Resistance to *Fusarium oxysporum* at Different Developmental Stages of Asiatic Hybrid Lilies

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**Abstract.** Tests for determining resistance to *Fusarium oxysporum* f.sp. *lilii* in different developmental stages of *Lilium* were developed. Under standardized conditions, commercial bulbs, yearling bulbs, scale bulblets, and scales of 16 Asiatic lily cultivars were tested for *Fusarium* resistance. Disease ratings were analyzed statistically using a threshold model for ordered categorical data. For each cultivar in each stage the resistance level was calculated as the disease severity score (DSS). Disease severity score values of cultivars in the four stages were reproducible between experiments, although some variation in cultivars was found. This variation may be attributed to the origin of plant material. Disease severity score values among cultivars in each developmental stage were correlated with the average DSS over all four stages, although some specific cultivar-stage deviations were found. The scale test is recommended for early selection while the scale bulblet test is recommended as a final check.

Production of the bulbous ornamental lily (*Lilium L.*) involves vegetative propagation followed by cultivation over several years. Bulb production is reduced by bulb rot, caused by *Fusarium oxysporum* f.sp. *lili* Imle.

During propagation and growth lily bulbs pass through three developmental stages. The first stage involves the induction of scale bulblets (Griffiths, 1933), whereby scales of commercial bulbs are incubated in soil, soilless substrate (e.g., vermiculite), or artificial media in vitro. Scale bulblets are then grown in the field for one season to form yearling bulbs. Yearling bulbs can also form during cultivation of commercial bulbs on the below-ground portion of the flower stem. The stem bulblets that develop are considered to be yearling bulbs by the end of the season. Yearling bulbs are grown for a further season in order to form commercial bulbs (Blaney and Roberts, 1966). Commercial bulbs are used in the production of cut flowers, pot, and garden plants.

*Fusarium* can cause severe losses, especially in scale bulb and yearling bulb stages. Preventing bulb rot depends mostly on fungicide application (Bald and Chandler, 1957; Bald et al., 1983; Boontjes, 1974; McRae, 1987). Increasingly, the reduction of fungicide application is considered desirable in order to limit environmental pollution. An alternative method of disease management is the cultivation of resistant cultivars. A reliable screening test is needed to breed for resistant varieties.

A screening test requires that observations reflect the ‘true’ level of resistance in the plants considered. Field experiments can provide this information, but results can vary greatly because of widely ranging environmental conditions. Greenhouse tests are preferred because environmental conditions can be more closely controlled and monitored. This is the more important since standardization is required to test with sufficient accuracy between genotypes because partial resistance is involved (Imle, 1942; Löfler and Mouris, 1989; Maginnes and Smith, 1971; Smith and Maginnes, 1969; Straathof et al., 1993; Van Tuyl, 1980).

Van Tuyl (1980) described a *Fusarium* screening test using lily scale bulblets; this test was optimized by Straathof et al. (1993) and was carried out under standardized conditions. Resistance levels were determined using a disease rating in combination with statistical analysis based on a threshold model (Jansen, 1990). There is no information in the literature concerning tests with yearling and commercial bulbs. Disease resistance between developmental stages may differ, so the levels of resistance in different stages must be compared.

Smith and Maginnes (1969) and Maginnes and Smith (1971) described a screening test using lily scales; this test was optimized by Löfler and Mouris (1989). A scale is a bulb organ and might be useful in screening for resistance, because of a more efficient use of greenhouse space.

The aim of this study was to determine resistance to *Fusarium* in a range of Asiatic lily cultivars, and to compare cultivar resistance in the four stages of development. Tests were also evaluated for their efficiency.

**Materials and Methods**

Commercial bulbs of 16 Asiatic lily cultivars (Aristo, Connecticut King, Enchantment, Esther, Golden Melody, Hilde, Milano, Mont Blanc, Montreux, Napoli, Orlito, Pirate, Prominence, Snow Star, Sterling Star, and Yellow Blaze) were obtained from Dutch growers in Autumn 1988. Plant material (Table 1) was cultivated under standardized conditions without using fungicides. For each experiment, bulbs or scales were selected for uniformity of weight. Scales of commercial bulbs were harvested from the stems of cultivated commercial bulbs. Both groups of yearling bulbs were cultivated for 2 more years to produce new commercial bulbs. Those new commercial bulbs were screened for *Fusarium* resistance in 1991.

Yearling bulbs. Yearling bulbs were produced by the cultivation of scale bulblets for 1 year, or were harvested from the stems of cultivated commercial bulbs. Both groups of yearling bulbs were screened for *Fusarium* resistance in 1990.
Table 1. Origin, year of the experiment, experimental design (number of blocks and number of plants per block), and duration of the experiment (weeks) used in Fusarium screening tests at different developmental stages of lilies.

| Developmental stage | Origin     | Year | Experimental design |
|---------------------|------------|------|---------------------|
|                     |            |      | Blocks | Plants/block | Weeks |
| Commercial bulbs    | Growers    | 1989 | 4      | 3           | 22    |
| Commercial bulbs    | CPRO-DLO   | 1991 | 5      | 4           | 20    |
| Yearling bulbs (stem)| CPRO-DLO   | 1990 | 5      | 4           | 18    |
| Yearling bulbs (scale)| CPRO-DLO   | 1990 | 5      | 4           | 18    |
| Scale bulblets      | Growers    | 1989 | 10     | 4           | 6     |
| Scale bulblets      | CPRO-DLO   | 1990 | 10     | 4           | 8     |
| Scales              | Growers    | 1989 | 4      | 10          | 12    |
| Scales              | CPRO-DLO   | 1990 | 8      | 10          | 12    |

Scale bulblets. Scale bulblets were induced on scales from commercial bulbs obtained from growers (1989 test) and from cultivation at CPRO-DLO (1990 test) as described by Straathof et al. (1993).

Scales. Scales were broken from commercial bulbs obtained from growers (1989 test) and from cultivation at CPRO-DLO (1990 test) and further treated according to Löffler and Mouris (1989).

Fungus. Two highly aggressive isolates of Fusarium oxysporum f.sp. lilii (CPRO-Fol4 and CPRO-Fol11) (Löffler and Mouris, 1989; Löffler and Rumine, 1991) were used to evaluate cultivar resistance. For soil infestation, the fungi were incubated for 3 weeks at 23°C in an autoclaved (120°C, 2 h) 1 oatmeal : 4 soil mixture (w/w). The fully grown cultures were ground and mixed in a 1:100 ratio with nonsterile potting soil (Löffler and Mouris, 1989). The number of propagules in soil was determined by dilution plating on a modified Komada medium (Komada, 1975; Löffler and Mouris, 1989) at planting time, which was 2 weeks after soil infestation (±10^5 propagules per gram of soil).

Experimental design. Plant material of different developmental stages were tested in separate experiments. Commercial bulbs, yearling bulbs, and scale bulblets were planted in 7.0-, 1.9-, and 1.1-liter pots, respectively. Several bulbs (Table 1) of one cultivar were planted in one pot per stage. Scales of all 16 cultivars were planted in a 45 × 30 cm flat tray containing 12 liters of soil. Per tray, ten scales of each cultivar were planted in a row. Experiments were laid out using a randomized block design, in which each block (Table 1) consisted of one pot or one row of all 16 cultivars. The 16 cultivars in a block were randomly assigned to the pots or rows. Several noninfested pots and trays were included as controls. Pots and trays were placed in a temperature-controlled greenhouse at 18/14°C (16-h day/8-h night). Because of the partial resistance of the cultivars, and to achieve optimal discrimination between resistance levels in each stage, bulbs were dug up and observations were carried out 6 to 22 weeks after planting (Table 1).

Disease assessment. Cultivars were evaluated for decay of the infested plant material visually according to a scale with categories 1 through 6: 1 = healthy; 2 = slightly rotten; 3 = moderately rotten; 4 = heavily rotten; 5 = very heavily rotten; and 6 = completely decayed (Straathof et al., 1993).

Statistical analysis. Disease ratings were analyzed according to a threshold model for ordered categorical data (Jansen, 1990; McCullagh, 1980), using a probit link function. The between-pot/row variation was estimated from the data (Jansen, 1990). For each experiment, the disease severity score (DSS) of a cultivar was calculated as the mean of disease ratings of that cultivar on an underlying linear scale (Straathof et al., 1993).

Conclusions concerning block and cultivar effects were based on deviance statistics (McCullagh and Nelder, 1989), which have to be compared with the table of the chi-squared distribution. The computer package Genstat (Payne et al., 1987) was used for all calculations.

To investigate reproducibility within developmental stages and to compare between stages, correlation diagrams of DSS values were made.

Results

Disease ratings. Disease severity score values for the 16 cultivars, corresponding to differences (relative to the resistant ‘Connecticut King’), deviations, and corresponding degrees of freedom for each experiment are given in Table 2. The analysis of deviance shows that cultivars significantly differ in their resistance to Fusarium (P < 0.001). Significant block effects (P < 0.05) occurred in three tests. The estimated between-pot/row variances were small except for the scale test in 1989. For ‘Esther’, DSS could not be calculated using the threshold model in three of the tests because almost all plants had the same disease rating. There were no plants in the disease rating category 6 (completely decayed) in the commercial bulb test of 1989 or in categories 1 (healthy) and 6 in scale tests of 1989 and 1990, leaving either four or three thresholds to be estimated. Disease severity score values for a cultivar and the deviance for cultivar effects differed from test to test and from year to year. For all developmental stages, the second experiment gave a larger deviance for cultivar differences.

Reproducibility. To study the reproducibility of the screening tests, results from replicate experiments for each stage were compared (Fig. 1). The DSS values for cultivars in the two experiments were correlated in all four stages but correlation was highest for the scale test. In the three bulb tests some cultivars showed variation in DSS values between replicates.

Comparison of stages. To compare DSS values of cultivars between the four developmental stages, the replicate with CPRO-DLO cultivated bulbs was chosen from each stage. For the yearling bulbs the replicate with the largest deviance for cultivar effect was used. Disease severity score values of cultivars at each stage were compared with the DSS values averaged over all four stages (Fig. 2). Cultivar resistance at each stage was correlated with the average over all stages. Correlations were highest for commercial bulbs and some specific cultivar-stage deviations occurred.

Discussion

The standardized methods developed to screen for Fusarium resistance in scale bulblets (Straathof et al., 1993) and scales...
Table 2. Disease severity score (DSS), corresponding standard error of differences (SED, relative to ‘Connecticut King’), deviances (dev), and corresponding degrees of freedom (df) obtained by the threshold model for different developmental stages of 16 Asiatic lily cultivars planted in Fusarium-infested soil.

| Cultivar              | Commercial bulbs | Yearling bulbs 1990 | Scale bulblets | Scale bulbs | Scales |
|-----------------------|------------------|----------------------|----------------|-------------|--------|
|                       | 1989 (DSS) | SED     | 1991 (DSS) | SED     | 1989 (DSS) | SED     | 1990 (DSS) | SED     | 1989 (DSS) | SED     | 1990 (DSS) | SED     |
| Connecticut King (CK) | 1.73      | 1.26    | 1.91      | 1.77    | 0.73      | 1.29    | 1.73      | 0.03    |
| Orlito (OR)           | ---       | -0.30   | 0.4       | 1.64    | 0.3       | 0.42    | 0.2       | 1.75    | 0.2       | ---     | 0.50    |
| Prominence (PR)       | 3.53      | 0.6     | 1.37      | 0.4     | 2.60      | 0.4     | 1.58      | 0.3     | 0.66      | 0.2     | 1.65      | 0.2     |
| Yellow Blaze (YB)     | 1.68      | 0.5     | 1.70      | 0.4     | 3.25      | 0.4     | 0.91      | 0.3     | 1.45      | 0.2     | 2.35      | 0.2     |
| Napoli (NA)           | 2.43      | 0.6     | 1.51      | 0.4     | 3.04      | 0.4     | 2.37      | 0.3     | 1.09      | 0.2     | 1.79      | 0.2     |
| Mont Blanc (MB)       | 2.75      | 0.6     | 1.48      | 0.4     | 1.71      | 0.4     | 1.95      | 0.3     | 0.54      | 0.2     | 1.52      | 0.2     |
| Hilde (HI)            | 4.67      | 0.6     | 1.99      | 0.4     | 3.71      | 0.4     | 2.14      | 0.3     | 0.55      | 0.2     | 2.10      | 0.2     |
| Snow Star (SN)        | 1.66      | 0.5     | 1.99      | 0.4     | 3.57      | 0.4     | 3.37      | 0.3     | 1.02      | 0.2     | 2.85      | 0.3     |
| Golden Melody (GM)    | 3.93      | 0.6     | 3.22      | 0.4     | 3.47      | 0.4     | 4.43      | 0.4     | 1.84      | 0.2     | 2.43      | 0.2     |
| Montreux (MO)         | 4.25      | 0.6     | 3.85      | 0.5     | 2.88      | 0.4     | 2.96      | 0.3     | 0.96      | 0.2     | 2.33      | 0.2     |
| Milano (MI)           | 4.66      | 0.6     | 3.11      | 0.4     | 4.84      | 0.4     | 5.96      | 0.6     | 1.28      | 0.2     | 2.21      | 0.2     |
| Sterling Star (ST)    | 5.08      | 0.6     | 4.08      | 0.5     | 5.33      | 0.5     | 4.03      | 0.4     | 0.89      | 0.2     | 2.72      | 0.3     |
| Enchantment (EN)      | 4.03      | 0.6     | 5.44      | 0.5     | 4.62      | 0.4     | 4.96      | 0.4     | 1.94      | 0.2     | 3.33      | 0.3     |
| Pirate (PI)           | ---       | 0.8     | 4.79      | 0.5     | 5.60      | 0.6     | 4.70      | 0.4     | ---       | 0.7     | 4.99      | 0.9     |
| Aristo (AR)           | 7.04      | 0.8     | 5.49      | 0.5     | 4.80      | 0.8     | 5.09      | 0.4     | 1.93      | 0.2     | 4.39      | 0.3     |
| Esther (ES)           | 7.04      | 0.7     | 7.04      | 0.5     | 5.96      | 0.6     | 1.97      | 0.2     | 3.37      | 0.3     | 4.54      | 0.5     |

| dev | df | dev | df | dev | df | dev | df | dev | df | dev | df | dev | df | dev | df |
|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|
| 1.1 | 3  | 10.3 | 4  | 3.2 | 4  | 10.4 | 4  | 3.7 | 8  | 4.7 | 9  | 1.3 | 3  | 17.9 | 7  |

| Cultivars | 115.2 | 130.3 | 15   | 145.4 | 15  | 188.4 | 15  | 128.0 | 14 | 221.2 | 15  | 91.3 | 13  | 414.5 | 15 |

aNot included in the experiment.
bDSS not calculated.
cNonsignificant or significant at P = 0.05 or 0.001, respectively, by χ2 test.

(Löffler and Mouris, 1989) were used to determine and compare resistance in different developmental stages of lily cultivars. Between replicates, similar results were obtained for all four stages, indicating that the tests are reproducible. Some variation between the replicates was, however, detected. The experiments with yearling bulbs were not replicates in time; bulbs differed in their derivation. Using scales and commercial bulbs, a higher number of plants were tested in the second experiment. Especially in the second scale test, a high level of discrimination between the cultivars was found.

The commercial bulbs used in the 1989 experiments to induce the scale bulblets and to obtain the separate scales originated from different growers. Plant material for the other experiments was cultivated under identical conditions without fungicides. An increased supply during the growing season or fungicide treatment (e.g., Benomyl, Captan, Prochloraz) used by some growers could have influenced resistance of the bulbs in the 1989 tests (Linderman, 1977). Because the cultivation conditions were standardized the year before the second tests, results of those experiments are assumed to be more reliable.

If the DSS values of the four lily stages are compared with the average DSS, generally a high correlation is found. This is in agreement with results of testing daffodils and tulips for resistance to Fusarium oxysporum. Daffodil bulbs derived from chips or twin scales and 1-year-old bulbils (Linfield and Price, 1986) and young and old daffodil bulbs (Bowes et al., 1992) showed similar levels of resistance. In tulips resistance in adult bulbs correlated with the resistance identified in juvenile bulbs (Van Eijk et al., 1979; Van Eijk and Eikelboom, 1983; Van Eijk and Leegwater, 1975).

The results from commercial lily bulbs were in close agreement with the average values. In the other developmental stages, however, some specific cultivar-stage deviations were found. The yearling stage of the cultivar Milano was more susceptible than at other stages. In the repeat experiment, however, ‘Milano’ was found to be less susceptible. Since this deviation is not consistent it may be ascribed to variation in the plant material. Scale bulblets of the most susceptible cultivars (e.g., ‘Pirate’ and ‘Aristo’) gave results that deviated from the average. This, however, did not affect the ranking of those cultivars.

In the scale test some cultivars (e.g., ‘Golden Melody’, ‘Mont Blanc’) differed from the average DSS. Those deviating results were reproducible (Fig. 1). This might be explained by differing mechanisms of infection and/or defense in scales compared with other developmental stages. A scale is broken from the basal plate of a bulb, resulting in a large wound. This may facilitate entrance of the fungus (Roebroeck et al., 1987). Scales planted in Fusarium-infested soil never formed any scale bulblets in contrast with control scales, which produced scale bulblets with roots and leaves within 6 to 8 weeks. Due to the high inoculum concentration, the basal part of the scales was always rapidly infected in all cultivars, preventing the formation of new bulblets (Straathof and Inggamer, 1992). Wounding is absent in the other stages, thereby biasing the results from the scale tests. To determine whether a different infection and/or defense mechanism is involved in scales, further investigation is required.

Clear cultivar differences were noticed when scales, scale bulblets, yearling bulbs, and commercial bulbs were tested. Fusarium resistance in cultivars ranged from partially resistant to completely susceptible. Although most bulbs were affected at harvest time, only commercial bulbs of the most susceptible cultivars were heavily diseased. All commercial bulbs except...
'Aristo' and 'Esther' gave normal flower production when compared with the control. In practice, flower production using commercial bulbs is less affected by *Fusarium* than using scale bulblets or yearlings for bulb production.

Scale bulblets were much more sensitive to *Fusarium* than larger bulbs. Scale bulblets had to be harvested within 8 weeks to be able to discriminate between partially resistant and susceptible cultivars. Imle (1942) has noted that lily seedlings are much more sensitive to *Fusarium* than larger bulbs. In daffodils, 1-year-old bulbs had a lower survival rate against *Fusarium* than 2- and 3-year-old bulbs (Bowes et al., 1992). In tulips, however, Van Eijk and Leegwater (1975) found that juvenile bulbs were less easily infected than adult bulbs. Most of the scale bulblets were infected in our experiments. The resistance found in some of the cultivars, however, is highly valuable to breeders and growers. The high levels of disease development after 6 to 8 weeks on scale bulblets is partly due to the high inoculum concentration used to obtain optimal discrimination in a short time period.

Determination of *Fusarium* resistance in lily using disease ratings in combination with a threshold model (Jansen, 1990) is very useful (Straathof et al., 1993). The calculated disease severity score (DSS) is not an absolute value but, rather, depends on the experiment. Disease severity score values cannot be calculated if most of the ratings for a certain genotype fall in the same category. This occurs if a certain cultivar is completely healthy or, conversely, heavily diseased (e.g., 'Esther'). Disease severity score values are more useful in screening tests if the performance of new genotypes is compared with a group of standard cultivars.

In conclusion, the standardized tests described can be used to detect levels of *Fusarium* resistance in Asiatic lily cultivars at different stages of development. For early selection of resistant genotypes in breeding programs, a scale test can be very useful because it is efficient in time, space, and labor, and only a small amount of plant material is needed. Because of some deviations in the scale tests compared with evaluations of bulbs, a final check of selected bulbs at the scale bulblet stage (which is also efficient in time) is suggested.

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