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Morphological traits related to western flower thrips resistance in the ornamental Gladiolus

Dinar Sari C. Wahyuni[1,2], Kirsten A. Leiss[3], and Peter G.L. Klinkhamer[1]

[1]Plant Sciences and Natural Products, Institute of Biology (IBL), Leiden University, Sylviusweg 72, 2333BE, Leiden, The Netherlands
[2]Pharmacy Department, Faculty Mathematics and Natural Sciences, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia
[3]Business Unit Horticulture, Wageningen University and Research Centre, Postbus 20, 2665ZG, Bleiswijk, The Netherlands

ABSTRACT

Host plant resistance can be based on morphological traits or chemical defense compounds. Understanding the mechanisms involved in host plant resistance opens the way for improved resistance breeding programs by using the traits involved as markers. Pest management is a major problem in cultivation of ornamentals. Gladiolus (*Gladiolus hybridus* L.) is an economically important ornamental in the Netherlands. Gladiolus is especially sensitive to attack by Western flower thrips [*Frankliniella occidentalis* (Pergande) (Thysanoptera:Thripidae)]. The objective of this study was, therefore, to investigate morphological markers for resistance breeding to western flower thrips in Gladiolus varieties. We measured thrips damage of fourteen Gladiolus varieties in a whole plant thrips bioassay and related this to morphological traits. Thrips damage varied strongly among the varieties: the most susceptible variety showed 130 times more damage than the most resistant one. Varieties with low thrips damage had smaller mesophyll cells, smaller epidermis cells, and a higher density of epicuticular papillae. In contrast, plant dry mass and leaf length were not correlated with thrips damage. All three traits that were related to thrips damage were highly correlated with each other. In fact, almost without exception all epidermis cells had one papilla. We assume that papillae may inhibit thrips movement or hinder penetration of the epidermis by the cell sucking thrips. In addition, papillae may serve as storage or production sites for plant defense compounds. Our results show that the density of papillae is an important morphological trait related to resistance to thrips. Papillae are easily visualized and may, thus, be used as thrips resistance markers in breeding programs for Gladiolus.

KEYWORDS: Gladiolus, *Frankliniella occidentalis*, host plant resistance, morphological markers, mesophyll, epidermis, papillae

INTRODUCTION

Plants have to defend themselves against a myriad of herbivores and have thus developed different ways of resistance. Host plant resistance can be based on morphological traits or chemical defense compounds. Plant morphological defence traits include smaller size, thorns, trichomes, leaf surface waxes and toughened cuticles (Scott Brown and Simmonds, 2006 and references therein). Chemical defences include the production of toxic and repellent compounds as well as digestibility reducers (Fürstenberg-Hägg *et al.*, 2013). Understanding the mechanisms involved in host plant resistance opens the way for improved resistance breeding programs by using the traits involved as markers. In this respect especially morphological markers can be important because they are mostly easily measured at low costs.

Pest management is a major problem in cultivation of ornamentals, as was again shown by the Russian boycott of Dutch ornamentals because of thrips infestation. Presently, the use of synthetic insecticides is the method of choice for controlling insects in ornamentals. Fumigation or dipping of bulbs and corms during storage and insecticide sprays on foliage and flowers is often applied. The widespread and excessive use of insecticides may cause negative impacts on human health, non-target beneficial organisms and the environment. In addition, it has led to a build-up of insect resistance (Carriere *et al.*, 2012). Therefore, new European Union regulations call for a reduced application of pesticides (Coelho, 2009). Integrated pest management (IPM), using different complementary control tactics is the way forward for bulb flower production (Rossing *et al.*, 1997; Benschop *et al.*, 2010). One of the main strategies to be included is natural host plant resistance.

The Netherlands are a leader in the production of flower bulbs worldwide. They generate $ 756 million in value with 21,000 ha of production area (Benschop *et al.*, 2010). Tulip, lily, narcissus, gladiolus, hyacinths, crocus and iris are the major bulbs produced. Gladiolus, comprises 5% of the total production (Benschop *et al.*, 2010). *Gladiolus hybridus* L. (Iridacaeae), are perennial bulbs belonging to the Iridaceae family. Gladiolus is marketed as cut flowers in summer. The latter are especially sensitive to attack of thrips.

Western flower thrips [*Frankliniella occidentalis* (Pergande (Thysanoptera:Thripidae)] is commonly found on gladiolus (Terry and Lewis, 1997).

Thrips are piercing-sucking insects causing damage to the corms and growing plants. Damage on the corms results in smaller corms, retardation of growth, and poor flowering. Some corms may even fail to germinate. Damage on the leaves, buds and flowers generate the characteristic silver damage (Denmark and Price, 1998). Damage in the buds and flowers may lead to distorted flower formation and opening. Moreover, severely damaged flowers may desiccate and fall off. Variation in gladiolus thrips resistance, primarily to *Thrips simplex* and *F. occidentalis,* has been reported by Terry and Lewis (Terry and Lewis, 1997). Nothing is known yet about the mechanism causing these differences. According to Dutch gladiolus breeders (personal communication), thrips are able to discriminate between different Gladiolus varieties, infesting certain varieties but not others.

The aim of this study is to obtain morphological markers for resistance to western flower thrips that can be used in breeding programs of Gladiolus. We focused on the following traits: plant dry mass, leaf length, size of the epidermis cells, size of the mesophyll cells and the density of papillae at the leaf surface. In particular we wanted to address the following questions: (1) Does thrips damage vary among Gladiolus varieties? (2) Do the varieties differ in potential morphological resistance traits of the leaves? (3) Is thrips damage related to morphological traits?

MATERIAL AND METHODS

Plant Materials

Fourteen different gladiolus varieties differing in size were used. Six small varieties (Charming, Charming Beauty, Nymph, Alba, Elvira and Robinetta obtained from Gebr P. & M. Hermans, Lisse, The Netherlands), eight medium to large size varieties (Ben Venuto, Red Balance, V-29, Chinon, Live Oak, Deepest Red, Green Star, and Essential obtained from VWS B.V., Alkmaar, The Netherlands). Each bulb was planted into a 9 x 9 cm pot filled with a 1: 1 mixture of potting soil and dune sand. Six to ten replicates of each variety were randomly placed into a growth room (L:D, 18:6, 20 ^{0}C) and grown for 10 weeks. Three to five replicates of each variety were used for a whole plant thrips bioassay while the remaining replicates were used for measuring the morphological parameters.

Plant Resistance to Thrips

A non-choice whole plant bioassay was conducted as described in Leiss *et al.* (2009). Plants were placed individually in a thrips proof cage, consisting of a plastic cylinder (80 cm height, 20 cm diameter), closed with a displaceable ring of thrips proof gauze. The cages were arranged in a fully randomized design in a climate chamber (L18: D6, 20 °C). Two male and 18 female adult western flower thrips were added and left for two weeks. Thereafter, silver damage, expressed as the leaf area damaged in mm^2, was visually scored for each plant.

Morphological Measurements

Morphological resistance traits were measured on the longest leaf of each replicate. In addition, plant dry mass was obtained after drying plants for 3 days in an oven at 50 °C. We measured the length of the leaves and of epidermis- and mesophyll cells as well as the density of the epicuticular papillae which form a convex outgrowth of epidermal cells (Koch *et al.*, 2008). The density of papillae was measured as number of papillae per 2100 μm^2. To measure these traits cross sections of fresh leaves were examined under a confocal laser scanning and a visual light microscope (Zeiss LSM Exciter) with a 20x magnification. Measurements were conducted using image J software. To visualize the leaf surface of Gladiolus varieties, we choose Charming Beauty and Robinetta as representatives of a variety with high and low thrips damage respectively, for scanning-electron microscopy (SEM). We used a JSM6400 scanning electron microscope (JEOL, Tokyo, Japan). Leaf discs were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7) followed by dehydration with a graded series of acetone solutions (70%, 80%, 90% 96% and 100% acetone) for10 min each. Before imaging, specimens were oriented, mounted on metal stubs and sputter-coated with gold (Polaron 5000 Sputtering System).

Statistical Analysis

Differences between varieties in plant dry mass and morphological traits were analyzed with one-way ANOVA and subsequent post-hoc analysis with Bonferroni correction. Silver damage did not fit a normal distribution and was, therefore, Ln-transformed. Correlations between thrips silver damage and morphological traits were analyzed using Pearson correlations.

RESULTS

Differences in Resistance to Thrips

Thrips silver damage in the whole plant bioassay differed significantly among varieties (F = 11.445, df = 13, P = 0.000). Charming Beauty and Charming as the most susceptible ones showed significantly more damage compared to all other varieties, while Robinetta and Alba showed almost no damage at all (Fig. 1). Charming, displaying the highest amount of damage, (mean 3159.3 ± 434.8 mm^2), showed 130-times more damage than Robinetta (mean 23.8 ± 8.9 mm^2).

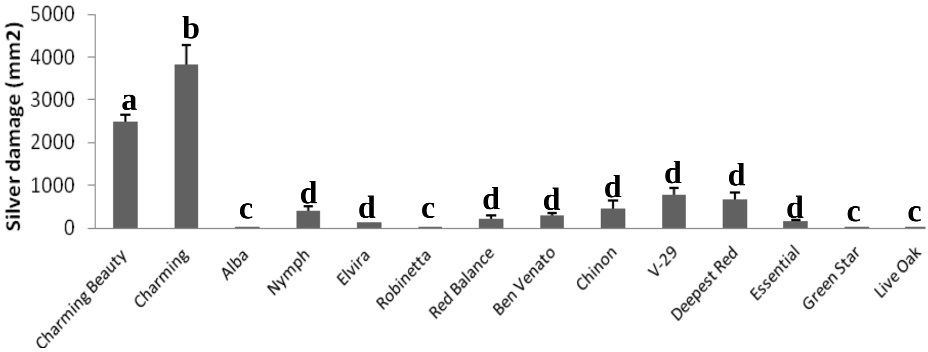


Figure 1. Silver damage, (mm^2) in fourteen Gladiolus varieties as measured by a whole plant thrips non-choice bioassay. Data represent mean and standard errors for three to five replicates. Different letters indicate significant differences between varieties at $p \leq 0.05$.

Morphological Differences

Leaf length (F = 15.522, df = 13, P = 0.000) differed significantly among varieties. Nymph, Elvira and Robinetta were significantly shorter than the other varieties (Fig. 2A). The dry mass of varieties differed significantly (F = 70.531, df = 13, P = 0.000) with large size varieties yielding more than double the dry mass compared to the small ones (Fig. 2B). The length of epidermis- (F = 125.459, df = 13, P = 0.000) (Fig. 3A) and mesophyll cells (F = 90.136, df = 13, P = 0.000) (Fig. 3B) also differed significantly between varieties. For both cell types the cells in the most susceptible varieties, Charming and Charming Beauty, were two times longer than those of the resistant varieties. The density of epicuticular papillae differed between varieties in a similar way as both cell lengths (F = 29.363, df = 13, P = 0.000) (Fig. 3C). Charming and Charming Beauty had two times less papillae compared to the resistant varieties, Alba, Robinetta, Green Star and Live Oak. The lengths of the epidermis and mesophyll cells were strongly correlated to the density of papillae (Table 1). This strong correlation is explained by the fact that as a rule each epidermis cell produces one papilla (Figs. 4E-F). The different leaf cell forms and the density of papillae of the thrips susceptible variety Charming Beauty compared to the thrips resistant variety Robinetta are depicted as microscopy images in Fig. 4A-F.

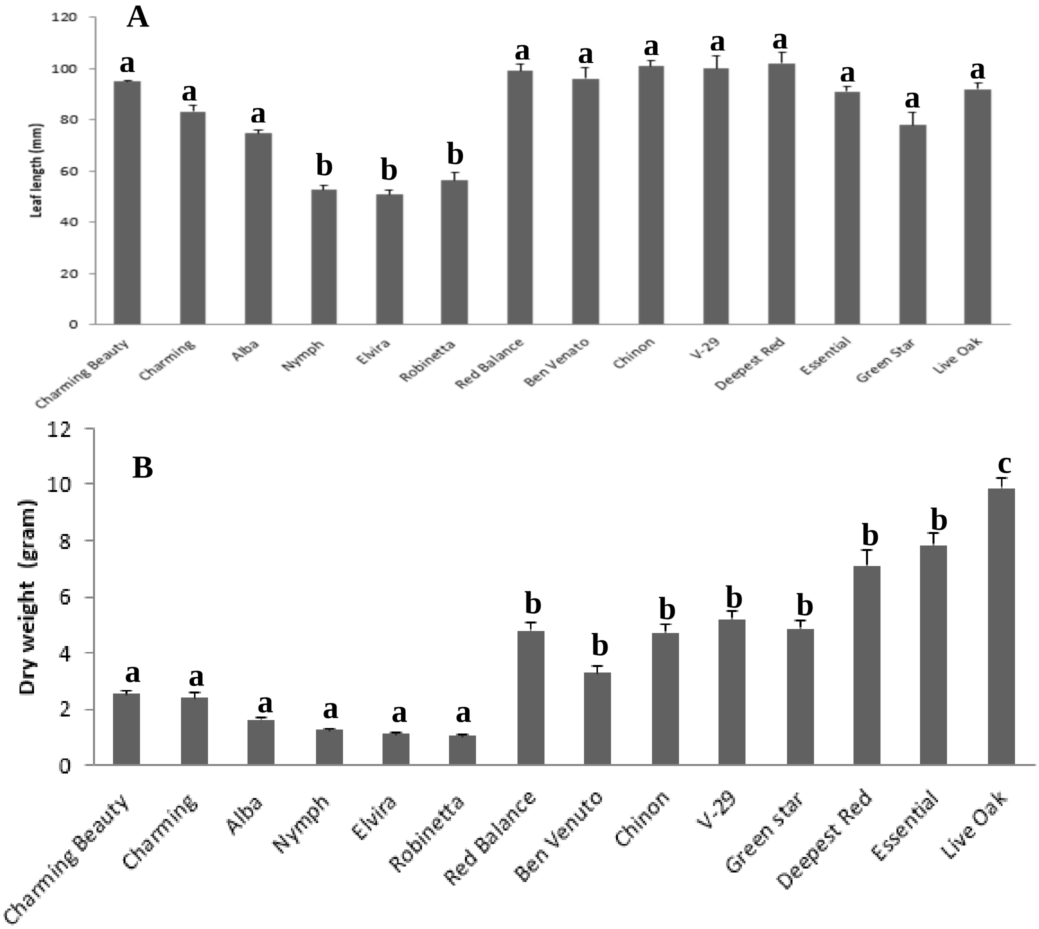


Figure2. Leaf length (A) and dry mass (B) of fourteen Gladiolus varieties. Data represent means and standard errors for three to five replicates. Different letters indicate significant differences between varieties at $p \leq 0.05$.

Table 1. Pearson correlations (*N* =14) between Ln-thrips silver damage (mm^2) and epidermis length (µm), mesophyll length (µm), density of papillae (per 2100 $µm^2$), leaf length (cm) and dry mass (gram) in Gladiolus varieties (*N* = 14). Data represent means of three to five replicates. Data in bold shows significant level at p ≤0.05.

	Epidermis length (µm)	**Mesophyll length (µm)**	**Density of papillae (per 2100 $µm^2$)**	**Leaf length (cm)**	**Dry mass (gram)**
Ln Silver damage	**r = 0.596 P = 0.024**	**r = 0.603 P = 0.022**	**r = -0.628 P = 0.016**	r = 0.320 P = 0.264	r = -0.222 P = 0.445
Epidermis length		**r = 0.931 P = 0.000**	**r = -0.873 P = 0.000**	**r = 0.704 P = 0.005**	r = 0.310 P = 0.281
Mesophyll length			**r = -0.909 P = 0.000**	**r = 0.777 P = 0.001**	r = 0.315 P = 0.273
Denstiy of papillae				**r = -0.669 P = 0.009**	r = -0.389 P = 0.170
Leaf length					r = 0.441 P = 0.114

The Relationship between Thrips Damage and Morphological Characteristics

Silver damage was significantly positive correlated with the lengths of the epidermis (r = 0.596, N = 14, P = 0.024) (Fig. 5A) and mesophyll cells (r = 0.603, N = 14, P = 0.022) (Fig. 5B) while it was significantly negatively correlated with the density of papillae (r = -0.628, N = 14, P = 0.016) (Fig. 5C). Silver damage did not correlate with leaf length (r= 0.320, N = 14, P = 0.264) and not with plant dry mass (r = -0.222, N = 14, P = 0.445).

DISCUSSION

Gladiolus varieties showed a broad range of variation in thrips resistance as demonstrated by the more than 130-fold difference in silver damage between the most resistant and the most susceptible varieties. Such a large variation is not uncommon for ornamentals. Chrysanthemum varieties also exhibited around 100-fold variation in thrips damage (de Jager *et al.*, 1995 and Kos *et al*., 2014). Gaum *et al.* (1994) found that variation of thrips resistance was 6 times lower in resistant varieties compared to susceptible ones in a study with 25 rose varieties.

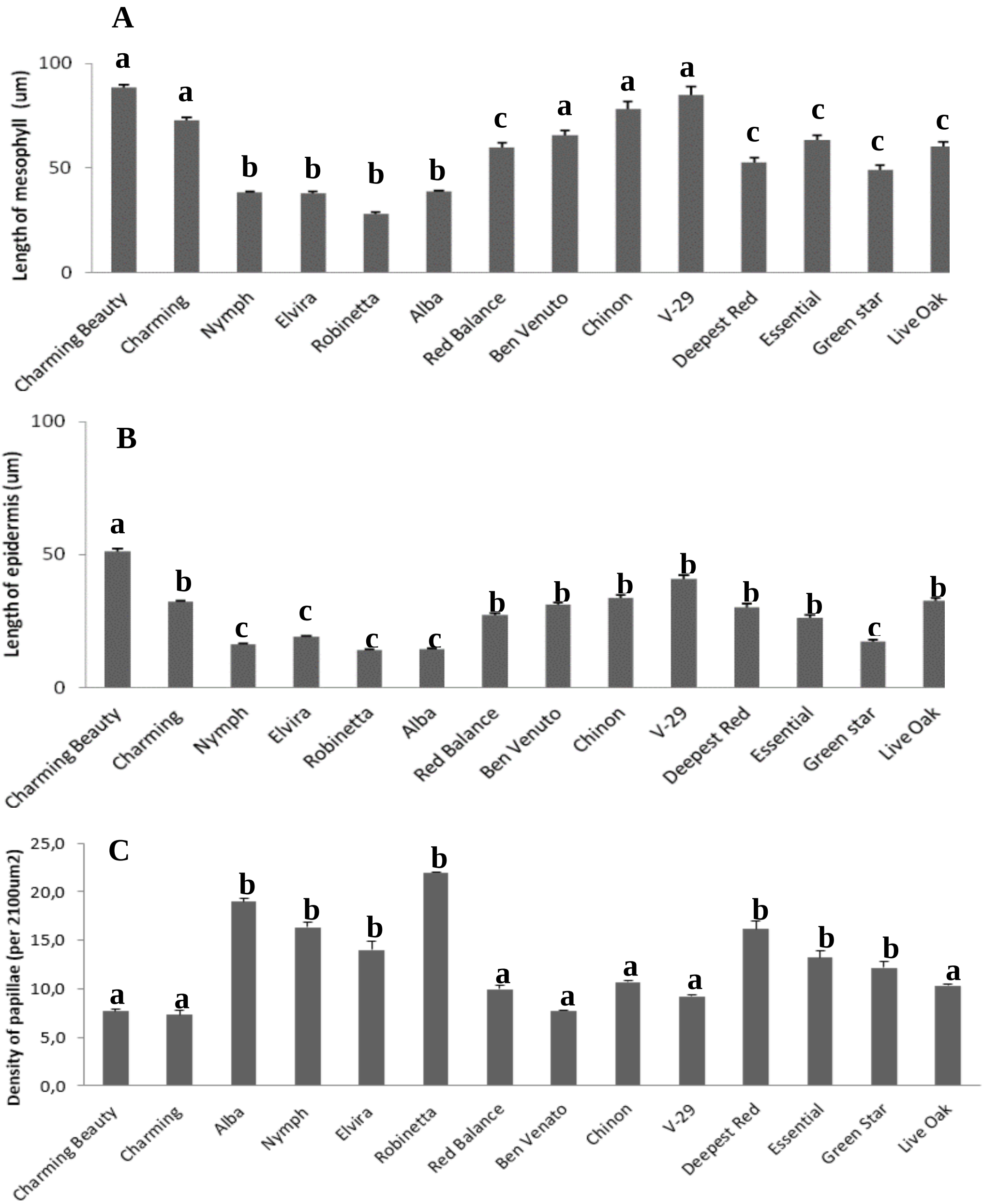
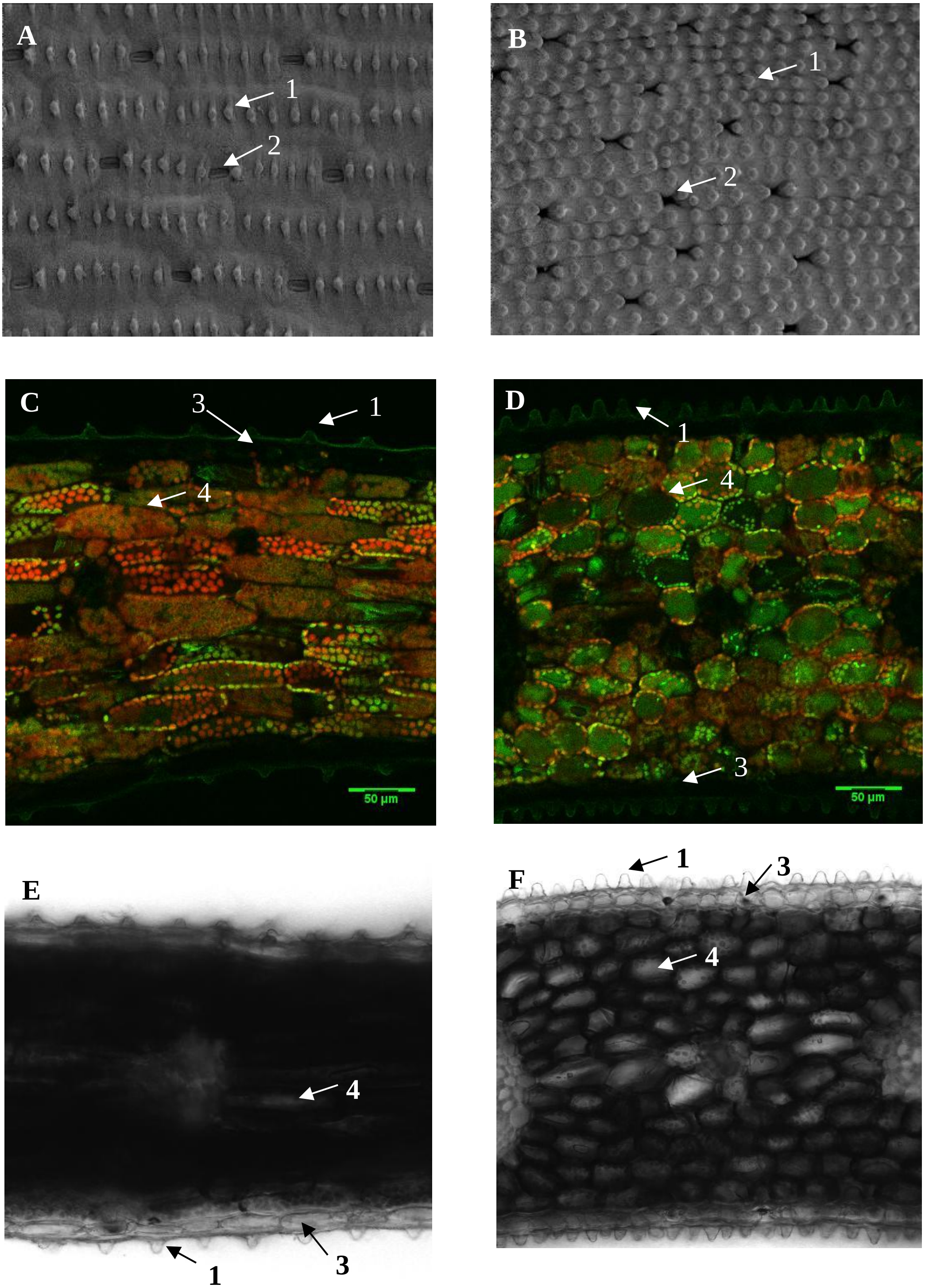


Figure 3. The length of mesophyll (A), epidermis (B) cells and the density of papillae (C) in fourteen Gladiolus varieties. Data represent means and standard errors for three to five replicates. Different letters indicate significant differences between varieties at p ≤0.05.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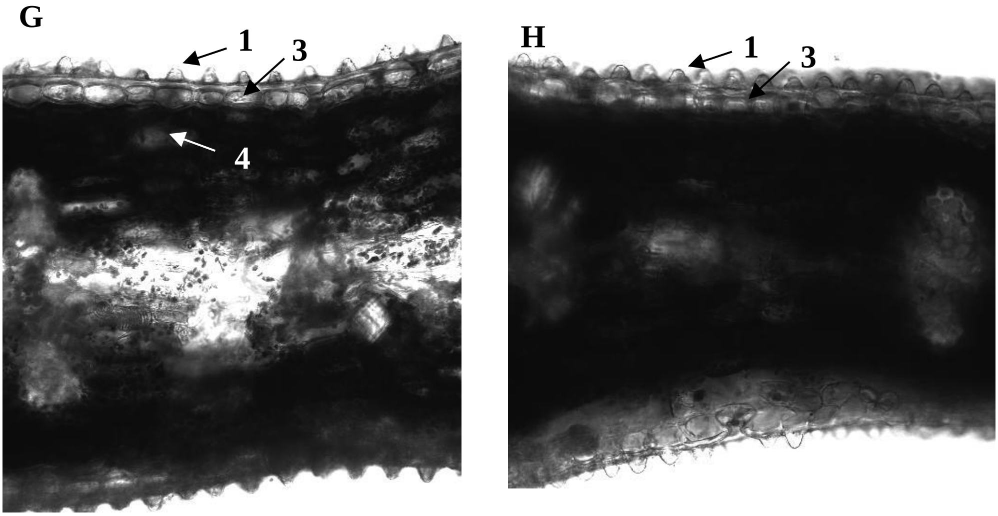

Figure 4. Leaf surface scanning electron photomicrographs of the thrips susceptible Gladiolus variety Charming Beauty (A) and the thrips resistant variety Robinetta (B). Cross leaf sections of Charming Beauty (C) and Robinetta (D) with confocal laser scanning, respectively. Cross leaf sections of Charming Beauty (E), Robinetta (F), Live Oak (G) and Green Star (H) with visual light microscopy, respectively. Arrows indicate papillae (1), stomata (2), epidermis (3) and mesophyll (4).

The density of papillae was negatively correlated to thrips damage while the lengths of the mesophyll and epidermis cells were positively correlated with thrips damage. As a rule, an epidermis cell produced one papilla. Thus, varieties with smaller leaf cells had a higher density of epicuticular papillae. Statistically it is not possible to make a distinction between the effects of cell length and the density of papillae on silver damage. Papillae may inhibit the movement of thrips or hinder penetration of the epidermis by the cell sucking thrips. However, Prüm *et al.* (2013) reported that papillae may slightly enhance adhesity to leaves in Colorado beetle. In line with our study, Scott Brown and Simmonds (2006) who studied effects of leaf morphology on *Heliothrips haemorrhoidalis* reported that this thrips had a preference for leaves with smooth surfaces, while trichomes and leaf surface wax structures inhibited thrips. Trichomes were also implicated to be related to thrips resistance in tomato (Boughton *et al.*, 2005) and chili peppers (Yadwad *et al.*, 2008).

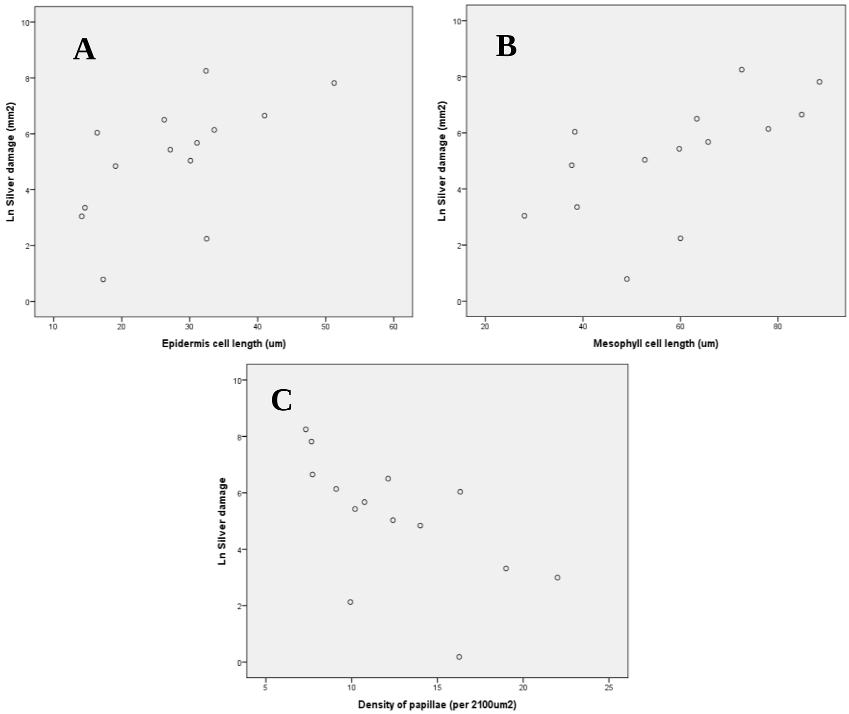

Figure 5. Relationships between Ln-thrips silver damage, measured in a whole plant non-choice bioassay, and cell length of the epidermis (A) (r = 0.596, N = 14, P = 0.024), cell length of the mesophyll (B) (r = 0.603, N = 14, P = 0.022) and density of epidermal papillae (C) (r = -0.628, N = 14, P = 0.016) in fourteen Gladiolus varieties.

Besides forming a physical barrier, papillae may store plant secondary compounds. The epidermal papillae of *Pandanus amaryllifolius* Roxb. are the storage site of the basmati rice aroma compound, 2-acetyl-1-pyrroline (Wakte *et al.*, 2007). Cardinosin A, an aspartic proteinase, suggested to be involved in plant defence against pathogens, is stored in the stigmatic papillae of *Cynara cardunculus* L. (Ramalho-Santos *et al.*, 1997). Similarly, Gladiolus varieties with higher densities of papillae may contain higher amounts of defence compounds. The density of papillae explained 39% of the variation in silver damage. From Fig.1 it becomes clear that the density of papillae sets an upper limit to silver damage. However, other factors may be involved as well as can be seen from the two varieties with low silver damage in relation to their density of the papillae. This will lead to false negatives when this morphological marker would be the only marker used in breeding programs for thrips resistance in Gladiolus. Next to papillae, chemical traits are likely candidates to be involved in thrips resistance. This needs to be further studied to develop additional markers.

In conclusion: our study revealed that morphological traits play an important role in thrips resistance in the ornamental Gladiolus. Varieties with low thrips damage exhibited high densities of epicuticular papillae. Papillae, in contrast to the correlated cell length, are easily visualized and counted. They, therefore, constitute promising morphological thrips resistance markers facilitating host plant resistance breeding in Gladiolus.

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