Effects of Chrysanthemum Stunt, Chlorotic Mottle, Aspermy and Mosaic on Flowering and Rooting of Chrysanthemums

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We wish to thank Robert W. McBride for technical assistance.

Accepted for publication 8 June 1976.

ABSTRACT

HORST, R. K., R. W. LANGHANS, and S. H. SMITH. 1977. Effects of chrysanthemum stunt, chlorotic mottle, aspermy, and mosaic on flowering and rooting of chrysanthemums. Phytopathology 67: 9-14.

Chrysanthemum stunt, chlorotic mottle, mosaic, and aspermy were investigated for their effect on fresh weight, flower diameter, and stem length of 10 cultivars of *Chrysanthemum morifolium*. Chlorotic mottle, mosaic, and aspermy significantly reduced growth of tested cultivars; however, stunt reduced growth more severely than did the other diseases. Vegetative propagation of cuttings from inoculated chrysanthemums was quite variable; however, root initiation consistently was reduced in stunt-affected plants. Visible symptoms of infections generally were not apparent in the vegetative growth of chrysanthemums, which emphasized the continued need for virus- and viroid-indexing programs conducted by commercial propagators.

Virus diseases in *Chrysanthemum morifolium* (Ramat.) Hemsl. were not recognized prior to 1945. Chrysanthemum stunt first was reported to be a virus problem in 1947 by Dimock (10). By 1949, it had become a widespread and serious threat to the chrysanthemum industry. In 1973, Diener and Lawson (9) described chrysanthemum stunt as a disease caused by a viroid (ChSV). By 1950, other virus pathogens were discovered that caused problems in chrysanthemum production. Chrysanthemum aspermy virus (ChAV) was reported by Hollings in England (13) and by Brierley and Smith in the United States (2). In 1950, Keller (18) described chrysanthemum mosaic virus Q (ChMV) and this was followed by a report of chrysanthemum mosaic virus-B by Noordam (19) in the Netherlands. Mosaic viruses-Q and -B now generally are assumed to be similar strains (5). More recently in 1971, Dimock et al. (11) described the chrysanthemum chlorotic mottle pathogen as a viruslike agent affecting chrysanthemum. Romaine and Horst (20) now have shown that this agent is a viroid (ChCMV) which is similar to, but distinctly different from, ChSV. Although viroids are physically and chemically different from viruses (8), symptoms produced by both are similar. To simplify the discussions in this manuscript, all viral and viroid agents will be referred to as infectious agents.

Serious losses which nearly destroyed the commercial chrysanthemum industry (4, 22) were caused by ChSV for 3-4 years after its discovery. The damaging effects of ChAV and ChMV on the chrysanthemum also have been described (2, 6). However, vigorous control programs by specialist propagators largely have reduced losses from these infectious agents in commercial chrysanthemum cut- and pot-flower production. Control consists chiefly of using indexing procedures for obtaining a nucleus of disease-free propagating stock (3, 17). The threat of these infectious agents to the industry continues and their general significance to commercial chrysanthemum flower production has not been fully investigated. The purpose of this study was to examine the effect of these infectious agents on flower diameter, stem length, plant fresh weight, and on the vegetative propagation of several chrysanthemum cultivars.

MATERIALS AND METHODS

The chrysanthemums were grown following the accepted cultural procedures outlined in the 1974 Cornell Recommendations for Commercial Floricultural Crops (7). Rooted cuttings generously supplied by the California-Florida Plant Corp., Fremont, California, were planted 30 Jan 73, 19 Oct 73, and 2 Mar 74 to produce winter, spring, and summer flower crops. Rooted cuttings for the summer flower crop (planted 29 Mar 74) were supplied from the revegetated winter flower crop (planted 19 Oct 73). Plants were kept vegetative by supplemental lighting with approximately 100 lux of incandescent light from 2000 to 0200 hours each evening. The vegetative period was 1-5 weeks depending on the time of year and cultivar. Flowers were harvested when the flower heads were judged to be saleable. Thus, flower evaluations were made during winter, spring, and summer months. Stem lengths, flower diameters, and fresh weights were measured and recorded immediately upon harvest.

Twenty-five plants each of chrysanthemum cultivars

Bonnie Jean, #2 Blue Chip, #3 Indianapolis White, Albatross, #3 Improved Albatross, #2 Good News, Goldburst Mefo, Improved Mefo, #2 Yellow Fred Shoesmith, and #2 Yellow Iceberg were inoculated 4 weeks after the transplanting of rooted cuttings by implanting them (12) with stem tissue from either ChSV-infected cultivars Blanche, ChCMV-infected Deep Ridge, ChAV-infected Fred Shoesmith, or ChMV-infected Blanche. The revegetated summer crop plants were not reintroduced because source plants for these cuttings were inoculated prior to the winter crop. Controls consisted of 25 noninoculated plants for each cultivar. Evaluation of the effects of these infectious agents on the various cultivars was made at the time of flowering. Observations also were made of foliar symptoms and flower distortion and/or color break in flowers.

Tissue plug samples were removed from each inoculation treatment of each cultivar prior to flower evaluations to determine if the inoculations were successful. Control plants for each cultivar also were checked by the same technique. Index plant cultivars which were used to test for the specific infectious agents were as follows: Blanche chrysanthemum for ChSV, Deep Ridge chrysanthemum for ChCMV, Fanfare chrysanthemum for ChMV, and *Nicotiana tabacum* 'Samsun' for ChAV. Tissue implantation inoculations were used to test for ChSV, ChCMV, and ChMV on their respective index plants. Leaf samples from plants to be checked for ChAV were homogenized in Na_2SO_3 (0.5 g/1,000 ml distilled water), and mechanically inoculated to Carborundum-dusted tobacco leaves which were approximately 3 cm across the base of the leaf blade. Serological assays also were used to check for ChAV (16). Bioassays for ChSV and ChCMV and bio- and sero-assays for ChAV indicated that tissue implant inoculations were successful. Tissue implant inoculations with ChMV were approximately 30-40% successful. Test plants for ChSV and ChMV were maintained for 16 weeks to assure a reliable bioassay; those for ChAV and ChCMV were held 4 and 8 weeks, respectively. All test plants were maintained in a greenhouse with temperatures of approximately 27 C. Supplemental light of 10,000 lux and 14-hr photoperiod was supplied during winter months for all test plants to increase light intensity (15) and shading

TABLE 1. The effect of viral and viroid infections on flower production of pompon and standard chrysanthemum cultivars. Data from three seasons of flowering

	ChSV[a]	ChCMV	ChMV	ChAV	Control
Pompons:					
Bonnie Jean FW	57**[b]	77**	82	81**	88
#2 Blue Chip FW	77**	87**	84**	81**	112
#2 Yellow Iceberg FW	63**	93**	80**	89**	107
Standards:					
Albatross FW	161**[c]	69	54**	59**	74
FD	11.2	11.4	10.7	10.9	11.1
SL	75	79	69	73	81
#3 Improved Albatross FW	57**	68**	66**	69	76
FD	10.9	11.4	11.3	11.4	11.9
SL	68	77	73	75	82
Improved Mefo FW	73**	87	70**	81**	94
FD	11.8	12.3	11.7	12.0	
SL	81	93	85	85	96
Goldburst Mefo FW	62**	85**	80**	64**	93
FD	11.4	12.6	12.4	11.4	13.1
SL	70	85	83	75	92
#2 Yellow Fred Shoesmith FW	12**	93	87**	87**	98
FD	11.5	13.6	13.7	13.0	13.9
SL	79	88	84	81	93
#3 Indianapolis White FW	85**	108	104**	92**	111
FD	12.8	14.2	13.8	13.7	13.8
SL	77	85	85	85	87
#2 Good News FW	42**	67	55**	61**	74
FD	10.1	11.4	9.7	11.4	11.7
SL	62	77	67	70	77

[a]Abbreviations: ChSV = chrysanthemum stunt viroid; ChCMV = chrysanthemum chlorotic mottle viroid; ChMV = chrysanthemum mosaic B virus; and ChAV = chrysanthemum aspermy virus.

[b]Honestly significant difference (hsd) = 7.07 for differences in infected pompons compared to noninoculated control at $P = 0.01$. FW = fresh weight.

[c]Honestly significant difference (hsd) = 6.77 for differences in infected standards compared to noninoculated control at $P = 0.01$ FW = fresh weight, FD = flower diameter, SL = stem length.

was provided for tobacco used to test for ChAV during summer months to reduce the maximum light intensity to approximately 16,000 lux (16).

Tests to determine the effect of these infectious agents on vegetative propagation of chrysanthemum cuttings removed from previously inoculated plants were carried out on a wooden frame covered with black plastic (21). Chrysanthemum cuttings were placed through holes made in the plastic so that the base of the cutting extended below and the leaves above the plastic to receive moisture during the mist cycle. Cuttings were kept under intermittent mist for 2 wk after which the rate of root initiation on each of 10 inoculated cultivars was compared with that for noninoculated controls. The plastic cover around the edges of the wooden frame could be lifted to observe root initiation around the base of the cutting. Number of cuttings exhibiting roots was recorded on alternate days for 14 days. The accumulative sum was used to evaluate the effect on root initiation and was termed a rooting index. The experimental design was a randomized block and three replications of each treatment were used in the first experiment and five in the second.

RESULTS

The effect of ChSV, ChCMV, ChMV, and ChAV on the 10 chrysanthemum cultivars can best be described by (i) comparing flower development on inoculated plants with its noninoculated control and (ii) dividing chrysanthemum cultivars into flower types (standards and pompons) since the commercial method of marketing the two is different. The pompon type has many flower heads and is sold by weight, whereas the standard type has a single flower per stem and is marketed by flower diameter and stem length with weight an indication of quality. Statistical analysis of flower shoot fresh weight revealed there were highly significant reductions due to these infectious agents (Table 1). There also were highly significant interactions of the infectious agents with season of flowering which indicated that infections were more severe when flowering occurred in different seasons. For clarity, data in Tables 2, 3, and 4 are presented as percent reduction in the mean flower shoot fresh weight, stem length, and flower diameter compared to the noninoculated control. Infection with ChSV resulted in a 24% reduction in fresh weight on pompons that flowered in winter and a 65% reduction in fresh weight on pompons that flowered in the summer (Table 2). The effect of these infectious agents on flowering was generally more severe on crops flowered in winter than those flowered in spring (Table 2). Infection by ChSV caused dramatic reductions in fresh weights, flower diameters, and stem lengths in cultivars flowered in the summer. Although increased reduction in growth was found in the summer crop infected with ChMV and ChAV as compared to that for the spring and winter crop, the reduction was not as dramatic as that found with ChSV. Flower production losses caused by ChCMV were similar in cultivars flowered in summer and spring but more severe in those flowered in the winter.

Although no viral or viroid resistance was exhibited by any cultivar, there were differences in response by individual cultivars based on flower shoot fresh weight, stem length, and flower diameter (Table 1). For example, ChSV and ChCMV caused highly significant reductions in fresh weight ($P = 0.01$) of Bonnie Jean but ChMV did not (Table 1 and Fig. 1-A). Variation in cultivar response to these infectious agents also was observed in standard-type cultivars. Reductions in stem length of #2 Good News were caused by ChSV and ChMV, but the effect of ChCMV and ChAV was not so apparent (Table 1 and Fig. 1-B). Infection of #2 Good News with ChMV also resulted in flower breakdown as shown in Fig. 1-B.

The most severe flower production losses were caused by ChSV. Fresh weight reductions of pompon cultivars due to ChSV were 24, 30, and 65% respectively for spring, winter, and summer flower crops (Table 2). Infection by ChSV also reduced the stem lengths, flower diameters, and fresh weights by 30, 14, and 40% respectively for the summer flower crop of standard cultivars (Table 2).

Growth of pompon chrysanthemums generally was reduced more by the infectious agents than was the growth of standards (Table 2). When the results of the three seasonal experiments were combined and grouped according to flower type (pompon and standard), it was

TABLE 2. The effect of viral and viroid infections on growth of pompon and standard chrysanthemum cultivars during three growing seasons

	30 Jan 73[a]				19 Oct 73				2 Mar 74			
	ChSV[b]	ChMV	ChAV	ChCMV	ChSV	ChMV	ChAV	ChCMV	ChSV	ChMV	ChAV	ChCMV
Pompons[c]:												
Fresh weight	24[d]	16	3	10	30	18	30	30	65	25	30	17
Standards[e]:												
Fresh weight	14	11	4	2	16	16	16	16	40	22	36	8
Flower diameter	7	2	. . .	. . .	5	8	7	3	14	4	9	1
Stem length	8	3	1	3	6	9	5	4	30	20	26	6

[a]Fresh weight reductions in both pompons and standards due to infections were highly significantly different with respect to season ($P = 0.01$).

[b]ChSV = chrysanthemum stunt viroid; ChMV = chrysanthemum mosaic B virus; ChAV = chrysanthemum aspermy virus; and ChCMV = chrysanthemum chlorotic mottle viroid.

[c]Pompons–Bonnie Jean, #2 Blue Chip, and #2 Yellow Iceberg.

[d]Data are presented as percent mean reduction from noninoculated control.

[e]Standards–#3 Indianapolis White, Albatross, Goldburst Mefo, #2 Yellow Fred Shoesmith, #3 Improved Albatross, and #2 Good News.

evident that pompons were more sensitive than standards to ChSV and ChCMV, whereas the two flower types were equally sensitive to ChMV and ChAV (Table 3). Combining the results of the three seasonal experiments and grouping according to infectious agent type revealed that all the infectious agents tested can reduce chrysanthemum flower production but ChSV infections caused the most serious yield reductions (Table 4).

Cultivars Albatross, #2 Yellow Fred Shoesmith, and #2 Good News were suspected of carrying a latent infectious agent ChCMV-NS (14), which protects against ChCMV. Attempts to recover ChCMV from these inoculated cultivars were unsuccessful. Recovery inoculations to Deep Ridge (used as a test plant for ChCMV) were challenged with ChCMV after 10 days and no symptoms were expressed after 30 days. This would indicate that #2 Yellow Fred Shoesmith, Albatross and #2 Good News contained ChCMV-NS. No other cultivars carried the latent strain. The effect of ChCMV-NS on chrysanthemum growth is not known.

The results of two experiments to determine the effect of infection on vegetative propagation of chrysanthemum are presented in Table 5. Root index is the accumulative summation of number of cuttings exhibiting roots recorded on alternate days for 14 days. Root systems capable of supporting vegetative growth developed after 14 days on infected cuttings; however, root initiation was retarded by ChSV in both experiments whereas variable results on the effect of ChAV, ChMV, and ChCMV were obtained between the two experiments. The rooting index of the noninoculated control was high in both experiments which would indicate that infections apparently reduced the initiation of roots on cuttings. The variability from experiment to experiment may have been due to an interaction between effects of infection, the time of year the experiments were performed, and the rooting conditions (i.e., water, temperature, and minerals).

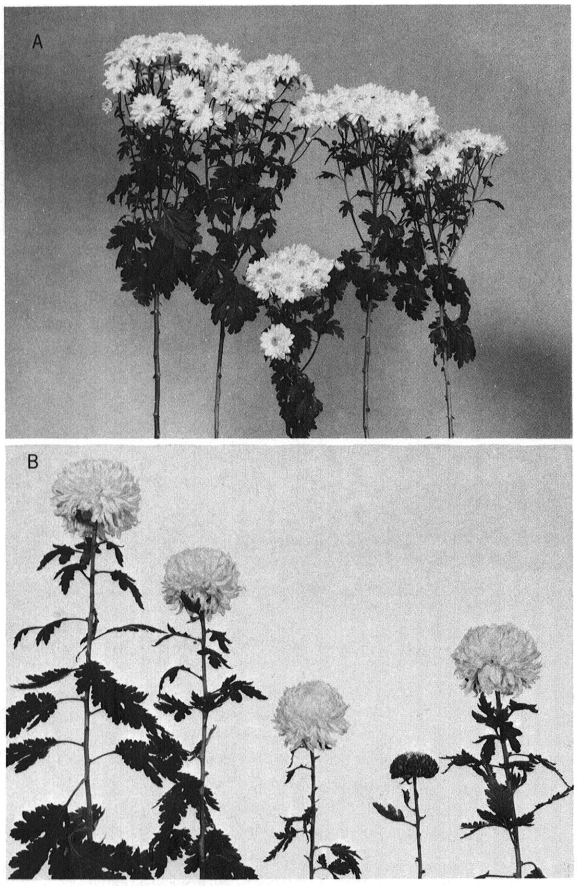

Fig. 1-(A, B). Symptoms on chrysanthemum flowers infected with various infectious agents. L to R: noninoculated, inoculated with chlorotic mottle, stunt, mosaic and aspermy. **A)** Cultivar Bonnie Jean and **B)** Cultivar #2 Good News.

DISCUSSION

The effect of ChSV on reduction in flowering on all tested cultivars was far more severe than any of the other tested infectious agents. Mean calculations of flower diameter and stem length on all cultivars showed that ChSV caused a 9% reduction in flower diameter and a 15% reduction in stem length, whereas ChMV and ChAV caused 4 and 5% reduction, respectively, in flower size and 10 and 11% reduction, respectively, in stem length (Table 4). Reductions in flower diameter and in stem length caused by ChCMV (1% and 5%, respectively) were less than those recorded for the other infectious agents.

Grafting procedures were used in earlier transmission studies of ChSV (3); however, tissue implants have been used more recently (1, 12). The tissue implant procedure was very efficient in transmitting ChSV and ChCMV in the studies reported herein; 100% recovery of the

TABLE 3. Effect of viral and viroid infections on fresh weight of pompon and standard flower-type chrysanthemums

	Flower types	
	Pompons	Standards
Stunt viroid	36[a]	24
Chlorotic mottle viroid	17	7
Aspermy virus	19	17
Mosaic virus	20	15

[a]Mean percent reduction in fresh weight from noninoculated control. The percentage is based on weights of 75 plants from three flower crops.

TABLE 4. Total reduction in fresh weight, stem length, and flower diameter of 10 chrysanthemum cultivars due to viral and viroid infections during three growing seasons

	Stunt viroid	Chlorotic mottle viroid	Aspermy virus	Mosaic virus
Fresh weight	29[a]	10	18	17
Stem length[b]	15	5	11	10
Flower diameter[b]	9	1	5	4

[a]Percent reduction from noninoculated control of fresh weight, stem length, and flower diameter.

[b]Stem length and flower diameter of only standard-type cultivars.

infectious agents was ascertained from inoculated plants. Tissue implantations of ChAV also were successful; however, ChMV was not so efficiently transmitted by implantation procedures. Approximately 65% of the implant inoculations made with ChMV were not successful which lends support to a previous report of inefficient transmission of ChMV with tissue implants (11). Recovery of ChMV from inoculated plants was not readily attained, but data for cultivars such as #2 Good News, which exhibited severe foliar and flower symptoms in response to ChMV, indicate that 30 to 40% of the ChMV inoculations were successful. Attempts to recover infectious agents other than ChMV from these plants were unsuccessful.

Flower damage resulting from infection was much more severe in the crop flowered in the summer; whereas, reduction in growth was not so pronounced in the crop flowered in the spring and winter (Table 2). Whether this effect is due to incubation time of the various infectious agents in the host plant or whether it is due to revegetation and/or seasonal effects is not known; moreover, the experiments were not designed to make this determination.

That these infectious agents fail to induce symptoms until flowering of the crop poses a serious threat to the chrysanthemum industry. Thus, a grower with a crop of plants without visible external symptoms until flowering may then sustain a significant reduction in quality and saleability of the crop. The latent infectious agent which protects against ChCMV also may be a potential threat to the industry since at the present time little is known about its ability to cause damage to the various chrysanthemum cultivars. There is no good test for ChCMV-NS other than the test for protection against ChCMV in the test plants (Deep Ridge) used to index ChCMV (14). Since all these infectious agents can be present in chrysanthemum cultivars yet not cause symptoms in the vegetative growth of the plants, commercially propagated chrysanthemums can be carriers. Because it also was demonstrated that these infectious agents can reduce root initiation in some cultivars, their presence in chrysanthemum stock plants can be vitally important to the commercial propagator. The presence of ChSV in #3 Indianapolis White and #2 Improved Albatross resulted in a 2- to 5-day delay in rooting which for commercial propagators can be a significant factor. In addition, the presence of ChCMV-NS in Albatross and #2 Good News may be important in vegetative propagation since the rooting index of the noninoculated controls in one experiment in which the latent strain was present was much lower than the rooting index of cuttings known to be infected (Table 5). Although root initiation was not reduced as much by the infectious agents in experiment #1 as in #2, ChSV reduced the root index in both experiments from all cultivars. In addition, it is very difficult to identify infections during rooting since symptoms are not readily detected. These results underscore the importance of indexing programs performed by commercial propagators in providing the chrysanthemum industry with plant material that is free of these infectious agents.

TABLE 5. Effect of viral and viroid infections on root initiation of 10 chrysanthemum cultivars

	Root index		
	Exp. #1	Exp. #2	Avg.
Control	77[a]	190	134
Stunt viroid	59	168	114
Chlorotic mottle viroid	79	172	126
Aspermy virus	66	189	128
Mosaic virus	84	150	117

[a]The accumulative sum of number of cuttings on which roots were initiated and which were recorded on alternate days for 14 days and are averages of 10 cultivars.

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