Breeding for Resistance to *Fusarium oxysporum* in Flower Bulbs

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Abstract

Cultivation of the major flower bulb crops, e.g., lily, narcissus, gladiolus and tulip, is threatened by the soil-borne fungus *Fusarium oxysporum*. *Fusarium* infected bulb lots have lower yields and cause significant problems for bulb export and cut flower production. Besides cultivation practices and chemical protection, resistant cultivars can play an important role in preventing this disease. To breed *Fusarium* resistant cultivars, screening and selection tests have to be developed and genetic variation in the host and in the pathogen determined. Information about the inheritance can be helpful in selecting the parents with the best breeding value for *Fusarium* resistance.

Clonal screening tests under standardized conditions (e.g., concerning temperature, inoculum concentration and duration of the test) are developed for lily, narcissus, gladiolus and tulip using artificially infested soil or bulbs. By using a clone of a genotype, levels of resistance can be determined accurately. Severity of the disease is mainly observed by using disease ratings or the percentage of diseased bulbs.

Partial resistance to *Fusarium* is present in all four crops. In *Narcissus* and *Gladiolus* even species with absolute resistance were identified. Resistant species are used in interspecific breeding programmes with commercial cultivars. Variation in virulence of *Fusarium* was investigated for f.sp. *lili*, f.sp. *gladioli* and f.sp. *narcissi*. In all these *formae speciales* indications for race-formation were observed.

Comparison of different sizes of bulbs within genotypes showed that screening immature bulbs is possible. Tests of individual plants in the seedling stage also gave promising results for a more efficient selection system. The use of indirect selection by molecular markers was investigated in the lily-*Fusarium* interaction. Inheritance of resistance in all four crops was mainly of a polygenic nature. Breeding values of parents determined after diallel analysis correlated well with resistance levels determined in clonal tests.

Key words: *Gladiolus*, *Lilium*, *Narcissus*, screening, selection, *Tulipa*, genetic variation
1. **Fusarium**

*Fusarium* belongs to the class of the Deuteromycetes (Fungi Imperfecti) and *F. oxysporum* Schlecht. is classified in the section *Elegans* (Snyder & Hansen, 1940). The haploid fungus can produce three types of asexual spores, uni (bi-)cellular microconidia, multicellular 3-4-septate macroconidia, and chlamydospores. Genetic exchange by a parsexual cycle may possibly occur following cell fusion when heterokaryons are formed (Puhalla, 1981; Molnar *et al.*, 1990). Under unfavourable conditions chlamydospores (cells with a thick cell wall) are formed which can survive for long periods in soil (Schipper & Van Eck, 1981). Infection occurs by germinated spores which penetrate the roots of a host (MacHardy & Beckman, 1981) or *via* wounds and stomata in bulbs (Baayen, 1992).

Within *F. oxysporum* more than 75 *formae speciales* are defined which are distinct from each other by their host plant range (Armstrong & Armstrong, 1981). Most of these *formae speciales* cause wilting and colonize the vessels of the host plant (MacHardy & Beckman, 1981). In flower bulbs, however, the dominant symptom is rotting of the bulb or corm.


2. **Control of Fusarium in Flower Bulbs**

In flower bulbs damage by *Fusarium* occurs mainly during bulb propagation and cultivation. *Fusarium*-infected bulb lots have lower yields and can be rejected by the Inspection Service. Contaminated soil cannot be used for bulb cultivation for several years and infected bulb material causes major problems for bulb exporters. Infected bulb material and intensive soil use can also cause major problems during flower forcing. No survey of the financial damage caused by *Fusarium* in flower bulbs has been reported. This is due to the fact that most of the damage is indirect and difficult to determine.

Prevention of infection by *Fusarium* depends on a combination of measures (McRae, 1987). Besides cultivation practices like crop rotation, a low nitrogen supply (Woltz & Magie, 1975), steaming of soil, hot water treatment (Roebroek *et al.*, 1991), and the use of healthy plant material (Bald & Chandler, 1957), prevention is mostly based on chemical disinfection of bulbs (Hanks, 1992) and soil. However, resistance of *Fusarium* to fungicides can occur (Bollen, 1972). Furthermore, insufficient protection is often obtained by fungicides. Chemical disinfection of soil used for control of nematodes, also has a fungicide effect. A decrease in the use of nematicides in bulb
cultivation can lead to an increase in damage caused by *Fusarium*. In the Netherlands, a reduction of the application of chemicals for disease control is necessary to reduce environmental pollution. For soil disinfection, with respect to 1985, a reduction of 60-85% in the flower bulb industry and approximately 75% in the bulb flower industry must be accomplished before the year 2000 (Van Aartrijk *et al.*, 1990).

An environmentally friendly alternative would be disease resistant cultivars. Breeding for resistance has proven to be successful in many crops and for many pathogens, including soil-borne diseases (Tinline *et al.*, 1989) such as *F. oxysporum* (Shaner, 1981). In flower bulbs, breeding for *Fusarium* resistance is being investigated for the major crops, i.e., lily, narcissus, gladiolus and tulip.

3. Breeding for *Fusarium* Resistance in Flower Bulbs

Several steps are necessary in order to start a successful breeding programme for *Fusarium* resistance in flower bulbs. First, a useful screening test has to be developed. Next, the occurrence of genetic variation for *Fusarium* resistance is required. Besides variation in resistance in the host, variation in virulence may exist in the pathogen. Finally, screening tests that preferably can be applied at individual level at the seedling stage are required to select resistant genotypes efficiently. Information about the inheritance can be helpful in selecting parents with the best breeding value for *Fusarium* resistance.

3.1 Screening methods

Before breeding can be conducted, development of a screening method is required to determine the level of *Fusarium* resistance in flower bulbs. Screening tests can be performed at clonal level and under standardized conditions. Special attention has to be paid to resistance measurements, the developmental stage of the plant material and to the influence of testing conditions.


In this context, repeatability is defined as the agreement of results between different screening tests. Highly significant correlations between the results of different years were found in lily (Straathof *et al.*, 1993), in narcissus (Tompsett, 1986) and in gladiolus (Löffler *et al.*, *in preparation A*). A significant effect of origin of plant material was found in a gladiolus-*Fusarium* test (Van Rijbroek *et al.*, *in press*). Nitrogen supply during cultivation, infection of other pathogens (e.g., virus), and the use of fungicides after harvest can influence the *Fusarium* sensitivity of the plant material in screening tests. For the estimation of the *Fusarium* resistance in flower bulbs, the cultivation of bulbs under standardized conditions in the year before testing is recommended.
Accuracy determines which size of differences in resistance can be measured between genotypes. Measurement of resistance to a bulb rot pathogen is difficult, because the major symptoms are not visible before harvest. In gladiolus the length of the shoots can be used to estimate *Fusarium* resistance levels (Löffler et al., in preparation A). After harvest, the number of diseased bulbs (Van Eijk et al., 1978; Linfield, 1986; Linfield, 1992a) or weight measurements (Straathof et al., 1993) can be used to estimate *Fusarium* resistance levels. An alternative is provided by qualitative non linear disease ratings (Jones & Jenkins, 1975; Straathof et al., 1993; Löffler et al., in preparation A). Weight measurements and disease ratings were highly correlated and provided similar precision (Straathof et al., 1993). All measurements can be analyzed by analysis of variance (ANOVA). Testing conditions e.g., temperature, inoculum concentration and duration of the experiment can effect the accuracy (Straathof & Inggamer, 1992). To obtain high accuracy, testing under standardized conditions is preferred. Interim harvest of some additional bulbs of genotypes with a well-known resistance ranging from susceptible via intermediate to resistant can be helpful to determine the most suitable harvest date. Furthermore, the use of these reference cultivars in screening tests, will make it possible to compare different tests.

The practical application depends on the amount of time, labour and resources (e.g., greenhouse capacity) needed for testing. The input of a screening method can be reduced by choosing a simple disease measurement; such as qualitative disease rating, using small bulb material (Van Eijk & Leegwater, 1975; Linfield & Price, 1986; Straathof & Löffler, 1994a) or conducting short experiments by using a higher temperature and/or inoculum concentration (Straathof & Inggamer, 1992). Löffler et al. (in preparation A) described an in vitro *Fusarium* test in gladiolus.

Reliability is defined as the agreement between screening results and resistance under growers conditions. Field experiments could provide information comparable with growers conditions. Environmental variation, however, makes control of the progress of the disease in the field difficult. Modified field experiments were carried out in lily (Imle, 1942), narcissus (Tompsett, 1986), gladiolus (Jones & Jenkins, 1975) and tulips (Van Eijk et al., 1978). There is no evidence that results under field conditions will differ from screening tests under standardized conditions.

### 3.2 Genetic variation

When screening methods are available, genetic variation in host resistance can be traced. Variation can be found in (old) cultivars or wild species. Interspecific breeding techniques are necessary to allow for introgression of resistance genes from various species (Van Creij et al., 1990). Resistance levels can vary from susceptible via partial resistance to absolute resistance. Especially if absolute resistance is found, variation in virulence in the pathogen has to be investigated. When different physiologic races of the pathogen occur, resistance might not hold for all isolates. Before intensive and expensive interspecific breeding programmes are carried out, the variability of the pathogen should be known.
In lily, partial resistance to *Fusarium* was detected in cultivars as well as in wild species (Imle, 1942; Smith & Maginnes 1969; Straathof & Van Tuyl, 1994). No absolute resistance was found in lily. In the Asiatic hybrids high levels, in the Oriental hybrids only low levels and in *L. longiflorum* intermediate levels of *Fusarium* resistance were found (Straathof & Van Tuyl, 1994). Also in narcissus genetic variation for resistance was found (Tompsett, 1986). In several species absolute resistance was detected (Linfield, 1986; Linfield, 1992a; Linfield, 1992b). Variation in *Fusarium* resistance was found in gladiolus (McClellan & Pryor, 1957; Palmer & Pryor, 1958; Jones & Jenkins, 1975; Chandra et al., 1985; Straathof et al., in preparation A). In the large flowered gladioli partial resistance was found, but all small flowered gladioli tested were susceptible. In an accession of *G. dalenii* absolute resistance was found (Straathof et al., in preparation A). New resistant large flowered cultivars were released (Wilfret & Magie, 1979; Wilfret, 1981; Wilfret, 1986). Partial resistance was also found in tulips (Van Eijk et al., 1978; Van Eijk et al., 1979).

Knowledge of genetic variation in virulence (sensu Van der Plank) is important for estimation of durability of the *Fusarium* resistance. Imle (1942) found no evidence for the existence of physiological races of f.sp. *lili* in lily. Bald et al. (1971) showed that isolates of *F. oxysporum* f.sp. *lili* from roots, bulbs and stems differed in tissue specificity. Löffler et al. (1995) found only significant interactions when low aggressive *Fusarium* isolates were tested on *L. longiflorum*. For lily breeding purposes, it is sufficient to test with the most aggressive isolates. Löffler et al. (in preparation B) found no adaptation of the pathogen to resistance in the host. Based on these results, durability of *Fusarium* resistance in lily is expected. The occurrence of physiological races of f.sp. *gladioli* in gladiolus has been reported. Two races were defined, one virulent for only a small flowered gladiolus cultivar and one virulent for a large flowered as well as for a small flowered gladiolus cultivar (Roebroek & Mes, 1992; Mes et al., 1994). Races could not be identified on basis of VCG or RFLP patterns. Differences in virulence have also been observed in f.sp. *narcissi* (Linfield, 1994; Linfield, in press).

Although formae speciales can be distinguished by their host range, cross-infection is reported. Imle (1942) reported that an isolate of *F. oxysporum* f.sp. *lili* was pathogenic on crocus, and a *Fusarium* isolated from crocus was pathogenic to *L. formosanum*. Löffler & Mouris (1992) and Löffler et al. (1995) showed that *Fusarium* isolates from gladiolus and tulip were pathogenic for lily. Apt (1958) found that cross-infection only occurred within the *Iridaceae*. Valásková (1976) demonstrated that freesia could be infected by fusaria isolated from gladiolus, lily and tulip. Roebroek & Mes (1992), Mes et al. (1994) and Straathof et al. (in preparation A) found that fusaria isolated from iris, crocus and freesia were able to infect gladiolus. This can have a strong influence on crop rotation systems for bulb and flower production.

### 3.3 Selection techniques

To obtain new resistant genotypes from populations, efficient selection techniques have to be developed. High efficiency can be obtained by using immature bulbs at clonal or at individual level (seedlings). First, different sizes of bulbs within genotypes have to
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be compared. Secondly, individual seedlings can be tested. If a seedling test is carried out using several populations from an (incomplete) diallel, breeding value of parents can be estimated.

The ranking of lily genotypes according to their resistance was very similar in four different developmental stages. Smaller bulbs, however, were generally much more sensitive to the pathogen than larger bulbs (Straathof and Löfler, 1994a). Tests with bulbils derived from chips and twin scales of narcissus bulbs gave similar results (Linfield & Price, 1986), but it was suggested that sensitivity to Fusarium decreased with maturity (Bowes et al., 1992). Gladiolus seedlings showed a lower sensitivity than corms (Straathof et al., in preparation B). In tulips ranking of juvenile and adult bulbs for Fusarium resistance was similar but juvenile tulip bulbs were less sensitive than adult bulbs (Van Eijk & Eikelboom, 1983).

Development of a lily seedling test was unsuccessful, since direct sowing of seeds in infested soil caused heavy losses by damping-off (Imle, 1942). Transplanting young seedling bulbs after a low temperature treatment to infested soil was more successful, although some susceptible plants were selected (escapes) and some resistant seedlings were discarded (missings). Large variation in resistance within and between populations was found, thus making selection possible (Straathof & Löfler, 1994b). Direct infection of narcissus seedlings was unsuccessful (Linfield & Price, 1986). The use of one, two and three year old seedlings was more promising (Bowes et al., 1992). Selecting for Fusarium resistance in gladiolus is possible at seedling level. Seeds were sown in a 2 cm layer of non-infested soil on top of Fusarium infested soil (Straathof et al., in preparation B). Seedling tests were also successful in tulips using one year old bulbs (Van Eijk et al., 1979).

To study the inheritance of (partial) resistance in flower bulbs, a large number of descendants of related populations (e.g., back crossings and selfings) are necessary. As an alternative, diallel analysis can be helpful. Analysis of the results of the flower bulb seedling tests led to the conclusion that several genes are involved. This is based on the fact that the general combining ability (GCA) was the most important component (Van Eijk et al., 1979; Bowes et al., 1992; Straathof & Löfler, 1994b; Straathof et al., in preparation B). Furthermore, a good association between the GCA value of a parent (breeding value) and its resistance level was determined in lily (Straathof & Löfler, 1994b) and gladiolus (Straathof et al., in preparation B). GCA's of parents of adult progenies showed good agreement with GCA's obtained of juvenile progenies in tulips (Van Eijk & Eikelboom, 1983). Some variation in GCA's of parents in different narcissus trials shows that caution is needed when interpreting a single experiment (Bowes, 1992). Molecular markers can be helpful in genetic studies and can be used for indirect selection when a close linkage between marker and resistance gene is obtained. Three RAPD markers linked with Fusarium resistance were detected in lily (Straathof et al., 1996). The three markers explain approximately 85 percent of the total variance of the resistance (Jansen, 1996).
References


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