches, each composed of a minimum of 20 excised cuttings.

A small number of the shoot tips produced roots in the modified White's medium in approximately 1 month. Unrooted shoot tips were removed from the medium when the largest leaves were 1 to 2 cm. long. After careful washing to remove the nutrient-agar medium followed by treatment with a commercial root-inducing preparation, the shoot tips were placed in a medium composed of #2 vermiculite. Root formation occurred within 2 weeks.

The rooted shoot tips were planted in a sterile soil mix in separate containers and greenhouse grown for production of cuttings. They were provided short days (9 hours) to sustain constant vegetative growth.

The young plants were laboratory tested and found to be free of *Verticillium* and other soil-borne pathogenic fungi. Several months after initial propagation, a number of the greenhouse stock plants became infected with a disease called bacterial fasciation, caused by *Corynebacterium fascians*. The source and method of infection has not been determined. The problem of maintaining propagation stock free of bacterial fasciation requires further work.

SUMMARY

Micro-cuttings of Shasta daisy produced under aseptic conditions can be used to establish a stock of plants for continuous production of cuttings. Under greenhouse isolation and generally accepted sanitation procedures, stock plants can be maintained free of *Verticillium*, a source of economic loss to field producers of Shasta daisy flowers.

LITERATURE CITED

1. Hackett, W. P., and J. M. Anderson. 1967. "Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices." *Proc. Amer. Soc. Hort. Sci.*, 90: 365-369.

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TABLE 1. Shasta Daisy Experimental Stem Section Surface Cleansing Treatments.

Chemical	Concentration	Treatment Time With Agitation
Sodium hypochlorite (6%) (household bleach)	Full strength, 1:5 and 1:10 dilution	0.5, 2, 5, 20, 30, and 40 minutes
Amphyl®	0.5%, 1.0%	5, 20, 30, and 40 minutes
Consan® (a quaternary ammonium compound)	200 ppm, 800 ppm, and 1,600 ppm	5, 20, 30, and 40 minutes