FUSARIUM RESISTANCE IN *Gladiolus*: EFFECTS OF PLANT MATERIAL AND TESTING CONDITIONS ON THE INFECTION OF CULTIVARS

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Abstract

Fusarium oxysporum f.sp. *gladioli* is the most important pathogen of *Gladiolus*. Cultivation of both corms and flowers is hampered by this fungus. Resistant cultivars are therefore much desired and partial resistance was found within the commercial gladiolus assortment. To assess the *Fusarium* resistance of cultivars for breeding purposes a standard assay is in use at CPRO-DLO. Genotypes are planted in infested soil. The resistance level is determined six to eight weeks after planting, by calculating the relative length (RL) of the shoots, i.e., length of shoots in infested soil divided by length of shoots in control soil. No information about effects of corm origin, corm size, corm cleaning, temperature or inoculum concentration is known. For Values for Cultivation and Use purposes (VCU) more information is needed about these conditions. Therefore this was investigated in the present study. The disease incidence of a number of cultivars with differing Fusarium resistance was assessed with various corm treatments.

In all our experiments, a significant cultivar effect was found. No cultivar by treatment interactions were detected. A significant corm origin effect was also found. For VCU purposes, corms from different growers (mostly breeders), must be cultivated during one year under equal conditions. The corm size had no significant effect on the level of Fusarium infection. Significant corm cleaning effects were found. Dehusking and disinfection of the corm gave a significantly higher infection level. For further tests corm cleaning is not advised, since by damaging the corm secondary infections may occur. Significant temperature and inoculum concentration effects were found. A higher temperature and a higher inoculum concentration gave a higher disease incidence. Both parameters can be manipulated to influence the time course of an assay.

Key words: Screening, *Fusarium oxysporum*, origin, corm size, corm cleaning, temperature, inoculum concentration, VCU.

1. Introduction

Various *formae speciales* of the soilborne fungus *Fusarium oxysporum* cause bulb- or corm rot in most species of the families *Liliaceae*, *Amaryllidaceae* and *Iridaceae*. In gladiolus, the forma specialis *gladioli* has become one of the major problems during cultivation. A way to avoid problems is the use of cultivars with a high resistance level (Löffler *et al.*, 1996). A large variation in Fusarium resistance was found in cultivars and species of gladiolus (Palmer and Prior, 1958; Jones and Jenkins, 1975; Garcia-Jimenez *et al.*, 1986; Bajaj *et al.*, 1989; Straathof *et al.*, 1996). Löffler *et al.* (1996) developed a standardized and sensitive assay to screen gladiolus genotypes for Fusarium resistance. However, differences in infection may not only be due to a cultivar effect but also to cultivation conditions. Sciortino and Roxas (1994) compared corms of various cultivars grown at various places, reporting significant differences in flower production, flower quality and sensitivity for *F. oxysporum*. Nitrogen supply (Woltz and Magie, 1975; Groen, 1990) as well as corm damage (Palmer and Prior, 1958; Jones and Jenkins, 1975) lead to a higher level of infection by the pathogen. Effects like temperature, corm damage (as a result of corm cleaning) and inoculum concentration could influence the disease incidence, the accuracy and the cultivar sequence. These factors are of great importance for VCU-purposes. Results of assays in various years must be comparable to each other. The aim of this study was to investigate effects like corm origin, inoculum concentration, greenhouse temperature, corm cleaning (dehusking and disinfection) and corm size on the results obtained from a screening assay.

2. Material and Methods

2.1 Screening assay

For soil infestation, a mixture of two aggressive isolates of *F. oxysporum* f. sp. *gladioli*, designated LBO-G2 and LBO-G15, was used in a 1:1 ratio for all experiments (Löffler *et al.*, 1996; Straathof *et al.* 1996). The fungi were kept at -80^{0}C on Protect Bacterial Preservers and before use grown on Czapek-Dox agar medium for inoculum preparation. The fungus was grown in glass jars at 23 ^{0}C, for about two weeks in an autoclaved oatmeal-soil mixture (1:5 w/w) (Löffler and Mouris, 1989; Straathof and Inggamer, 1992; Löffler *et al.*, 1996). The fully grown culture was then ground and mixed in a 0.1% concentration into the soil. The number of propagules was determined on a modified selective Komada-medium (Komada, 1975; Löffler and Mouris, 1989; Löffler *et al.*, 1996) just before planting. The fungus population was allowed to equilibrate for two weeks and then the corms were planted.

The experiments were set up in three replications. Each replication contained a 10-liter pot with control and infested soil containing six corms of each cultivar. All experiments, except temperature and corm cleaning, were carried out as a completely randomized block design with three replications. The corm cleaning and temperature experiment was carried out as a latin square. The pots were placed in a greenhouse at 14/18^{0}C night/day-temperature and the corms were harvested about six weeks after planting.

Two parameters, the measurement of the longest shoot and the Average Disease Rating (ADR) were used to assess the infection of each corm. An ADR value is a visual evaluation of the infection according to a scale of 1 to 5 (1: no infection; 2-5: 0-5%, 5-15%, 15-50% and 50-100% of the corm surface covered with mycelium). For each pot the ADR was calculated. The Relative Length (RL) for each pot with infested soil was calculated by dividing the shoot length in this pot by the average shoot length in the corresponding control pot (Löffler *et al.* 1996). Control corms, showing a latent infection after harvest, are left out of the calculations. The results were statistically analyzed with the computer package Genstat (Payne *et al.*, 1987).

2.2 Corms from various "origins"

Following an inquiry among growers (Van Keulen and Van Aartrijk, 1993) ten moderately resistant cultivars, 'Amsterdam', 'Applause', 'Eurovision', 'Jacksonville Gold', 'Jester', 'Praha', 'Rose Suprême', 'Victor Borge', 'White Goddess' and 'Wind Song' were selected. These cultivars were grown at five different places (origins) under equal conditions before testing. The soil conditions at the various "origins" are given in Table 1. Before planting, corms were dehusked, disinfected in a 2% formalin solution for ten minutes, rinsed in flowing tap water for half an hour and dried over overnight. This experiment, with an inoculation level of 0.01% started in march 1994 at a disease pressure of about 3 800 propagules per gram soil.

Table 1: Soil conditions at the various places of origin

Origin	Soil type	% humus	pH(KCl)
Nederweert	sand	4.4	5.1
Hengelo (Gld)	sand	4.2	5.1
Wageningen	sand	3.7	5.7
Baexem	corse grained sand	1.1	5.4
Creil	sandy clay	0.9	7.3

2.3 Corm size

The test comparing corm sizes, was carried out with untreated corms of the cultivars 'Eurovision', 'Peter Pears', 'Priscilla', 'Red Beauty', 'Victor Borge', 'White Goddess' and 'White Prosperity'. For this test and the test with inoculum concentration effects, cultivars with a greater difference in resistance against *F. oxysporum* were chosen than in the "origin" test. Of the desired cultivars not all corm sizes were available so we had to choose others. The test was started in March 1995 at an inoculation level of 0.1% resulting in a disease pressure of about 24 500 propagules per gram soil.

2.4 Inoculum concentration effects

In the assay with different inoculum concentrations, untreated corms of the cultivars 'Amsterdam', 'Applause', 'Eurovision', 'Fidelio', 'Hunting Song', 'Peter Pears', 'Rose Suprême', 'Spic and Span' and 'White Goddess' were tested. These cultivars were chosen because of the great differences in their resistance level against *F. oxysporum*. In March

and April 1995, three inoculation levels of 0.3, 0.1 and 0.01%, resulting in a disease pressure of about 37 000, 24 500 and 11 000 propagules per gram soil were compared.

2.5 Corm cleaning and temperature effects

An assay with corm cleaning and temperature effects was carried out in May and June 1994. Three different treatments (an untreated corm, a dehusked corm and a dehusked/disinfected corm) and three different temperatures (15, 20 and 25^{0}C) were applied to corms of 'Applause' and 'Victor Borge'. Corms were planted in infested soil at an inoculation level of 0.1% resulting in an infestation level of about 32 000 propagules per gram soil.

3. Results

In all tests the parameters RL and ADR showed similar results. Therefore only RL-data are presented.

In the assay of various "origins" a high infection occurred, which is shown in the low RL for a number of cultivars in infested soil (Table 2). Large differences between cultivars were found. To a lesser extent differences between "origin" were observed. The differences between cultivars and origins were highly significant, but no significant interaction between both effects was found (Table 3).

Table 2: Relative Length for various gladiolus cultivars and their various origins after infestation with *Fusarium oxysporum*. The infestation level was 3.800 propagules per gram soil. (The cultivars and places are given in decreasing order of infection level).

Corm origin* Cultivar	Ned	Hen	Wag	Bax	Cre	Means
Applause	0.78	0.65	0.45	0.58	0.41	0.58
Eurovision	0.59	0.47	0.58	0.55	0.38	0.58
Rose Suprême	0.49	0.58	0.60	0.53	0.34	0.51
Amsterdam	0.43	0.62	0.27	0.46	0.25	0.41
Praha	0.50	0.35	0.52	0.32	0.22	0.38
Jacksonville Gold	0.43	0.41	0.49	0.21	0.32	0.37
Victor Borge	0.47	0.53	0.18	0.24	0.24	0.33
Wind Song	0.25	0.25	0.43	0.29	0.19	0.28
White Goddess	0.42	0.19	0.28	0.22	0.25	0.27
Jester	0.07	0.11	0.04	0.05	0.12	0.08
Means	0.44	0.42	0.38	0.34	0.27	0.37

*: Corm origin: Ned = Nederweert; Hen = Hengelo; Wag = Wageningen; Bax = Baexem; Cre = Creil.

In the assay with different corm sizes the cultivar effect was highly significant. Corm size and the two way interaction were not significantly different (Table 3).

In the comparison of different inoculum levels, highly significant cultivar and inoculum concentration effects were found. The inoculum concentration effect was linear (Table 3). Only the highest inoculum level gave a significant higher infection level for all

cultivars. The lower inoculum concentrations (11 000 and 24 500 prop.) showed small differences between the two infection levels. No significant interaction was found.

When comparing differences in corm cleaning and greenhouse temperatures, large main effects for corm cleaning ($p < 0.001$) as well as for temperature ($p = 0.035$) on shoot length reduction were found (Figure 1).

Table 3: Analysis of variance calculated for Relative Length, of gladiolus cultivars, after an infection with *Fusarium oxysporum* for corm "origin", corm size and infestation level.

effect	d.f.	SS	MS	p-value
Corm origin				
Cultivar	S	2.81671	0.31297	<0.001
Origin	4	0.53264	0.13316	<0.001
Cultivar.origin	36	1.08514	0.03014	0.062
Residual	97	1.95282	0.02013	
Corm size				
Cultivar	6	1.17912	0.19652	<0.001
Size	3	0.07419	0.02473	0.340
Cultivar.size	18	0.22751	0.01264	0.895
Residual	54	1.16756	0.02162	
Infestation level				
Cultivar	8	1.005581	0.125698	<0.001
Infestation	2	0.396891	0.198445	<0.001
linear	1	0.382730	0.382730	<0.001
deviations	1	0.014161	0.014161	0.193
Cultivar.infestation	16	0.057178	0.003574	0.964
Residual	52	0.424231	0.008158	

4. Discussion

Differences in resistance level between cultivars are known to exist (Palmer and Prior, 1958; Jones and Jenkins, 1975; Garcia-Jimenez, 1986; Bajaj *et al.*, 1989; Straathof *et al*, 1996). Highly significant differences in resistance between cultivars were also demonstrated in all our assays. A comparison of the two observed parameters (ADR and RL) showed a good correlation ($r = 0.87$). This was confirmed by Löffler *et al.* (1996) who also reported a good correlation between these parameters ($r = 0.93$). Since elongation growth stops and corm infection goes on, the differences between cultivars become smaller. Therefore results are discussed on the basis of the parameter RL. Nevertheless latent infection in corms, which sometimes occurs (Palmer and Prior, 1958; Löffler *et.al.*, 1996) and influences elongation growth in the control pot has to be eliminated.

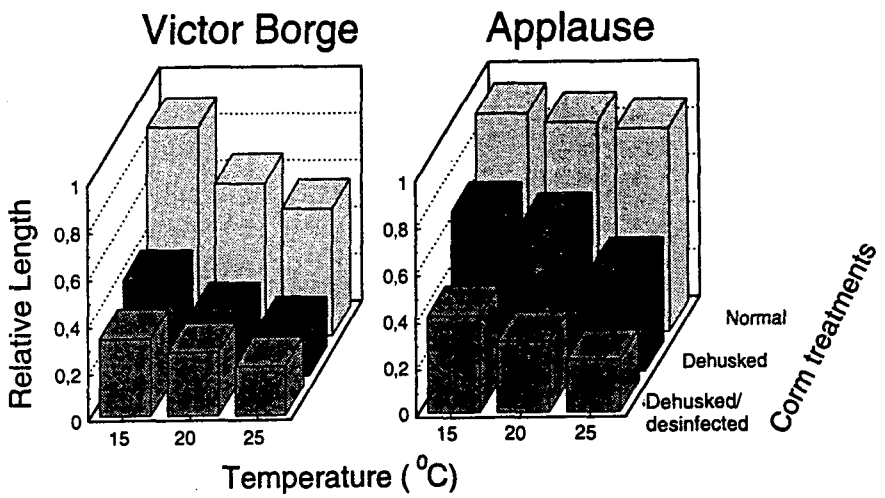

Fig. 1: Effects of greenhouse temperature and corm cleaning on the disease level of gladiolus cultivars after infection with *Fusarium oxysporum*

A large origin effect but no significant interactions were found in the test comparing gladiolus cultivars from different corm origins. This origin effect was confirmed in two other tests. The growing conditions of the corm resulted in differences in disease severity of the corm. The observed origin effect may be due to factors like pH and humus level of the soil. The two origins with the lowest infection level, Nederweert and Hengelo, both contained a high humus level. The origin Creil contained a low humus level, a high pH and showed a high infection level. At this origin a high level of N-fertilizer is applied which stimulates the infection of the corm (Woltz and Magie, 1975; Groen, 1990). It is necessary to cultivate corms of various cultivars under equal conditions, suitable for gladiolus, at the different sites of origin.

Biochemical differences between various susceptible cultivars do exist (Garcia-Jimenez *et al*, 1986; Bajaj *et al.*, 1989). Resistant plants contained higher amounts of phenols compared with susceptible ones. No reports of environmental effects on phenol level have been published but it is plausible that they exist.

No significant differences were found for corm size or interaction with cultivar so all corm sizes can be used for these tests. The results as described in this article were confirmed in another experiment with corms used for the origin test.

No influences of inoculum concentration on the sequence of cultivars was found. These results were confirmed by Löffler *et al.* (1996). The results of the two lowest infestation levels showed little difference whereas the higher infestation level led to a higher disease level of the plant. So for testing cultivars the application of an inoculum pressure between 0.01 and 0.1% is desired. A higher inoculum pressure level results in a higher disease level and might result in smaller differences between cultivars.

Nevertheless, the inoculum concentration can influence the time course of an assay. A high disease level can shorten the length of a test period. This was also confirmed in lily scales infected with *F. oxysporum* f.sp. *lilii* (Straathof and Inggamer, 1992). Using untreated corms, a lower inoculum concentration than 0.01% inoculum pressure resulting in an infestation level of less then 10 000 propagules per gram soil is not advised, because little or no elongation growth reduction occurrs (Löffler *et al.*, 1996).

Both dehusking and disinfection of the corm resulted in a great increase of plant infection (Figure 1). A two way interaction for the two cultivars used was not found ($p=0.192$). Unlike our results, Jones and Jenkins (1975) found an interaction between cultivar and wounding, indicating a surface barrier for the fungus. Since interactions may occur and the risk of a secondary infection is present wounding should be avoided, and therefore, corm cleaning and disinfection are not advised.

Temperature affects the speed and extent of invasion by the fungus (Figure 1). A higher temperature leads to a higher infection level. These results confirm those of McClellan (1952), Bald *et al.* (1971) and Wilfret and Woltz (1973). A temperature above 27^0C results in lower corm infection (Wilfret and Woltz, 1973). As reported for *Narcissus* inoculated, with *F. oxysporum* f.sp. *narcissi*, at 22-29^{0}C infection proceeds rapidly while at soil temperatures under 13^0C hardly any infection is observed (McClellan, 1952). In lily scales both a higher inoculum concentration and higher temperature increase disease development (Straathof and Inggamer, 1992).

In conclusion, the described test can successfully be used for VCU-purposes, using corms grown at the same place. Testing conditions like temperature and inoculum concentration, as they might occur in an assay, have no effect on the cultivar sequence. Both instruments can be used for shortening the assay length. Conclusions about the resistance level of cultivars can be based on the parameter length reduction (RL). To trace a latent infection the disease rating has to be observed in control pots.

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References

Bajaj, K.L., Aurora, J.S., and Kaur, P.P., 1989. Biochemical differences in tolerant and susceptible varieties of gladiolus to *Fusarium* wilt. J. Res. Punjab agric. Univ. 26 (4): 585-587.

Bald, J.G., Suzuki, T., and Doyle, A., 1971. Pathogenity of *Fusarium oxysporum* to Easter lily, narcissus and gladiolus. Ann. App. Biol. 67: 331-342.

Garcia-Jimenez, J., Piera, V.J., and Alfaro, A., 1986. An improved method to evaluate *Fusarium oxysporum* f.sp. *gladioli* pathogenicity. Acta Hort. 177: 541-545.

Groen, N.P.A, 1990. Measurement before application is important. Nitrogen encourages Fusarium infection in gladioli (in Dutch). (Stikstof bevordert Fusariumaantasting bij gladiolen.) Bloembollencultuur 4 (101): 35.

Jones, R.K., and Jenkins Jr, J.M., 1975. Evaluation of resistance in gladiolus species to *Fusarium oxysporum* f.sp. *gladioli*. Phytopathology 65: 481-484.

Komada, H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soils. Rev. Plant Prot. Res 8: 114-125.

McClellan, W.D., 1952. Effect of temperature on the severity of *Fusarium* basal rot in *Narcissus*. Phytopathology 42:407-412.

Löffler, H.J.M., and Mouris, J.R., 1989. Screening for *Fusarium* resistance in lily. Mededelingen Faculteit Landbouwwetenschappen Rijksuniv. Gent 54(26): 525-530.

Löffler, H.J.M., Straathof, Th. P., Van Rijbroek, P.C.L., and Roebroeck, E.J.A., 1996. *Fusarium* resistance in *Gladiolus*: The development of a screening assay. (in preparation).

Palmer J.G., and Prior, R.L., 1958. Evaluation of 160 varieties of gladiolus for resistance to *Fusarium* yellows. Pl. Dis. Rep. 12 (42): 1405-1407.

Payne, R.W., Lane, P.W., Ainsly, A.E., Bicknell, K.E., Digby, P.G.N., Harding, S.A., Leech, P.K., Simpson, H.R., Todd, A.D., Verrier, A.D., White, R.P., Gower J.C., Tunnicliffe Wilson, G., and Paterson, L.J., 1987. Genstat 5, Reference manual. Claredon Press, Oxford. 749 pp.

Sciortino, A., and Roxas, U.A., 1994. Biological evaluation of gladiolus corms obtained in different sites (in Italian). (Valutazione biologica di bulbi di gladiolo ottenuti in località diverse.) Sementi elette 6: 35-46.

Straathof, Th.P., and Inggamer, H., 1992. Influence of temperature, inoculum concentration and time course in a scale test for Fusarium resistance in *Lilium*. Acta Hort. 325: 695-701.

Straathof, Th.P., Roebroeck, E.J.A., and Löffler, H.J.M., 1996. *Fusarium* resistance in *Gladiolus*: Studies on resistance and virulence. In preparation.

Van Keulen, H., and Van Aartrijk, J., 1993. Disease susceptibility of flower bulb cultivars (in Dutch). (Ziektegevoeligheid van cultivars van bloembolgewassen.) Milieuplatform Bloembollensektor Hillegom: 29-30.

Wilfret, G.J., and Woltz, S.S., 1973. Susceptibility of corms of gladiolus cultivars to *Fusarium oxysporum* f.sp. *gladioli* Snyd. & Hans. at different temperatures. Proc. Fla. State Hort. Soc., 86: 376-378.

Woltz S.S., and Magie, R.O., 1975. Gladiolus *Fusarium* disease reduction by soil fertility adjustments. Proc. Fla. State Hort. Soc. 88: 559-562.