Infection of *Curvularia gladioli* on different gladiolus genotypes

Denise P. Torres, Mariana A. Silva & Gleiber Q. Furtado

Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil

Author for correspondence: Gleiber Q. Furtado, e-mail: gfurtado@ufv.br

ABSTRACT

In this study the susceptibility of different genotypes of gladiolus (*Gladiolus callianthus* and the genotypes of *G. grandiflorum* T-704, Red Beauty, Tradehorn, Verônica, Amsterdam, Yester Gold, and Rose Friendship) was evaluated and correlated with the development of *Curvularia gladioli* infective structures. The plants were inoculated with a 3 x 10⁴ conidial suspension of the pathogen, and the severity of infection was assessed eight days after inoculation. Leaf samples of *G. callianthus* and *G. grandiflorum* var. Amsterdam, Red Beauty, and T-704 were collected 24 hours after inoculation. They were cleared and the rates of spore germination and appressorium formation of *C. gladioli* were quantified. *G. grandiflorum* var. T-704 and Red Beauty were the most susceptible genotypes, followed by *G. callianthus* and *G. grandiflorum* var. Tradehorn. The remaining genotypes did not differentiate among them. No differences related to the conidial germination (93.5%) were found for the genotypes. However, the highest values for appressorium formation were observed for *G. callianthus* (66.5%) and *G. grandiflorum* var. Amsterdam (55.7%), and the lowest values were observed for T-704 (32.4%). Thus, appressorium formation of *C. gladioli* was higher on leaf surfaces of resistant genotypes.

Key words: *Gladiolus callianthus*, *Gladiolus grandiflorum*, appressorium, curvularia leaf spot, flower bulb.
The conidial suspension was sprayed on the lower surface of gladiolus leaves until the maximum number of drops was retained. Twenty-one-day-old G. callianthus and G. grandiflorus vars. Amsterdam, Red Beauty, Friendship Rose, T-704, Tradehorn, Verônica, and Yester Gold were used. The inoculated plants were moistened, wrapped in plastic bags to maintain a saturated environment, and kept in a growth chamber at 25°C with a photoperiod of 12 h of light for 30 h. The plastic bags were removed, and the plants were transferred to a growth chamber at 22°C with a photoperiod of 12 h of light.

The assessment of the severity of the curvularia spot was performed eight days after inoculation. Leaf sections of 17 cm, from the edge of the leaf, were scanned, and the images were analyzed for the proportion of healthy and diseased tissue using the QUANT software v. 1.01 (Universidade Federal de Viçosa, 2003).

The experiment used a completely randomized design with four replicates, and each replicate consisted of two leaves, one fully expanded leaf and one young leaf, per plant. The experiment was repeated once. Data were analyzed by analysis of variance, using the arcsine root transformation of the proportion of severity. The means were compared by Tukey test at 5% probability using SAS software Version 9.0 (SAS Institute). The results of the two trials were pooled because homogeneity of variance was confirmed by Bartlett’s test (Gomes & Garcia, 2002).

The percentages of conidial germination and appressorium formation were evaluated in G. callianthus and G. grandiflorus vars. Amsterdam, Red Beauty and T-704. Twenty leaf samples of approximately 1 cm² were randomly collected from each treatment 24 h after inoculation and transferred to glass vials containing a 1 g mL⁻¹ solution of chloral hydrate (Vetec). The leaf samples were stained with lacto-fuchsin (0.1%), transferred to glass slides containing a drop of glycerinated water (50/50 v/v) and observed with a light microscope. The data were analyzed by analysis of variance, and the means were compared using the Duncan test at 5% probability. The relationship between the percentages of appressorium formation and the severity of curvularia leaf spot was assessed by Pearson’s correlation using SAS software v. 9.0.

The genotypes showed different levels of susceptibility to C. gladioli (Table 1). G. grandiflorus vars. T-704 and Red Beauty were the most susceptible, followed by G. callianthus and G. grandiflorus var. Tradehorn. The G. grandiflorus vars. Amsterdam, Rose Friendship, Verônica, Tradehorn and Yester Gold varieties were the most resistant, and there were no significant differences among them. There are several varieties of gladiolus that are highly resistant to leaf spot caused by C. gladioli (Magie, 1953). The susceptibility of 46 varieties of gladiolus to Fusarium rot and leaf spots caused by Botrytis and Curvularia were evaluated by McClellan & Pryor (1957), who found that, on a scale of 0 to 4, only four varieties showed a severity of curvularia leaf spot less than or equal to one, and ten were very susceptible to disease. However, the conditions in which plants were incubated after inoculation were different from those used in this work. McClellan & Pryor (1957) kept the gladiolus at 29°C for 48 h and subsequently transferred them to a greenhouse until evaluation of disease symptoms.

On fully expanded leaves, the first lesions were observed between 3 and 4 days after inoculation. There were differential responses among the genotypes. The most susceptible varieties showed circular to oval leaf spots, which were gray to dark brown with or without a yellow halo. The spots became irregular in shape and extended along the vein of the leaf. In G. grandiflorus var. T-704, the spots became necrotic, and the leaves acquired a wilted aspect (dry). In G. grandiflorus var. Red Beauty, the spots also became necrotic; however, the spots were always surrounded by yellow halos. The spots in G. callianthus were round and always surrounded by yellow halos. In the less susceptible genotypes, G. grandiflorus varieties Amsterdam, Friendship Rose, Tradehorn, Verônica and Gold Yester, small, light brown to dark brown spots were observed.

McClellan & Marshall (1950) reported that C. gladioli infects the Picardy variety in a temperature range from 7°C to 30°C, with the optimal temperature between 24°C and 30°C. Jackson (1961) reported that infection of the leaves of the Corona, Florida Pink and Valeria varieties occurred from 24°C to 36°C. In this study, leaf infection occurred in all varieties tested at 22°C; however, there was no sporulation of the pathogen. Lack of sporulation was most likely due to the lower temperature used in this experiment.

Conidia showed polar germination (Figure 1A), frequently occurring at both ends simultaneously. The germ tubes grew in random directions, and sometimes ramifications occurred from 24°C to 36°C. In this study, leaf infection occurred in all varieties tested at 22°C; however, there was no sporulation of the pathogen. Lack of sporulation was most likely due to the lower temperature used in this experiment.

**Table 1 - Severity of curvularia leaf spot in Gladiolus callianthus and seven varieties of Gladiolus grandiflorus incubated in a climatic chamber at 22°C eight days after inoculation with Curvularia gladioli**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gladiolus grandiflorus var. T-704</td>
<td>21.46 A</td>
</tr>
<tr>
<td>G. grandiflorus var. Red Beauty</td>
<td>11.92 B</td>
</tr>
<tr>
<td>Gladiolus callianthus</td>
<td>5.20 C</td>
</tr>
<tr>
<td>G. grandiflorus var. Tradehorn</td>
<td>1.96 CD</td>
</tr>
<tr>
<td>G. grandiflorus var. Verônica</td>
<td>1.15 D</td>
</tr>
<tr>
<td>G. grandiflorus var. Amsterdam</td>
<td>0.68 D</td>
</tr>
<tr>
<td>G. grandiflorus var. Yester Gold</td>
<td>0.34 D</td>
</tr>
<tr>
<td>G. grandiflorus var. Rose Friendship</td>
<td>0.05 D</td>
</tr>
</tbody>
</table>

Means followed by the same letters in the column do not differ by Tukey test at 5% probability. The data were transformed to arcsine root of the proportion of severity.
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were observed. The majority of conidia (93.5\%) germinated 24 h after inoculation, but this did not differ between the genotypes. Therefore, there was no relationship between susceptibility to curvularia leaf spot and the percentage of spore germination. Similar results were observed in rice (\textit{Curvularia tuberculata} B.L. Jain and \textit{C. oryzae} Bugnic.); corn (\textit{Exserohilum turcicum} (Pass.) K.J. Leonard & Suggs); and apple (\textit{Colletotrichum gloeosporioides} (Penz.) Penz. & Sacc.) (De Luna et al., 2002; Muiru et al., 2008; Araújo & Stadnik, 2011). Magnani et al. (2007) reported that in leaves of different soybean cultivars inoculated with \textit{Phakopsora pachyrhizi} Syd. & P. Syd., the germination of conidia on the surface of susceptible genotypes was higher than that on the surface of resistant genotypes and that the resistance of the genetic material tested was observed in the pre-penetration phase.

Hyaline appressoria (Figure 1B) formed mainly in the epidermal cells, but some formed on stoma. The percentages of appressorium formation differed between the genotypes. The highest values were observed in \textit{G. callianthus} (66.5\%) and \textit{G. grandiflorus} var. Amsterdam (55.7\%) and the lowest values were observed in \textit{G. grandiflorus} var. T-704 (32.4\%) (Figure 2). Thus, we observed a negative correlation ($r = -0.64$) between the severity of curvularia leaf spot and the percentage of appressorium formation. In most pathosystems, the early events of pathogen infection, conidial germination and appressorium formation are similar on susceptible and resistant surfaces (Bell, 1981). However, a direct relationship between the percentage of appressorium formation and the susceptibility to pathogen has been found in guarana (\textit{Colletotrichum guaranicola} F.C. Albuq.); tomato (\textit{Alternaria solani} Sorauer); and soybean (\textit{P. pachyrhizi}) (Bentes & Matsuoka, 2002; Araújo & Matsuoka, 2004; Magnani et al., 2007). The results of this study indicate that different genotypes of gladiolus grown in Brazil have different reactions to curvularia leaf spot. \textit{G. grandiflorus} var. T-704 is the most susceptible, and \textit{G. grandiflorus} var. Amsterdam, Friendship Rose, Veronica, Tradehorn and Gold Yester are the least susceptible. Resistance to this disease is not related to the percentage of conidial germination; however, it is positively correlated with the percentage of appressorium formation.

Considering the high susceptibility of some species and varieties of \textit{Gladiolus} to curvularia leaf spot, further studies encompassing a larger number of varieties and isolates of this pathogen must be performed to detect sources of resistance to \textit{C. gladioli} in this ornamental.
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