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FUSARIUM DISEASE OF GLADIOLI CONTROLLED BY INOCULATION OF CORMS WITH NON-PATHOGENIC FUSARIA[1]

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Abstract. **Several experiments conducted in field plots showed that inoculation of gladiolus corms with certain isolates of** Fusarium moniliforme **'Subglutinans' Snyd. & Hans. and** F. solani **protected the corms from infection by the Fusarium yellows and corm rot pathogen,** F. oxysporum **f. sp.** gladioli **(Massey) Snyd. & Hans. Protection with** F. moniliforme **isolate M-685 was equal to that obtained with benomyl (Benlate) corm dip treatment. M-685 was superior to benomyl where corms were also inoculated with the pathogen either before or after inoculation with M-685. All experiments were conducted with commercial corm stocks from flower farms. Inoculations were made within a week after corm harvest. The results indicate that Fusarium control programs should include consideration of soil conditions and corm inoculations that may provide biological control. The protection of pathogen-free propagules by inoculation with nonpathogens can be important in controlling soil-borne pathogens on clonally propagated stocks.**

Corm rot caused by *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyd. & Hans. (3, 8, 11) is the most damaging disease of gladiolus in warm growing areas of the world. In addition to corm rot, the disease causes root rot, stunted plants and blind flowers (2, 3, 5, 10). Corm infections tend to remain dormant until activated by warm weather, ammonium fertilizers and high levels of nitrogen (5, 9, 13, 14). Soils become infested where corms are planted (5, 7). Treatment of commercial corm stocks with carbon dioxide caused 80 to 100% of corms to rot before flowering due to activation of latent Fusarium infections (6). Because of the latent infections, the disease has been difficult to control (5).

Many gladiolus cultivars are tolerant but none are truly resistant to Fusarium disease (4, 12). In addition to *F. oxysporum,* some isolates of *F. roseum* (Lk.) Snyd. & Hans. and *F. moniliforme* (Sheld.) Snyd. & Hans. were pathogenic to gladiolus corms under laboratory conditions (15). In exploring the probability of other Fusarium species being primary pathogens of gladiolus, these and other Fusarium isolates from corms, caladium tubers and citrus root were used to inoculate corms by a procedure used previously (6). *F. moniliforme* 'Subglutinans' isolate M-685 produced healthier plants and better corms than noninoculated controls (15). This paper reports on the biological control of Fusarium corm rot with isolate M-685.

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General Methods and Materials

Corm stocks donated by flower growers[3] were used in all experiments. Corms at least 12-14 cm in circumference were used. Corms were cleaned (mother corms and roots removed) within 24 hours of harvesting. Inoculations with a pathogen or nonpathogen were made within 24 hours after corm cleaning.

Inocula were grown on a reciprocating platform shaker at 24°C. The liquid culture medium contained the following made to a liter in deionized water: 50 g glucose, 1.71 g NH_4NO_3, 1.36 g $NaH_2PO_4 \cdot H_2O$, 0.58 g KCl, 0.89 g $MgSO_4 \cdot 7H_2O$, and 0.5 g $CaCO_3$. Micronutrients were included at the rate of 0.05 ppm each of Cu and Mo and 0.5 ppm each of Fe, Mn and Zn. Good growth was obtained. Inocula were prepared for each batch of corms by using 1 liter of blended liquid culture suspension made to 12 liters with tap water. Corms were placed in the Fusarium suspensions, or in water for 2 seconds. Corms were then drained and placed in polyethylene bags for a 24-hour moist incubation at 24-28°C. The inoculated and noninoculated lots of corms were transferred to individual corm storage trays to air dry at 24-28°C for 1-2 days. After a one week curing period, corms were stored at 4°C with adequate ventilation until planting time.

Specific Methods and Results

Experiment 1

Methods and materials. 'Friendship' corms (16-20 cm) were harvested on December 29, 1975, then cleaned and inoculated on December 30. Inocula consisted of conidia (800,000 to 1,200,000/ml) prepared from 5-day old shake cultures of Fusarium isolates listed in Table 1. Freshly cleaned corms were dipped in the spore suspensions for 2 seconds, then held moist for 45 hours at 22°C in plastic bags. For each isolate, 210 corms were used. Another lot of 210 corms was dipped for 10 minutes in benomyl 1 g/liter plus captan ai 2 g/liter and the last lot was held as un-

[3]The author thanks the Manatee Fruit Company and Roman J. Claprood Company for the gladiolus corms used in these experiments.

Table 1. Gladiolus flower and corm production in relation to treatment of mother corms with nonpathogenic Fusarium isolates or fungicides. Experiment 1.

Treatment and inoculum source	Number flower spikes	Average length spike (cm)	Number corms	
			Sound	Rotted
Control uninoculated	98 bc[z]	101.2	130 b	61 b
F. moniliforme M-669 Gladiolus corm	112 ab	104.5	125 b	61 b
F. moniliforme M-685 Gladiolus corm	117 ab	105.4	166 a	15 c
F. solani 3505A Caladium tuber	117 ab	101.9	168 a	16 c
F. solani CF-12 Caladium tuber	89 cd	101.4	104 b	80 a
F. solani FTC-733 Citrus root	78 d	98.3	106 b	83 a
Benomyl 1 g + captan 2 g/liter (10 min.)	127 a	105.8	178 a	11 c

[z]Means in a column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).

treated control. After incubation, corms were held in open trays at 6°C until planted on March 3, 1976 in Fusarium-infested soil where 5 gladiolus crops had been grown since 1969. Treatments were replicated 4 times and were randomly assigned to plots within blocks. Each plot contained 50 corms. Numbers and lengths of flower spikes and numbers and weights of corms were recorded.

Results. No differences were found in time of sprout emergence, earliness of flowering, or length of flower spikes. Numerically, the highest production of sound corms and flower spikes came from corms treated immediately after harvest with benomyl and captan dip; however, yields of spikes and corms for M-685 and 3505A treatments and spikes for M-699 were not significantly different from those for the benomyl/captan treatment (Table 1). Isolates CF-12 and FTC 733 increased the number of diseased corms and the latter produced fewer spikes than noninoculated control. M-699 treatment was not effective in controlling corm rot.

Experiment 2

Methods and materials. 'Friendship' corms were harvested and cleaned on March 1 and 2, 1976. On March 2, 800 corms (10-12 cm) were inoculated with the gladiolus pathogen, *F. oxysporum* f. sp. *gladioli* isolate 001 (480,000 conidia/ml) and held moist for 3 days. On March 5, 200-corm lots were treated as follows: 1) control, untreated; 2) benomyl 50W 1 g ai + thiram 65W 3 g ai/liter; 3) inoculated with 4.25 million conidia/ml *F. moniliforme* 'Subglutinans' M-685, incubated 3 days; and 4) inoculated with 2.8 million conidia/ml *F. moniliforme* 'Subglutinans' M-669, incubated 3 days.

After 24 weeks storage in open trays at 6-7°C, diseased corms were discarded and 180 of the healthiest in each lot were planted on September 10 in replicated, randomized plots of 45 corms each. Data were obtained on severity of disease symptoms and on number of flower spikes and sound corms. Flowers were cut during December-January and corms were harvested on March 23.

Results. Benomyl/thiram treatment resulted in the most flower spikes and corms but not significantly more than were produced by the corms inoculated with M-685 culture (Table 2). Inoculation with M-699 gave results similar to those from untreated corms.

Table 2. Gladiolus flower and corm production from corms treated with nonpathogenic Fusarium isolates or fungicides.[z] Experiment 2.

Treatments	Index of disease symptoms[y]	Number flower spikes	Number sound corms	Weight corms, ounces
Untreated control	1.8 b[x]	99 b	116 b	226 b
Benlate/thiram	0.5 c	152 a	171 a	378 a
M-685 culture[w]	0.9 c	142 a	163 a	361 a
M-669 culture[w]	2.6 a	86 b	110 b	200 b

[z]For each treatment there were 180 'Friendship' corms planted in 4 replications.
[y]0 = no symptoms, 4 = most severe symptoms on plants at early bloom period.
[x]Means in a column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).
[w]Isolates of *F. moniliforme* 'Subglutinans' from gladiolus corms.

Experiment 3

Methods and materials. Corms of 'T-590' cultivar (12-14 cm) were dug on March 23, 1976, cleaned on March 24 and 800 were immediately inoculated with a 5-day old culture of *F. oxysporum* isolate 001 at 560,000 conidia/ml. Inoculated corms were held moist for 42 hours at 22-25°C. Another lot of 800 corms was held dry in a shed. On March 26, 200 inoculated corms and 200 noninoculated corms were used as experimental units for the following treatments: 1) placed in mesh sacks and held as controls; 2) dipped for 10 minutes in benomyl 50W 0.1% ai + captan 50W 0.2% ai; 3) dipped for 2 seconds in conidial suspension (2,000,000/ml) of culture M-685; and 4) in conidial suspension (2,200,000/ml) of culture M-669. The inoculated corms were held in plastic bags at 22-24°C until March 29. Corms were stored at 7-8°C from April 5 to September 17. The sound corms were planted on September 22, 1976. Flower spikes and corms were harvested and counted.

Results. Inoculation with M-685 was as effective in disease control and flower and corm production as fungicidal treatment on noninoculated corms and significantly better than benomyl/captan in protecting against Fusarium rot and in promoting flower production from corms inoculated with the pathogen (Table 3). Inoculation with M-669 gave results intermediate between M-685 and untreated.

Table 3. Gladiolus flower and corm production from corms treated with a pathogenic Fusarium and nonpathogenic Fusarium isolate or with a fungicidal dip. Experiment 3.

Treatments	Inoculated with pathogen[z]	No. sound corms after storage	Number flower spikes	Number corms harvested 2/16/77
Uninoculated check	Yes	58 d[y]	6 e	0
	No	112 bc	45 d	38 d
Inoculated M-669[x]	Yes	132 ab	82 c	62 c
	No	138 ab	116 b	88 b
Inoculated M-685[x]	Yes	146 ab	128 ab	114 a
	No	150 a	138 a	126 a
Benomyl 1 g + captan 2 g/liter dip	Yes	102 c	62 d	58 c
	No	142 ab	134 a	124 a

[z]Corms dug on March 24; 800 of 1600 corms inoculated with *F. oxysporum* isolate 001 on March 24, 1976.
[y]Means in a column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).
[x]*F. moniliforme 'Subglutinans'* isolates from gladiolus corms. These and the fungicides were applied to corms on March 26, 1976.

Experiment 4

Methods and materials. 'Friendship' corms (14-18 cm) were lifted and cleaned on May 7, 1976. The following day 500 corms were treated 15 minutes in benomyl 50W 1 g ai plus dichloran 75 W 2 g ai per liter. Another 500 corms were inoculated by dipping for 1 minute in a suspension of 800,000 conidia per ml of *F. moniliforme* 'Subglutinans' M-685. The third lot of 500 corms was held as untreated control. After storage at 4°C for 17 weeks, the corms were planted in 5 replications of 100 corms per plot. Data on number of flower spikes and corms and weight of cormels were obtained.

Results. Inoculation with culture M-685 was as effective as the fungicidal treatment in flower and cormel production and more effective in corm production (Table 4). Untreated controls produced fewer corms and less weight of cormels than did the fungicide or M-685 isolate treatment.

Experiment 5

Methods and materials. Corms and cormels harvested on January 26, 1977 in Experiment 4 were tested as follows to determine any carry-over effects of the previous season's treatments. Pieces of cores of 10 corms chosen at random from each treatment used in Experiment 4 were plated out on potato dextrose agar. The other corms and cormels were

Table 4. Gladiolus flower and corm production from corms treated with a nonpathogenic Fusarium isolate or fungicidal dip. Experiment 4.

Treatment	Number flower spikes[z]	Number sound corms	Weight of cormels (g)
Control, uninoculated	370 b[y]	391 c	415 c
Inoculum: M-685 of *F. moniliforme*	490 a	527 a	1405 a
Dip: benomyl 1 g + dicloran 2 g/liter	442 ab	479 b	1069 ab

[z]500 corms (12-14 cm) used for each treatment.
[y]Means in a column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).

inoculated with *F. oxysporum* isolate 001 as in Experiment 2. They were cured, held in storage at 6°C for 17 weeks, and 100 corms (size 16-20 cm) and 500 cormels harvested from each treatment were planted, without further treatment, in replications of 25 corms and 100 cormels per plot. Data on disease symptoms and flower and corm yields were obtained.

Results. F. moniliforme was cultured consistently from corms produced from the corms inoculated in the previous year with *F. moniliforme* M-685 (Table 5). Only *F. oxysporum* types were isolated from corms derived from those treated with fungicides or untreated. Daughter corms and cormels from the M-685 inoculated corms were protected from disease and produced more flowers and corms than the corms and cormels derived from corms that were not inoculated with M-685, indicating that the protection against disease development afforded by M-685 treatment was transmitted to daughter corms and cormels (Table 6).

Table 5. Fusarium species recovered from 'daughter' corms of 'Friendship' cultivar grown from the corms treated in Experiment 4. Experiment 5.

Treatments on mother corms	Number of daughter corms assayed	Number corms containing	
		F. oxysporum	*F. moniliforme*
Untreated control	10	8	0
Benomyl/dicloran dip	10	7	0
F. moniliforme M-685	10	1	10

Discussion

Inoculations of freshly harvested corms with *Fusarium moniliforme* 'Subglutinans' M-685 culture consistently improved the productivity of gladiolus corms. More gladiolus daughter corms were produced from corms treated with the M-685 culture than were produced from corms treated with fungicides when mother corms had also been inoculated with the corm rot pathogen. Corm inoculations with antagonists of the Fusarium pathogen offer a promising additional or alternative method of treating corms carrying latent infections of the Fusarium disease.

To be useful as biological control agents, nonpathogens must be able to colonize corms aggressively without reducing flower and corm production. In looking for biocontrol agents, laboratory tests alone were not sufficient. Corm inoculations, preferably with both pathogen and nonpathogen, were necessary to demonstrate the usefulness of a nonpathogen as a biocontrol. Antagonism between fungi demonstrated in a Petri dish (unpublished data) has not evaluated such action in corms. The mechanism of control has not been established as antibiosis, competition, or a host response to the nonpathogen as suggested by the work of Baker and Dottarar (1).

The most useful inoculations might be with nonpathogenic isolates of benomyl-resistant fungi, including Fusarium and Penicillium. Populations of benomyl-resistant fungi could be maintained in corm stocks easily by treating corms with benzimidazole fungicides. Such fungicides might help to control Fusarium disease by reducing the pathogen as well as other organisms that might compete with the benomyl-resistant fungi in corms. The colonizing of corms with more than one kind of fungus and a bacterium is suggested by the observance that two or more fungi and bacteria are usually isolated from most corms in commercial corm stocks.

Pathogen-free corms, bulbs, cuttings, etc. developed from tissue culture are most vulnerable to invasion or colonization by pathogens and nonpathogens. Inoculation with nonpathogens before subjecting such stocks to disease fungi would be important. Inoculation of hot-water-treated corms and cormels (7) with nonpathogens could be important also.

The application of biological control methods to corms appears to be practical and inexpensive. The disease control effects of corm inoculation persisted into the second season to protect the daughter corms and cormels. *F. moniliforme* 'Subglutinans' was recovered from the daughter corms. Additional inoculations may not be necessary, especially if the nonpathogens are resistant to fungicides used to treat corms.

An integrated Fusarium control program should include corm inoculations with antagonists. Pathogen-free stocks such as bulbs and cuttings for clonal propagation should probably be protected by nonpathogens rather than by fungicides alone. Research with nonpathogens in disease control may offer the possibility of significantly reducing dependence on fungicides.

Table 6. Second generation gladiolus flower and corm production resulting from a nonpathogenic Fusarium isolate or a fungicidal dip applied to first generation corms. All second generation corms were inoculated with *Fusarium oxysporum* pathogen before storage of corms. Experiment 5.

Treatments to first generation corms	Index of symptoms on plants[z]	Yields from 100 corms planted		Corms from 500 cormels
		Number of marketable flower spikes	Number of sound corms	
Control, untreated	2.4 a[y]	31 a	27 a	67 a
Benomyl/dicloran dip	2.2 a	38 a	36 a	81 a
M-685 inoculation	0.3 b	87 b	93 b	302 b

[z]0=no disease, 4=severe disease symptoms observed at flowering time on plants grown from the corms.
[y]Means in a column not followed by the same letter are significantly different at the 5% level (Duncan's multiple range test).

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FOLIAR CHLOROSIS OF KALANCHOE BLOSSFELDIANA POELLN. AS INFLUENCED BY TEMPERATURE, DARKNESS, AND ETHYLENE[1,2]

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Abstract. Kalanchoe blossfeldiana **Poelln. plants were held in simulated shipping environments with variables of air ± 0.5 ppm ethylene, temperature, and light exposure regimes for 2 and 3 days. Plants held in light (1.3 klx for 12 hr/day) for 2 days at 23.5°C did not become chlorotic, whereas those held in darkness became severely chlorotic. Ethylene increased the number of chlorotic leaves on plants held in the dark but had no effect when plants were held in the light. With each successive increase in temperature from 23 to 28 to 33°C, plants held in darkness with or without ethylene had increased leaf chlorosis. Plants held in darkness for 3 days contained less chlorophyll than those held for 2 days. These simulated shipping tests suggested that, in transit, leaf chlorosis of kalanchoe occurs when plants are packaged (held in darkness) and subjected to elevated temperatures for 2 or more days.**

Kalanchoe plants have increased in popularity in the last few years, and the increased demand for this flowering plant has resulted in an increase in the numbers of propagating specialists who produce the plants asexually (from cuttings) and ship them to growers, who force the plants to flowering size. Plants generally have been observed to arrive at their destination in poor condition, principally because of leaf chlorosis. Although some reports are available on the effects of environment during the growing period (8, 9, 10), little research has been reported on the shipping and handling environment of kalanchoe. Marousky and Harbaugh (5) reported that minute quantities of ethylene induced floret sleepiness, foliar chlorosis, and abscission in mature flowering plants. Sheehan and Nell (11) reported that light and fertilizer used during the plant growing phase influenced the degree of chlorosis during the postharvest phase.

In this paper, we report the effects of temperature, darkness, and ethylene on development of leaf chlorosis and loss of chlorophyll in *Kalanchoe blossfeldiana* Poelln. plants during simulated shipping conditions.

Materials and Methods

Kalanchoe plants in 6 cm (2.3 inch) square plastic pots were obtained commercially. Plants were 2-3 weeks old (after transplanting) and had been growing under a short night environment (i.e. vegetative). All experiments were started and terminated during the afternoon and repeated at least once. All treatments had a minimum of 6 plants. The general procedures for mixing ethylene and air and distributing gases in chambers were similar to those previously reported (6). Air and ethylene flow was regulated to provide one complete change of air per chamber every 30-35 min. After exposure, the number of chlorotic leaves on each plant was counted. In some experiments, chlorophyll levels were determined. One gram of tissue was collected from the lower leaf blades, and chlorophyll was extracted with 80% acetone and determined according to the technique of Arnon (2). Chlorophyll was expressed as milligrams per 100 grams of leaf tissue.

Experiment 1. 'Goddess' and 'Conquistador' kalanchoe plants were placed in chambers in a laboratory maintained at 23.5 ± 1°C. Plants were held in light (1.3 klx/12 hr/day) or in darkness in air ± 0.5 ppm ethylene for 2 days.

Experiment 2. 'Goddess' and 'Conquistador' plants were placed in chambers held in darkness in incubators maintained at 23, 28, and 33 ± 0.5°C. Each chamber contained air ± 0.5 ppm ethylene. The numbers of chlorotic leaves and chlorophyll content in plants were determined after a 3-day exposure.

Experiment 3. 'Tabasco' plants were held for 2 and 3

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