

Effect of Virus Infection on Susceptibility to Certain Fungus Diseases and Yield of Gladiolus

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ABSTRACT

Virus infection of *Gladiolus hortulans* L. decreased yield of flower spikes, corms, and cormels, and increased plant mortality when plants were grown in fungus-infested soil. Virus infection did not affect mortality or yield of plants growing in fumigated soil. Reduced survival and yield of virus-infected gladioli was correlated with increased susceptibility to foliage and soil-borne pathogens. In-

fection with cucumber mosaic virus or tobacco ring-spot virus increased the prevalence and/or severity of *Fusarium* and *Stromatinia* root rot diseases, *Curvularia* leaf spot disease, and storage rot of corms. Similar responses were noted with inoculated and chronic virus-infected plants. *Phytopathology* 60: 1809-1813.

Additional key words: Multiple-pathogen-interaction, synergism, predisposition, disease complex.

Numerous reports of yield losses due to virus infection of agronomic plants are available in literature (3, 4, 6). Losses may be quantitative or qualitative, may vary in extent depending upon value of crops and type of damage done, and may affect both annual and perennial plants (3, 5, 14, 15). Several fungal and bacterial diseases are known to increase in severity when the host plant is dually infected with a virus (4, 7, 12). It is becoming increasingly apparent that these virus-induced alterations in disease susceptibility are frequently associated with yield losses and may, in some instances, be a critical predisposing factor to disease (13).

Gladioli (*Gladiolus hortulans* L.) are hosts for a number of viruses (1). Symptoms range from a subtle dark to light green mottling to a pronounced orange and white flecking or blotching of leaves (1). Foliage symptoms are generally not expressed until the season following infection, and, in some instances, viruses are resident in the plant without causing symptoms (2). After infection occurs, the virus persists indefinitely in infected corms because of the vegetative nature of propagation. Loss of salable flowers resulting from virus-induced abnormalities in floret pigmentation is thought to be the primary threat of virus diseases in gladioli. Therefore, the research reported herein was directed at two aspects: (i) characterizing the effects of infection by certain viruses on gladiolus growth, yield, and reproduction; and (ii) evaluating the effects of virus infection on susceptibility of gladioli to other pathogens.

MATERIALS AND METHODS.—Plant culture.—Corms used in these tests were grown from hot water-treated cormels (11) produced in methyl bromide-treated soil (10). Dovicide B (85% sodium 2,4,5-trichlorophenoxide) was used as a prestorage treatment (2,400 ppm for 15 min) within 3 days of digging. All corms planted in field plots were treated with Ceresan L (methylmercury 2,3-dihydroxypropylmercaptide 2.89% + methylmercury acetate 0.62%) (2,500 ppm for 10 min) at time of planting. Corms were selected from previous-year plantings on the basis of a low percentage of total plants showing foliage virus symptoms. Excess numbers of corms were usually planted so that virus-infected plants could be rogued if necessary. The cultivars Beverly Ann,

Friendship, Traveler, Roman Holiday, Spic and Span, and White Friendship were used. Beverly Ann possesses moderate resistance to both root rot and leaf spot diseases. Spic and Span is highly susceptible to both root rot and leaf spot diseases, while the other three varieties are intermediate for resistance to both types of disease.

Four planting-stock corms (10-18 mm diam) were planted in moist, fine sandy loam in 4-inch plastic pots (1,200 g sand/pot). Larger corms (25-50 mm) were planted in 6-inch plastic or clay pots (2,400 g sand/pot). Field plots were treated with methyl bromide injected 15-20 cm into the soil at 336 kg/ha rates and sealed with a plastic covering for 48 hr. Methyl bromide was also used for sterilization of potting medium.

Source and maintenance of fungus and virus cultures.—Isolates of cucumber mosaic virus (CMV) and tobacco ringspot virus (Tob RSV) were obtained from G. V. Gooding, Jr., Raleigh, N. C. The viruses were maintained in a greenhouse at 20-32 C by periodic sap inoculation on tobacco (*Nicotiana tabacum* L.) and cucumber (*Cucumis sativus* L.). Viruses in infected, finely-chopped leaves were also stored over CaCl₂ at -10 C. *Curvularia trifolii* f. *gladioli* (Kauff.) Boed. and *Fusarium oxysporum* f. *gladioli* Snyd. & Hans. were obtained from R. D. Milholland, Castle Hayne, N. C. The fungi were maintained on potato-dextrose agar (PDA) slants.

Inoculation procedures.—Inoculum of each fungus was produced by growing on PDA in petri dishes at 24 C for 14-21 days. Conidia were removed from the agar surface by flooding with distilled water, followed by gentle agitation.

Plants were inoculated with *F. oxysporum* conidia by pipetting 10-20 ml of a standardized inoculum suspension (1.5×10^5 conidia/ml) uniformly upon the surface of the soil in the pot in which the plants were growing and watering to saturation. Disease severity was determined 2-3 months after inoculation on a scale of increasing severity from 0-9 in which the foliage, stems, and roots were each rated 0-3, then totaled (8). Ratings were made on 4 plants in each pot, and the average of four pots/treatment constituted the disease index. All experiments were repeated at least twice.

The inoculum of *C. trifolii* was standardized to a concn of 1.5×10^5 spores/ml and applied to leaves of rapidly growing gladiolus plants with an atomizer. Inoculated plants were placed in a moist chamber at 25-30 C for 48-72 hr, then transferred to a greenhouse bench at approx 25 C. Plants were rated for leaf spot severity 6-10 days after fungus inoculation. Excised leaves from field-grown gladioli were cut into 20-cm segments, placed in moist chambers at 25-30 C, and inoculated as previously described. Disease severity was determined 4 days after inoculation.

Virus inoculum was prepared by grinding virus-infected tobacco or cucumber leaves in a mortar containing 0.1 M phosphate buffer (pH 7.0) and 400-mesh Carborundum powder. Plants were inoculated by rubbing leaves with a polyurethane swab containing infective sap. Control plants were rubbed with only buffer and Carborundum. Separation and identification of viruses occurring in naturally-infected tissue was made using indicator host species in conjunction with serology, using the double diffusion technique in 0.8% agarose containing 0.02% sodium azide (1). Host plants used for virus identification were Pinto bean (*Phaseolus vulgaris*), Early Ramshorn Blackeye cowpea (*Vigna sinensis*), Burley 21 tobacco (*Nicotiana tabacum*), and National Pickling cucumber (*Cucumis sativus*). Antisera produced in rabbits against type cultures of CMV, Tob RSV, and tomato ringspot virus were provided by G. V. Gooding, Jr., Raleigh, N. C. Because repeated analyses of symptomless plants utilized in this research failed to detect any mechanically transmissible viruses, all symptomless plants were considered to be free of virus infection. Although virus-inoculated plants in these tests were not subsequently assayed for virus infection, previous tests (M. K. Beute, unpublished data) indicated that viruses could be reisolated the 2nd year following inoculation. Analysis of variance and multiple range tests were utilized to determine significant differences (5% level) between means.

RESULTS.—Effects of virus infection on emergence and survival.—Traveler corms produced the previous year from plants exhibiting orange and white flecking of leaves and symptomless plants were planted on Onslow fine sandy loam known to be infested with *P. oxysporum* and *Stromatinia gladioli* and a similar soil which had been broadcast-fumigated with methyl bromide. Separation and identification of viruses in representative plants from these plantings showed that CMV

was the most prevalent virus. Tomato ringspot virus and Tob RSV were also detected in a small percentage of plants. Virus-free plants emerged 99% and 98%, respectively, on the fumigated and nonfumigated soils. Virus-infected plants emerged 18% less than virus-free plants on fumigated soil; however, 56% of the virus-infected plants failed to emerge in the nonfumigated soil (Fig. 1).

All plants on the fumigated soil, both virus-infected and virus-free, were still living 40 days after emergence when the first flower spikes were harvested; however, 32% of the virus-infected plants which had emerged on the nonfumigated soil were dead at time of spiking. Only 9% of the virus-free plants growing on nonfumigated soil had died during this time (Fig. 1).

Effect of virus infection on yield.—Essentially every plant, both virus-infected and virus-free, growing on fumigated soil produced a flower spike. Sixty-one per cent of the virus-free plants still living on the nonfumigated soil 40 days after emergence produced a spike, whereas, only 10% of the living virus-infected plants produced a spike. Total yield of spikes for virus-free and virus-infected plants in the nonfumigated field was 55 and 3%, respectively (Table 1).

The quality of the flower spikes harvested, as reflected by spike wt, was also significantly reduced by virus infection. Flower spikes produced by either virus-infected or virus-free plants growing in fumigated soil did not differ in length. Flower spikes from virus-infected plants, however, weighed 23% less than those from virus-free plants (Table 1). The significant reduction in wt of all flower spikes harvested from the nonfumigated field was attributed to the extensive root damage caused by soil-borne fungus pathogens. Nevertheless, the limited number of flower spikes produced by virus-infected plants weighed less than flower spikes from virus-free plants.

Effect of virus infection on production of corms and cormels.—Virus infection significantly reduced production of new corms in both fumigated and nonfumigated fields. Some root and corm rot disease developed even in the fumigated field, and was reflected in percentage of new corms harvested. Ninety-five per cent of the virus-free plants growing in the fumigated soil produced a new corm, as compared with 84% for the virus-infected plants in the same soil. Total yield for virus-free and virus-infected corms planted in fumigated soil was 94% and 71%, respectively (Table 1).

TABLE 1. Effect of virus infection on yield of gladiolus

Yield ^b	Virus-free plants		Virus-infected plants ^a	
	Fumigated soil	Nonfumigated soil	Fumigated soil	Nonfumigated soil
% Spikes harvested	90 a	55 b	88 a	3 c
Spikes wt. (g)	53 d	18 f	41 e	12 f
% Corms harvested	94 h	79 i	71 i	18 j
Corm wt. (g)	33 k	10 l	15 l	10 l

^a Traveler corms were produced the previous year from plants exhibiting orange and white flecking of leaves. Values are the average of four replicates each containing 50 plants.

^b Values followed by the same letter did not differ significantly (5% level),

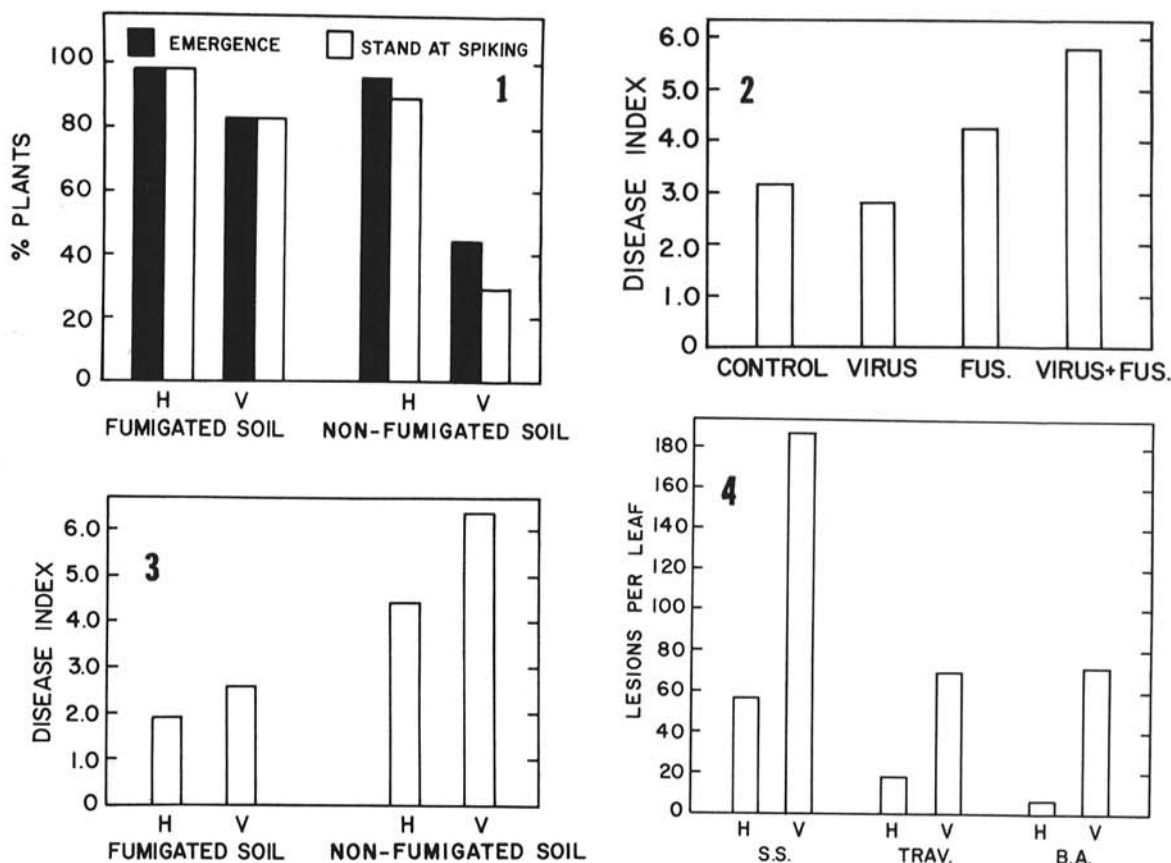


Fig. 1-4. 1) Effect of virus infection on growth and survival of gladioli. H = virus-free; V = virus-infected plants. 2) Effect of cucumber mosaic virus infection of gladioli on severity of *Fusarium* root rot. 3) Effect of chronic cucumber mosaic virus infection on *Stromatinia* root rot of gladioli following transplantation from fumigated into nonfumigated soil. H = virus-free plants; V = virus-infected plants. 4) Effect of cucumber mosaic virus infection of gladioli cultivars Spic and Span (S.S.), Traveler (TRAV.), and Beverly Ann (B.A.) on prevalence of *Curvularia* leaf spot lesions. H = virus-free plants; V = virus-infected plants.

Eighty-eight per cent of the virus-free plants alive at time of spiking in the nonfumigated soil produced a new corm, as compared with 60% for virus-infected plants in the same field. Total yield for virus-free and virus-infected corms planted in nonfumigated soil was 79% and 18%, respectively (Table 1). Virus-free plants growing in fumigated soil produced corms 33% larger in diam and twice as heavy (Table 1) as virus-infected plants growing in the same soil. All plants surviving in the nonfumigated soil produced corms of the same diam and wt as corms planted. General reduction in vigor of plants in the nonfumigated field appeared to mask the effect of virus infection on corm production.

Field-grown gladioli showing pronounced orange and white flecking of foliage produced significantly fewer cormels than comparable symptomless plants growing in the same field. Four cultivars grown from fungus-free planting-stock corms on fumigated soil were examined for cormel production. Although the cultivars differed in inherent cormel producing ability, virus infection resulted in a 36-50% reduction in cormels in each cultivar examined (Table 2).

Susceptibility of corms to storage rot.—Corms col-

lected from field-grown gladioli showing orange and white flecking of foliage were placed in storage with similar corms from symptomless plants growing in the same field. All corms were grown in fumigated soil and had originated from hot water-treated planting stock. In one test, corms were treated with a prestorage fungicide. Corms were left untreated in all other tests. Percentage of corms rotted was determined after 6 months in storage, using 100-182 corms/treatment.

When corms of four cultivars differing in susceptibility to *Fusarium* corm rot disease were placed into storage without fungicidal treatment, virus-infected corms rotted 2.0-4.5 times more frequently than did corms from virus-free plants (Table 2). In one test wherein corms of the cultivar Beverly Ann were treated with a prestorage fungicide, 62% more virus-infected corms rotted than did corms from virus-free plants. The efficacy of the fungicide in reducing all corm rot seemed to mask differences in inherent susceptibility to disease.

Effect of virus infection on susceptibility to root rot diseases.—Greenhouse-grown gladioli at the two-leaf stage were inoculated with CMV or Tob RSV, and 3

TABLE 2. Effect of virus infection on production of gladiolus cormels and incidence of corm storage rot disease

Cultivars	Cormels produced/plant ^a		% Corms rotted in storage ^b	
	Virus-infected plants	Virus-free plants	Virus-infected plants	Virus-free plants
Beverly Ann	15	19	38	12
Friendship	10	30	44	10
Roman Holiday	3	6	60	20
Spic and Span	6	9	52	25

^a Virus infection significantly (5% level) reduced cormel production in all cultivars examined. Values are the average of 20 plants.

^b Virus infection significantly (5% level) increased the incidence of storage rot disease in all cultivars examined. Values are the average of two tests using 100-182 corms/treatment.

days later soil was infested with either a suspension of conidia or mycelial fragments of *F. oxysporum*. Plants used as controls were rubbed with a buffer solution. After 90 days, root rot of plants inoculated with either virus was greater than that of virus-free plants. In a typical experiment, the disease index for *Fusarium* was increased from 4.2 to 5.7 by Tob RSV (Fig. 2). No increase in root rot occurred when plants were inoculated with CMV or Tob RSV alone. Compared with the normal discoloration of roots observed in the controls, inoculation with CMV or Tob RSV doubled the severity of *Fusarium* root rot in both cultivars Traveler and Spic and Span. Symptoms were increased in all parts of virus-fungus-infected plants, and were characteristic of severe *Fusarium* root infection.

To substantiate these results under field conditions, a test was conducted with field-grown gladioli. Traveler plants at the two-leaf stage of growth were carefully removed from fumigated field soil. All roots were removed while leaving the corm and foliage intact. Adhering soil was washed free from corms under running tap water. Plants showing pronounced orange and white flecking of foliage and plants with symptomless foliage were transplanted into 6-inch pots containing non-fumigated or fumigated field soil. Half of the pots were placed in water baths at 16 C, which is favorable for development of *Stromatinia* root rot (M. K. Beute, unpublished data). The other half were placed in water baths at 28 C, which prevents *Stromatinia* root rot but favors *Fusarium* root rot. Disease severity was determined 9 weeks after transplanting. Ten virus-infected plants used in this test were assayed for viruses and found to be infected with CMV.

New root systems developed and plants grew at both soil temp. Virus-infected and virus-free plants grew equally well in fumigated soil. Although the root rot which developed was characteristic for the fungi involved at the indicated temp, disease indices were comparable at both temp. Virus-infected plants at both 16 and 28 C had significantly more severe root rot disease than did the virus-free plants (Fig. 3). Compared with the control plants growing in fumigated soil, chronic

virus infection doubled the severity of both *Stromatinia* and *Fusarium* root rot diseases.

Effect of virus infection on development of Curvularia leaf spot disease.—Plants of the cultivars Spic and Span, Traveler, and Beverly Ann were inoculated with CMV or Tob RSV at the two-leaf stage of growth. Plants used as controls were rubbed with a buffer. A standard concn of *C. trifolii* spores was sprayed on all foliage 3 to 6 days later, and plants were placed in a moist chamber for 48-72 hr. Number of leaf spot lesions was determined 10 days after fungal inoculation. No virus symptoms were apparent during the experiment.

Inoculation with either virus significantly increased the prevalence of lesions in all 3 cultivars (Fig. 4). Spic and Span and Traveler had a 3-fold and 4-fold increase, respectively, in number of lesions, whereas lesions on the more resistant cultivar Beverly Ann increased 11-fold as a result of virus infection. The severity of leaf spot disease on virus-infected Traveler or Beverly Ann was increased to the level of severity observed on virus-free Spic and Span leaves. Lesions developed more rapidly and were larger on virus-inoculated than on noninoculated leaves of the same cultivar.

The effect of chronic virus infection on leaf spot susceptibility was also tested. Leaves of field-grown gladioli showing foliage virus symptoms as well as symptomless leaves were excised, cut into 20-cm lengths, placed in moist chambers, and sprayed with a standard concn of *Curvularia* spores. Number of lesions was determined after incubation at 28 C for 4 days. Susceptibility of the cultivars Spic and Span, Traveler, Friendship, and Beverly Ann was significantly increased by virus infection. In seven replicated tests, virus-infected leaves from these four cultivars consistently had 2 to 3 times more lesions/leaf area than did virus-free leaves.

DISCUSSION.—Virus-infected plants grown from fungus-free corms in fumigated soil produced a lower quality spike, fewer cormels, and a smaller increment of corm growth than did virus-free plants. Although no plants succumbed to the low level of inoculum present under these conditions, failure of a significant number of virus-infected plants to produce a new corm was attributed to the slight-to-moderately severe stem and root infection which developed. All detrimental effects of virus infection observed in the relatively disease-free, fumigated soil were increased several-fold when plants were grown in fungus-infested soil. Although virus-free plants were adversely affected by soil-borne pathogens when grown in their presence, more plants survived and produced a flower spike than did virus-infected plants. This contrasting effect of virus infection in fumigated and nonfumigated soil shows that the observed abnormalities of growth and yield are primarily the result of a disease complex, and not the result of virus infection alone.

A previous observation that virus-infected plants growing in the field were more severely diseased by *Botrytis* leaf spot (*Botrytis gladiolorum*) than were

virus-free plants (9) suggests that a synergistic relationship may exist between these two diseases. Indeed, all attempts to characterize the effects of virus infection on gladioli indicated a subsequent increase in severity and/or prevalence of several important root and foliage diseases. Dual inoculation with CMV or Tob RSV and *F. oxysporum* significantly increased root rot severity. Chronic infection with CMV also resulted in more severe *Fusarium* and *Stromatinia* root rot. Similarly, chronic virus infection of field-grown gladioli leaves consistently resulted in 2 to 3 times more lesions/leaf area when inoculated with *Curvularia* conidia than did virus-free leaves. Moreover, inoculation of 3 cultivars with either CMV or Tob RSV increased the subsequent prevalence and severity of *Curvularia* leaf spot disease. The increased incidence of storage rot in virus-infected corms may be a reflection of increased infection in the field. It is possible, however, that metabolic alterations resulting from chronic virus infection stimulates latent *Fusarium* infection to the extent that active rotting occurs.

The extent of virus damage is not immediately apparent but, without doubt, is a major factor in decline of gladiolus stocks. The role of virus infection seems to be primarily a predisposing effect increasing susceptibility to debilitating fungal pathogens. This hypothesis is supported by the fact that decreased survival and yield is correlated with, and apparently dependent upon, the increased incidence and severity of major fungal diseases.

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