Effect of Cultural Practices on Infection of Florists' Carnation by Gibberella zeae

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ABSTRACT

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Several cultural practices used in greenhouse production of carnations for cut flowers were examined for their effect on the incidence of infection by *Gibberella zeae*. Cold storage of rooted, but not of unrooted, cuttings prior to planting increased the incidence of infection by *G. zeae* when the cuttings were inoculated after planting. Shears used to cut flowers were effective inoculating devices. Vegetative stems were less susceptible to infection than reproductive stems. Shorter stubs were more susceptible to infection and infections in shorter stubs were more likely to expand into severe lesions. Fresh stubs were infected at higher rates than older stubs. Manipulation of these cultural practices may reduce incidence of Fusarium stub dieback.

Fusarium stub dieback is part of a stem-rotting disease complex of carnation, *Dianthus caryophyllus* L. The disease complex has been reported as being caused by *Fusarium culmorum* (W. G. Smith) Sacc., by *F. avenaceum* (Fr.) Sacc., and by *Gibberella zeae* (Schw.) Petch. (imperfect state = *F. graminearum*) (2, 9).

Control measures include protectant or systemic fungicides and sanitation (1, 5, 6, 7). Since the crop is managed intensively, changes in cultural practices may give some control without increasing production costs.

In commercial practice, carnations are propagated by rooting terminal cuttings taken from large stock plants. Nonrooted cuttings may be kept in cold storage for up to 6 mo and rooted cuttings for 3 to 12 wk (3, 7). Holley and Baker (3) reported that cuttings stored unrooted were more resistant to Fusarium stem rot during propagation than were nonstored cuttings. Factors related to the position on, and culture of, stock plants from which cuttings are taken affect the crop grown from these cuttings long after they have been planted (3, 7), but no effect of cold storage of cuttings on flower production has been found (3).

Breaking off rather than taking cuttings with a knife will minimize spread of *Fusarium* spores (7, 14), but it is not practical to break off flowers. Some growers have resorted to sterilizing the flower-cutting tools at frequent intervals to reduce inoculum carryover from stub to stub.

Several weeks after rooted cuttings are planted, they are pinched (the end of the shoot broken off) to induce branching. The stage of development of the main shoot when pinched is determined by the desired timing of the first crop (3, 7). Susceptibility of the plant to stub dieback at various stages of shoot development has not been reported.

The first crop of flowers is borne on the axillary side shoots produced after pinching the young carnation plants. After these flowers are cut, secondary side shoots develop to produce another crop.

The objective of this investigation was to determine the effect of cultural practices on the susceptibility of carnation to infection by *G. zeae*.

MATERIALS AND METHODS

Inoculum preparation and plant inoculation.—The original isolate of *G. zeae* used in these studies was #R-762 from the Fusarium Research Center, The Pennsylvania State University, University Park, PA 16802. Inoculum preparation and inoculations were done as described by Stack et al. (13).

Carnation culture.—Carnations were planted and grown as previously described (13). Some experiments were done in a greenhouse maintained at a minimum temperature of 15 C. Other experiments were done in a controlled-environment growth chamber (Model M-1, Environmental Growth Chamber, Integrated Development and Manufacturing Co., Chagrin Falls, OH 44022) set to maintain the following conditions: temperature 20.5 ± 1 C nights, 26 ± 1 C days, 14-hr photoperiod with illumination of 21 klx warm-white fluorescent plus 8% incandescent (40w, 220 v bulbs operated at 125 v) separated from the plants by a 0.7 cm Plexiglas barrier. Some inoculated plants were placed in a

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high-humidity chamber (4) after inoculation for a short period of time.

Disease ratings.—The ratings reported for these experiments are given as incidence of infection calculated on the basis of counts of plants showing lesions according to the method reported by Stack et al. (13).

RESULTS

Storage of cuttings.—In one experiment, cuttings of the cultivar, Improved White Sim, were stored unrooted in polyethylene bags in boxes at 0.5 C for 6, 8, or 10 wk. These cuttings together with fresh, nonrooted cuttings were rooted and planted in plastic pots in the greenhouse. After 5 wk, stems were cut and inoculated with *G. zeae*. Disease ratings were recorded 6 wk after inoculation.

In a second experiment, cuttings of Colorado White Pike's Peak were graded for uniformity by length and by weight, packed into polyethylene-lined boxes, and placed in a refrigerator at 3.5 C (stored) or rooted immediately (nonstored). Samples of cuttings were removed from storage after 4 and 10 wk, rooted, and treated the same as nonstored cuttings. All plants were grown in a chamber under identical conditions. Four wk after planting, the plant stems were cut and inoculated with *G. zeae*. Disease ratings were recorded 4 wk after inoculation.

Commercially-supplied rooted cuttings of Improved White Sim were placed in storage at 3.5 C. One month later these cuttings were removed and together with nonstored rooted cuttings, were planted and placed in the greenhouse. Plant stems were cut and inoculated with *G. zeae* 5 wk after planting; 5 wk later, disease ratings were made. This experiment was repeated once.

In a fifth experiment, rooted cuttings of Improved White Sim were stored at 0.5 C for 2 mo and then planted along with rooted cuttings that had not been stored. After 6 wk in the greenhouse, all plants were cut and inoculated with *G. zeae*. Eight wk after inoculation disease ratings were recorded.

The incidence of infection in young plants from stored rooted cuttings inoculated with *G. zeae* was significantly greater than that in plants from unstored rooted cuttings ($P = 0.05$) in two experiments (38% vs. 22% and 37% vs. 20%) and showed the same trend in the third (20% vs. 12%). When cuttings were stored before rooting, there was no effect of storage on subsequent infection by *G. zeae*.

Carriage of inoculum on cutting tool.—Plants of cultivars Improved White Sim and Scania were planted and grown in the greenhouse for 14 wk with supplemental lighting (6.3 klx warm-white fluorescent) that provided a 16-hr photoperiod. Plants were branched and had an average of three lateral flowering shoots when all these stems were cut with a pruning shears in the third or fourth internode above the origin of the branch. In noninoculated controls, the shears were dipped in 95% ethanol and flamed between cuts. In inoculated controls, shears were also dipped and flamed between cuts, and a droplet of a spore suspension of *G. zeae* was placed on each cut stub. To test the transmission of inoculum on shears, 12 stems were cut in succession with shears that had been dipped into a spore suspension (100 conidia/μliter) of *G. zeae*. This process was repeated five times, the shears being dipped in ethanol and flamed between each series. Following inoculation, all plants were placed in a high-humidity chamber for 1 wk, then returned to the greenhouse for 4 wk after which disease ratings were recorded. Noninoculated plants showed 24% lesions, plants inoculated by one drop of spore suspension had 53%, and those inoculated by infested shears had 54%. The latter values were significantly greater than the noninoculated controls at $P = 0.01$. There were no differences in incidence of infection among the serially cut stubs.

Stage of plant development.—Seven experiments were done to compare the susceptibility of carnation plants at different stages of plant development; five compared vegetative versus budded shoots and two compared budded shoots versus shoots with open flowers. Three of these were done in the greenhouse and four were done in a controlled-environment chamber. In each experiment plants were grown for 4 to 6 wk prior to cutting and inoculation. After inoculation, plants were placed in a high-humidity chamber for 1 wk. After 4 wk, disease ratings were recorded. Carnation plants increased in susceptibility to *G. zeae* as they developed from vegetative to reproductive through flowering (Fig. 1). Significantly higher incidences of infection were found in budded plants than vegetative ones and plants with open flowers had more infection than budded plants. This relationship was consistent in experiments carried out in different environments although specific infection level varied considerably from experiment to experiment.

Effect of length of stub left at pinching.—Observations on the effect of stub length after pinching were taken from eight separate experiments using cultivars that differed in susceptibility (12). The entire group of stubs exhibited a normal distribution for length with mean = 20.3 mm and SD = 4.5 mm based on a total of 2,626 separate stub measurements. Four of these experiments were done in the greenhouse, the other four in the chamber. Rooted cuttings were inoculated 4-6 wk after planting. Infection ratings were recorded after 4-5 wk.

Length of stubs was measured at the time that infection was determined and placed into one of four arbitrarily selected size classes (0-10, 11-20, 21-30, 31 + mm long) so that for each length class there was a count both of the total number of stubs and of the number of those stubs which showed lesions.

A significant negative correlation ($r = -.93$, $P = 0.05$) occurred between stub size and infection both for incidence of all infections and for incidence of severe lesions (Table 1). However, there was only a 5% difference from high to low infection incidences in the counts of all infections. The distribution of severe infections showed a highly significant concentration in the small-stub class ($P < 0.01$).

In a separate experiment, rooted cuttings of Improved White Sim were planted and grown in a chamber for 3 wk. At that time half of the plants were randomly selected and stems were cut leaving long stubs (mean = 34.7 $\pm$ 8.2 mm SD); the remaining plants were cut close to the node leaving short stubs (14.5 $\pm$ 3.3 mm SD). All plants were inoculated after cutting. Four wk later disease ratings were recorded. Infection was 25% in the long (35 mm) stubs and 46% in the shorter (15 mm) stubs. This difference was significant ($P = 0.05$).

Stub age.—Plants of Colorado White Pike's Peak were planted in a greenhouse bench and grown at 11 C at night and 15.5 C during the day and the crop brought into flower. Near the end of the cropping period, 116 plants were selected (about 30% of all plants) which met the following criteria: at least three cut flower stubs between 4 and 6 wk old but showing no dieback, and at least one remaining flower or bud yet to be cut. The plants were located at random throughout the bench (0.92 × 10.4 m). On each plant, two stubs of the same age were selected and one was recut at 0.5 cm below the original cut surface to expose fresh tissue. All of the paired stubs were inoculated with *G. zeae*. All other stubs on the plants were left as noninoculated controls. Four wk after inoculation, disease incidence was recorded. Isolations were made on potato-dextrose agar from noninoculated, and recut inoculated stubs.

There were significantly more infections ($P = 0.01$) in the recut stubs (98/116) than in those not cut but inoculated with *G. zeae* (52/116). There were also significantly ($P = 0.05$) more lesions in the old stubs inoculated with *G. zeae* than in the old, noninoculated stubs (52/116 vs. 73/235, respectively). Only *G. zeae* was isolated from inoculated stubs. *Gibberella zeae* was not isolated from the noninoculated stubs.

In another experiment, rooted cuttings of Improved White Sim were planted in the greenhouse. After 5 wk, the stems were cut off and the stubs inoculated with *G. zeae* at 0, 1, 2, or 4 wk after cutting. In addition some 4-wk-old stubs were recut before inoculation. All plants were placed in a high-humidity chamber for 1 wk following inoculation, then returned to the greenhouse for 4 wk after which disease ratings were recorded.

There were significantly fewer infections in stubs where inoculation was delayed 2 or 4 wk after cutting (Fig. 2). When 4-wk-old stubs were recut to expose fresh tissue, there was a significant increase in incidence of infection over similar stubs inoculated but no recut (Fig. 2).

DISCUSSION

From a horticultural standpoint (3), there is usually no discernible deterioration or reduction in quality of the

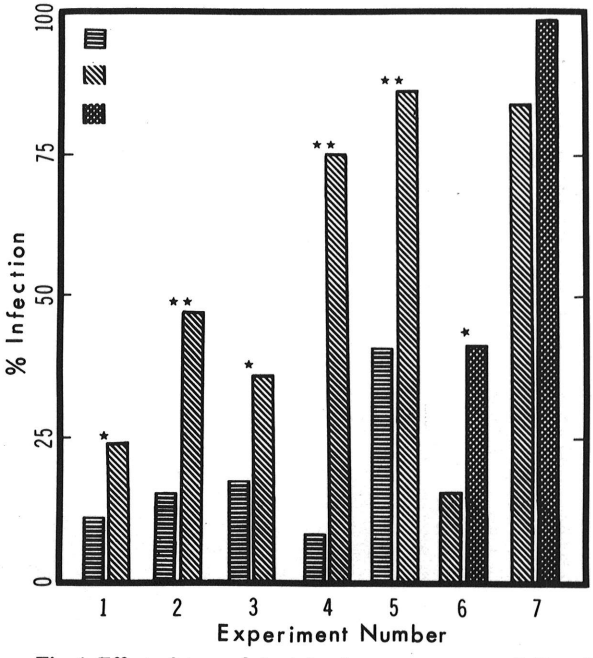

Fig. 1. Effect of stage of plant development on susceptibility of carnations to infection of *Gibberella zeae*. Percentage of plants that showed lesions when inoculated with *G. zeae* at different stages of development. Each experiment (1 to 7) was conducted separately. Statistically significant differences within experiments are indicated by asterisks (*, $P = 0.05$; **, $P = 0.01$) above the bars.

TABLE 1. Relationship between carnation stub length and susceptibility to *Gibberella zeae*

A. All Infections	Stubs per class (length in mm)				
	0-10	11-20	21-30	31+	Total
All stubs (no.)	96	1,100	1,007	189	2,392
Infected (no.)	37	398	338	64	837
Incidence of infection (%)	38.5	36.2	33.5	33.8	35.0

χ^2 (chi-square) = 1.55 N.S.[a]
r = −.93 (P = .05)

B. Severe infections (past 1 node)	Stubs per class (length in mm)				
	0-10	11-20	21-30	31+	Total
All stubs (no.)	44	392	274	38	748
Infected (no.)	29	161	74	10	274
Incidence of infection (%)	66.0	41.1	26.9	26.3	36.7

χ^2 = 20.5 (P = .005)[a]
r = .93 (P = .05)

[a]Chi-square calculation expected = (overall infection rate) × (number of stubs in class).

plants which develop from stored rooted cuttings, but susceptibility to *G. zeae* of plants produced from stored rooted cuttings is increased. Any tendency for changes to occur during storage was probably emphasized by our use of 3.5 C instead of the recommended 0 C; however, the same increase in susceptibility was seen after 2 mo of storage at 0.5 C. Storage of nonrooted cuttings did not affect the susceptibility of resultant plants. Storage of rooted cuttings should be kept to a minimum and eliminated if possible, particularly for growers in the eastern USA where stub dieback and stem rot are a continuing threat.

Flowering carnations are more susceptible to *G. zeae* than vegetative plants and our results are consistent with observed increases in susceptibility to *G. zeae* of flowering corn and wheat (8, 10).

Although crop considerations determine the timing of shoot cut (pinch), a grower faced with the likelihood of severe Fusarium stub dieback or stem rot might wish to plan his crop so that young plants are pinched as early in their development as possible. Young plants should be pinched by breaking off shoots, just as in taking cuttings, because an infested tool may disseminate inoculum. Since there was no reduction in infection after 12 successive cuts, it is likely that such an infested tool could serve to inoculate a large number of stems. A single nodal region in a carnation stem lesion may release 40,000 to 80,000 macroconidia after wetting for 1 min (11). A tool can easily pick up enough spores from one cut through such a stem for many inoculations.

One may theorize that the decreased susceptibility of large stubs occurs because longer stubs dry out more readily, or the distal part of the internode may somehow differ from the proximal. It is also possible that the distance from the node affects levels of hormones, photosynthates, or minerals. The conclusion for the grower is obvious; stubs should be left as long as possible, with a minimum length of 2 cm above a node to minimize the chance of infection.

The observation that older stubs are less susceptible than freshly cut ones is not surprising. If a stub is examined a few weeks after it has been cut, a dark green band can be seen across the stub just below the dried-out area. The region of cellular proliferation on its outer surface has a layer of distorted cells which take no aqueous stain in fresh section. This wound response may limit infection in older stubs. The infection rates will be fairly high with such "healed" stubs but lower than with fresh stubs.

The parallels between the effect of host-related factors on Fusarium dieback of carnation and Gibberella stalk rot of corn, also caused by *G. zeae*, are too frequent for coincidence. In corn increased susceptibility was related to sugar levels in the stalk (8). Whether the phenomenon in carnation is strictly physiological or a combination of morphological and physiological factors is not understood. We suggest that Fusarium stem rot of carnation may provide a good system for investigating the physiology of pathogenicity of *G. zeae*, with some implications for the development of Gibberella stalk rot.

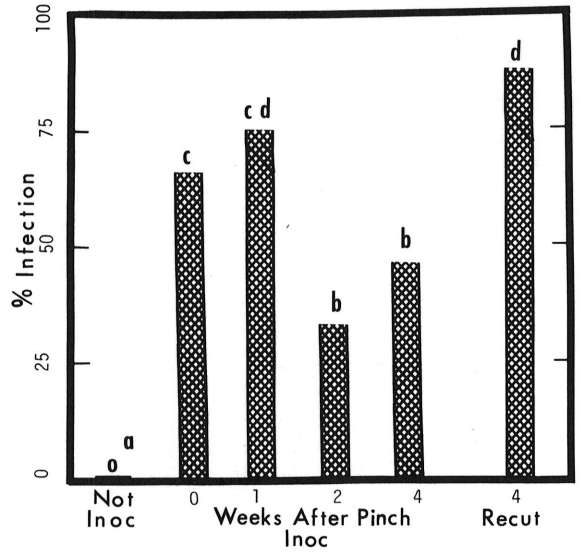

Fig. 2. Infection of carnation stubs by *Gibberella zeae* inoculated at several time intervals following cutting of the stem. Bars over which same letters appear are not significantly different, $P = 0.05$. Percent of stems with lesions in plants inoculated with *G. zeae* at four time intervals after cutting the stem or noninoculated.

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