Ecology and Control of Gladiolus Fusarium Wilt

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摘　　要

謝式坪鈺（1985）唐菖蒲萎凋病之生態及防治　（國立中興大學）

由 *Fusarium oxysporum* f. sp. *gladioli* 引起之萎凋病爲本省唐菖蒲生產最大的難題。病菌經常汚染種植材料，可由病株或外觀健全植株之種皮、球莖和根部分離到病菌。不過病菌在病株球莖之含量常比外觀健全者高出 10 倍以上。

根由球莖長出後即可分離到病菌，病菌在土壤內主要分佈在表土至深 20公分處，到 30 公分深乃能分離到，球莖種植在不同深度的土壤影響其發芽與發病度。

溫水處理（57.2C）30 分鐘可消除球莖內之病菌，但處理後球莖之發芽率減低很多，對木子之影響較小。

單獨用福爾馬林或蒸氣土壤消毒並不能減輕病害之發生，添加 S-H 混合物並無法降低土壤內之病菌，也無法減少病害之發生。

農藥貝芬替、腐絕、鋅銅四氯丹、撲拉克和普拔克可有效制菌絲之生長，但只有撲拉克能抑制球莖組織內之病菌，利用撲拉克（有效成份 0.0125%）在種植前處理球莖 3 小時以上，可消除球莖內大多數病菌，田間試驗顯示對感病品種之防治率高達 50% 以上。

ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *gladioli* is a serious problem to gladiolus production in Taiwan. No control measure has been ever practical to this disease previously. The pathogen was usually associated with planting materials, and was commonly isolated from root, corm, husks of diseased and apparently healthy gladiolus. About 10 times more of the fungus was obtained from macerated diseased corms than from apparently healthy ones.

Colonization or infection of the fungus on roots was detectable immediately

after growing out from corms. The pathogen was present in soil mainly from surface to a depth of 20 cm and was detectable down to 30 cm deep. Corms planted at differrent depths in soil affected the germination of corms and incidence of disease.

Hot water treatment of planting materials at 57.2C for 30 min resulted in good control of the disease. However hot water treatment drastically reduced the germinability of the corms. Small cormels were more tolerant to hot water treatment than corms. Soil treatments with formalin and steam alone did not reduced the incidence of the disease. Soil amendment with S-H mixture at 0.5 and 1% neither reduced the population of the fungus in soil, nor decreased the incidence of the disease.

In the chemical screening tests, Bavistin, Mertect, Previcur N, Sportak and Zincofol were effective in inhibition of mycelial growth *in vitro*, however only Sportak was able to eradicate the pathogen from corm tissues. Corms treated with Sportak (0.0125% a. i.) for more than 3 hr before planting gave good control of the disease, the incidence being reduced up to 50% or higher on the susceptible varieties.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder & Hansen is an old and serious problem to gladiolus production in Taiwan. When susceptible varieties are grown, especially under continuous cultivation, the fungus may cause up to 50% losses or even completely destroy the plantation.

Growth of resistant varieties is the most effective way of controlling the disease. Unfortunately, most of the good quality varieties introduced from abroad are susceptible. Before the resistant varieties is available, adequate control other than seeking resistant varieties has to be developed.

The objectives of this study were to investigate the ecology of the causal organism; distribusion of F. *oxysporum* f. sp. *gladioli* in soil and its host plant; and to evaluate the effectiveness of various control methods for control the disease.

MATERIALS AND METHODS

PCNB[5] was used to isolate *F. oxysporum* f. sp. *gladioli* from plant materials and the soils. Identification of the pathogen was made by inoculating the isolates on corms or leaves of gladiolus. Span, a susceptible variety was used throughout the experiments except specifically indicated.

Qualitative and quantitative estimation of populations of the fungus in corms

Qualitative assay: The corms were vertically dissected from the center, and about 1 mm thick of the tissue was sectioned from the cut surface. This tissue section was further cut into 5 mm^2, and placed onto PCNB plates for incubation at 30C. The appearance of colonies of the pathogen on tissue sections was recorded 10 days later.

Quantitative assay: Sterile distilled water and corm tissues at ratio 20:1

(V/W) were homogenized in a high speed Waring Blender for 10 seconds and then subjected for serial dilution planting on PCNB plates. The colony counts were made 10 days after incubation at 30C.

RESULTS

Colonization and/or infection of gladiolus roots by *F. oxysporum* f. sp. *gladioli*

Fifty corms after broken their dormancy were placed on wet scott papers. After 1-, 3-, 5-, 7- and 9- day incubation 10 corms each were sampled, and their roots were cut and placed on PCNB plates to isolate the fungus. It was found that 34% of root was infected with the fungus 24 hr after germination of corms, and 50, 79, 71 and 75% of roots were infected 3, 5, 7 and 9 days after germination, respectively.

Density of *F. oxysporum* f. sp. *gladioli* in symptomless and diseased corms

Diseased plants and apparent healthy plants were sampled from field 2 months after planting. Individual corm was quantitatively assayed for density of *F. oxysporum* f. sp. *gladioli* on PCNB plates. The fungal population in diseased corms were $7.1\times10^4-4.2\times10^5$ propagules/g fresh weight. On the other hand, the fungal densities in apparent healthy corms were much less, $0-6.4\times10^3$ propagules/g fresh weight.

Distribution of *F. oxysporum* f. sp. *gladioli* in soil

Soils were sampled from Pei-tou, Tien-wei and Fou-li where gladiolus has been cultivated for many years. To analyze the vertical distribution of the fungus in soil. five soil samples were collected from different depth of the soil from surface to 30 cm depth with 5 cm intervals. These soil samples were subjected to serial dilution plating on PCNB plates. The fungus was mostly present in the upper 20 cm in depth; populations ranged from 2×10^2 to 2.6×10^3 propagules/g. At a depth of 20-30 cm, the population of propagules decreased to 0-300 propagules/g of soil. (Table 1)

Teble 1. Vertical distribution of *Fusarium oxysporum* f. sp. *gladioli* in soil

Depth of the soil (cm)	No. of propagules/g soil ($\times10^3$)		
	Pei-tou	Tien-wei	Fou-li
0-5	1.6	1.8	2.2
5-10	1.5	2.6	1.1
10-15	2.3	0.2	2.3
15-20	1.1	0.8	1.0
20-25	0.3	0.3	0.1
25-30	0.1	0.1	0

Planting of gladiolus corms in different depth of soil in relation to the occurrence of Fusarium wilt

Corms of Valeria and Hei-ku without any treatment, were planted in soil depth 4, 8, 12 and 16 cm, each consisting of 300 corms in 4 replicates at Pei-tou in 1983. The germination of corms was

recorded one month after planting, and percent of infection was estimated at flowering stage.

Of these two cultivars tested, the percentage of ungerminated corms and occurrence of the disease were increased with the increasing depth of planting in soil (Table 2). Valeria planted at 4 cm deep grew better than rest of treatments, and Hei-ku planted at depth from 4-12 cm grew better than that planted at 16 cm deep.

Table 2. Occurrence of gladiolus Fusarium wilt in different depth of soil

Variety	% of diseased plants or ungerminated corms in soil depth (cm)			
	4	8	12	16
Valeria no germination	22.5	26.3	23.8	31.3
diseased	5.0	15.0	20.0	13.8
subtotal	27.5a	41.3b	43.8b	45.1b[1)]
Hei-ku no germination	3.8	11.3	7.5	15.0
diseased	6.3	2.5	3.8	11.3
subtotal	10.1a	13.8a	11.3a	26.3b

1) Subtotals followed by a common letter do no differ significantly (P=0.05).

Effect of hot water treatment on the germination of corms and cormels and on the incidence of Fusarium wilt

Corms and cormels were kept at 35C for about one week immedately after harvest for curing. They were presoaked for 2 hr in water at room temperature and then in hot water at 57.2C for 30 min[1]. After thoroughly dried, they were cool storaged for 2 months. Half of the corms and cormels were qualitatively assayed for the presence of the fungus, and the rests were planted in the field for observation of the germinability and

Table 3. Effect of hot water treatment on the germination of corm and cormel and the incidence of Fusarium wilt

		% *F. oxysporum* f. sp. *gladioli* isolated	% germination	% disease
Cormel	treated	3	48	25
	CK	28	58	51
Corm[1)]	treated	78	16	4
	CK	100	53	42
Corm[2)]	treated	26	23	7
	CK	43	87	34

1) From diseased plants; 2) From apparent healthy healthy plants.
Data are means from two experiment of 100 cormels or corms each.

the disease incidence.

Hot water treatment drastically reduced the germinability of the corms, but has only slight influence on the cormels. When hot-water-treated planting materials were grown in the field, disease incidence was decreased. (Table 3)

Effect of SH mixture amendment on the population of *F. oxysporum* f. sp. *gladioli* in soil and the occurrence of Fusarium wilt

S-H mixture[6] was amended in soil at rate of 0, 0.5 and 1% (W/W) in field of Fou-li just before planting. Soil were sampled 2, 4 and 6 weeks after amendent and the serial dilution plating was made to estimate the population of the fungus, The percent of infection was recorded 2 months after planting. Amendment of soil with S-H mixture did not affect the population of the fungus in soil, neither did reduce the incidence of the disease.

Effect of soil treatments on germination of gladiolus corms and disease incidence

Soils previously grown gladiolus for 3 years at Pu-li were disinfested with steam or formalin. After disinfestation, isolation was made on PCNB to ensure that there was no any *Fusarium* detectable in treated soil. The control was provided by using the same field soil without disinfestation, containing about 5×10^2 propagules/g of *F. oxysporum* f. sp. *gladioli*. The experimental design was completely randomized with four replicates, with 200 corms per replicate. Soil sterilization with formalin and steam to eliminate the pathogen did not affect the germination of corms and the incidence of the disease. There was no significant difference in percentage of germination and percentage of disease among formalin treatment, steam treatment and the control (Table 4).

Table 4. Germination of gladiolus in formalin and steam disinfected soils and incidence of Fusarium wilt

Treatment	% germination	% diseased
Formalin	83 a	53 a[1)]
Steam	85 a	60 a
CK	80 a	58 a

1) Based on 200 corms in each treatment. Colomn means followed by a common letter do not differ significantly (P=0.05).

Evaluation of fungicides for controlling Fusarium wilt of gladiolus

Paper discs in 5 mm diameter were soaked in 0, 0.1 or 0.067% suspension of Benlate (50% Benomyl), Bavistin (50% Carbendazim), Mertect (40% Thiabendazole), Difolatan (40% Captafol), Captan (50% Captan), Zincofol (50% Captafol+6% Zinc+12.% Copper), Previcur N. (70% Propamocarb) and Sportak (25% Prochloraz), for 1 hr., then removed and placed on a nylon screen for drying. After dryness, they were placed on Petri dish containing 10 ml of PSA to which approximately 100 macroconidia of the fungus were added. The

inhibition zone was measured from edge of paper discs 10 days after incubation at 28 C. Sportak was the most effective followed by Mertect, Previcur N, Bavistin and Zincofol (Table 5). In another experiment, corms were soaked in fungicides for 3 hr. and then qualitatively assayed for the presence of the fungus in corm tissues. Sportak was the most effective in eradicating the pathogen in corm tissues. Sportak at 125 ppm eliminated about 70% of pathogen in corm tissues, whereas other fungicices did not significantly reduce the percentage of isolation, as compared with the check (Table 6).

Table 5. Effect of fungicides on the inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *gladioli*

Fungicide	Inhibition zone (mm) at concentration		
	500X	1000X	1500X
Bavistin	14.4	14.0	12.8
Benlate	5.6	1.0	1.6
Captan	6.8	6.8	6.8
Difolatan	9.8	9.0	8.0
Mertect	18.2	13.0	14.4
Previcur N	17.3	15.6	10.2
Sportak	18.5	18.4	17.7
Zincofol	13.0	10.5	12.0

Table 6. Frequeny of isolation of *Fusarium oxysporum* f. sp. *gladioli* from corms treated with various fungicides for 3 hr

Fungicides	Concentration (a. i.)	% isolation[1)]
Bavistin	500 ppm	91
Mertect	400 ppm	87
Previcur N	800 ppm	86
Sportak	125 ppm	30
Zincofol	500 ppm	94
CK		99

1) Based on 100 isolations.

Isolation of the fungus from old corms or newly formed corms and cormels

To understand the effect of fungicidal treatment on the subsequent crop, corms after Sportak treatment for 3 or 24 hrs were planted in the field. After about 4 months, old corms as well as newly formed corms were qualitatively assayed for the presence of the fungus. The

fungus was present in 95% old corms and 75% newly formed corms in the control block-without fungicidal treatment. Whereas, in those treated with Sportak for 24 hr, it was isolated from 24% old corms and from 12% newly formed corms (Table 7). Sportak treatment for 24 hr gave better control than treatment for 3 hr only.

Table 7. Isolation of *Fusarium oxysporum* f. sp. *gladioli* from old and newly formed corms produced from plants previously treated with Sportak before planting

Duration of treatment before planting (hr)	% isolated from	
	Old corms	Newly formed corms
0	95	75
3	83	54
24	24	12

Duration of Sportak treatment and the occurrence of Fusarium wilt

Corms before planting were soaked in Sportak (0.0125% a. i.) for 3, 6, 12 and 24 hrs. Corms soaked in tape water for 24 hr, or without soaking served as control. The experimental design was completely randomized with five replicates. Germination of corms was observed one month after planting, and percentage of disease was recorded at flowering stage. The results showed that 3 hr treatment was sufficient to control the disease. and longer treatment did not further reduce the incidence of the diseases. Prolongation of treatment reduced the germination of corms (Table 8).

Table 8. Duration of treatment of corms in Sportak in relation to the gladiolus Fusarium wilt

Duration time (hr)	% Ungerminated	% Diseased	% Ungerminated + Diseased
3	15.0 a	2.5 a[3)]	17.5
6	13.0 a	3.0 a	16.0
12	14.5 a	3.5 a	18.0
24	24.5 b	1.5 a	26.0
0[1)]	22.0 b	48.5 b	70.5
0[2)]	29.5 b	54.5 b	84.0

1) Without soaking.
2) Soaking in water for 24 hr.
3) Column means followed by a common letter do not differ significantly (P=0.05).

Field trial of Sportak treatment on the control of the disease

Two field trials were conducted at Pei-tou and Fou-li in 1983. The experimental design was completely randomized with four replicates. Each replicate had 150 plants. Corms were soaked in Sportak (0.0125% a. i.) for 3 hr before planting. Corms soaked in irrigation water for 3 hr were used as control. The percentage of disease and number of harvestable cut flowers were recorded at flowering stage. The results showed that Sportak gave a satisfactory control of the disease, with only 17.4% plants infected with the fungus as compared with 68.7% infection in control (Table 9). The average number of cut flowers in treated block was 130, and it was 28 only in control block.

Table 9. Field trial of the effectiveness Sportak treatment on the control of gladiolus Fusarium wilt

Treatment	% Diseased	No. cut flowers
Sportak	17.4 a	130 a[1)]
CK	68.7 b	28 b

1) Data are means from two experiments of four replicates. Each replicate has 150 plants. Column means followed by a common letter do not differ significantly (P=0.05).

DISCUSSION

Fusarium oxysporum f. sp. *gladioli* was isolated from root, corm tissues, husks of diseased and apparently healthy gladiolus. The pathogen was carried over through the planting materials, a typical corm-borne disease. More than 50% of roots were infected by the pathogen immediately after growing out from the corms. The pathogen may enter into soil from infected husks and root tissues. This explains why the pathogen was not present in virgin soil but was detectable immediatdly after planting of gladiolus. (Unpublished data)

From the unpublished data, it was shown that the pathogen was also isolated from corm tissue of resistant varieties. This indicates that the corms of resistant varieties might be also infected by the pathogen, but remained symptomless. The symptoms would not appear at the early stage of infection when the density of fungus in the tissue was not high. The fungual population in corm from diseased plants was 10 times more than that from apparent healthy ones. The pathogen in corm tissues has to multiply up to certain levels before showing symptoms. The pathogen population in diseased corms is always more than 10^4 propagules/g fresh tissues. The bacterial population in diseased corm was also much higher than that in apparently healthy corm. The bacteria from corm tissue were mostly non-pathogenic, but occasionally *Xanthomonas gummisudans* was observed. The role of the bacteria in diseased corm tissue has to be identified.

The population of *F. oxysporum* f. sp. *gladioli* in soil was mostly distributed from surface to a depth of 20 cm, in concomitance with the distribution of root system of the gladiolus. Planting corms in shallower soil seemed to be better than those in deeper soil. However from practical point of view gladiolus has to be planted at deeper soil as plants may fall when planted at shallow soil.

Hot water treatment at 57.2C for 30 min effectly control the disease, however it reduced the germinability of the corms. These results were similar to that of Magie[3]. However, hot water treatment is not practical as the equipment is usually not available and it is time consuming.

Amendment of soil with S-H mixture at 0.5 and 1% did not reduce the population of the fungus in soil and the incidence of the disease. This was different from the results of Sun & Huang [6] who reported that S-H mixture amendment reduced *Fusarium oxysporum* f. sp. *niveum* population in soils.

Planting untreated corms in formalin and steam sterilized soils did not reduce the incidence of the disease. Similar results were obtained by Milholland[4], Ferguson and Markley[2] who showed that planting untreated corms in fumigated soil resulted in an increase rather than a reduction of disease. Therefore soil fumigation should not be used when untreated corms are to be planted. The best results can be expected through planting clean corms in sterilized soils.

Among various fungicides tested, Sportak not only effectively inhibited the mycelial growth *in vitro* but also eliminated most of the fungus in corm tissues. The corms treated with Sportak (0.0125 % a. i.) for 3 hr before planting was sufficient to control the disease, the incidence being reduced up to more than 50% on the susceptible varieties. Prolongation of treatment did not further increase the effectiveness of the disease control, although it reduced the fungal population in treated corms and newly formed corms of subsequent crop. Since Sportak is not a systemic fungicide, presene of Sportak in corm tissue mainly relies on passive diffussion. The treatment of Sportak would be better conducted when corm has partially lost its water content and has a greater water absorption capacity. When treated with Sportak immediately after harvest, corms were found to have more fungus uneradicated than those after storage. Sportak was unable to eradicate the fungus in corms completely; it is recommended that the corms be treated before planting at each crop.

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