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The role of plant physiology and cultivar of chrysanthemum in the resistance against Western flower thrips

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Abstract

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an economically important pest insect in vegetable and ornamental cultivation worldwide. Little is known about host plant resistance in the vegetative and generative phases of chrysanthemum [*Chrysanthemum* × *morifolium* (Ramat.) Hemsl. (Asteraceae)] and the role of resistance factors such as flavonols, flower color, and flower shape. We screened a broad range of chrysanthemum cultivars across two seasons to quantify resistance against Western flower thrips. Resistance was based on silver damage on the leaves, and relative flavonol content was measured using a Dualex Scientific 4 hand-held sensor. There was significant variation in silver damage between cultivars, indicating different levels of resistance. There was no correlation between the relative flavonol content in middle leaves and plant silver damage. A clearer resistance level discrimination by flavonols in resistant and susceptible cultivars would be possible in the future by comparing multiple leaf positions during the ontogenetic phases of plant development. Moreover, the influence of flower color and shape on resistance to thrips was investigated by counting the adult thrips and larvae on flowers. The results showed significant differences in flower color preference by adults, but not by larvae. Flower shape influenced thrips larvae and females, but not males. The importance of the results for resistance determination is discussed.

Introduction

Chrysanthemum [*Chrysanthemum* × *morifolium* (Ramat.) Hemsl. (Asteraceae)] is a popular ornamental plant worldwide. Today's international cut chrysanthemum trade is the second largest of all flowers after roses (*Rosa* spp.) (Spaargaren & van Geest, 2018). Flower color and shape are the most important traits in chrysanthemums, and there is an increasing demand by the flower sector for a wider range of cultivars (Datta, 2013; Spaargaren & van Geest, 2018). In addition to mites (e.g., *Tetranychus urticae* Koch), aphids [e.g., *Macrosiphoniella sanborni* (Gillette)], and leaf miners (*Liriomyza trifolii* Burgess), in particular the Western flower thrips [*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)] is a serious pest in chrysanthemum cultivation (Kos et al., 2014; Spaargaren & van Geest, 2018) and it is abundant in many field and greenhouse crops. This polyphagous insect is characterized by its rapid population increase, cryptic lifestyle, and feeding method. Thrips ingest cell contents from epidermal cells (adaxial and abaxial), as well as from palisade and spongy mesophyll cells (Kumar et al., 1995; Kindt et al., 2003; Fiene et al., 2013). Emptying cell contents leads to air-filled spaces, which cause the typical silver damage, as the cells appear silvery to the human eye (de Jager et al., 1993). Silver damage can occur anywhere on aboveground plant parts and can lead to a considerable loss of the cosmetic and economic value of the crop. It is more prevalent on mature leaves, whereas feeding on young leaves leads to a distortion of leaf tissue (Fung et al., 2002; Rhainds & Shipp, 2003; Kos et al., 2014).

Control of thrips in greenhouse production is difficult, because pesticide applications do not reach the various

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hiding places of this insect (Cloyd, 2009). Currently, there is an increasing public demand for the development of alternative control strategies for pest insects. A sustainable strategy for the prevention of thrips damage could be plant breeding with a focus on resistance characteristics (i.e., constitutive resistance) against thrips. For chrysanthemum, a combination of qualitative suitability and marketable flowers combined with resistance against insects would be the best option. A large amount of variability in resistance against Western flower thrips among leaves of chrysanthemum cultivars has been previously reported (de Jager et al., 1995; Kos et al., 2014), but more research is needed on host plant resistance against *F. occidentalis* at various plant growth phases for this host. Visschers et al. (2019) predicted a similar resistance level at different leaf positions within the ontogenetic stages (vegetative, generative, fruit ripening) of pepper (*Capsicum* spp.) accessions.

A very important factor in the development of Western flower thrips on chrysanthemum plants is that of flowers (Kirk, 1996). Investigations of the role of pollen as a food source for *F. occidentalis* have yielded inconsistent results in the literature. Some studies have implied a minor role of pollen or flower tissue for oviposition and development of *F. occidentalis* (Kiers et al., 2000), whereas others describe flowers and flower tissue as a good and important food source with high nutritional value (Kirk, 1985b; de Jager et al., 1995; Kirk, 1996; Gerin et al., 1999; Hulshof et al., 2003). De Jager et al. (1995) predicted better reproductive success for Western flower thrips on chrysanthemum plants with flowers than those on non-flowering plants. However, the feeding activity of *F. occidentalis* can lead to premature senescence of flower tissue, which lowers the nutrient quality of the host flower. Nonetheless, females prefer to lay eggs in healthy flowers. Assuming that aging flowers are low-quality hosts for larvae, increased dispersal of female *F. occidentalis* is expected to be a response to the deterioration of chrysanthemum flowers. This should increase their reproductive success (Rhainds & Shipp, 2003). Flower attributes such as color, shape, size, and scent are expected to exert a strong influence on host choice. It is not clear whether Western flower thrips show a preference for a certain shape or color of flowers. Most studies investigating the influence of color use only colored traps (Otieno et al., 2018; Stukenberg et al., 2020).

Potential resistance factors against insects are secondary metabolites, such as phenols, e.g., flavonols. These metabolites can act as insect growth inhibitors (Leiss et al., 2009a; War et al., 2013; Onkokesung et al., 2014; Liu et al., 2015; Silva et al., 2016). Flavonols are distributed in the flowers, fruits, stems, and leaves of all green plants (Taiz & Zeiger, 2007). They have been found within leaves in both the mesophyll and epidermal (adaxial, abaxial) cells (Weissenböck et al., 1986; Agati et al., 2011). Thrips-resistant chrysanthemums contained a higher amount of two phenylpropanoids, chlorogenic acid and feruloyl acid (Leiss et al., 2009b). Leaves of 21 Japanese chrysanthemum taxa could be divided into three chemotypes based on their flavonoid characteristics, but no differentiation according to resistance was examined (Uehara et al., 2012).

Direct analyses of flavonol content are time- and labor intensive. To determine potentially resistant chrysanthemum plants for follow-up breeding, an easy and rapid method for resistance detection is desirable. Additionally, using the plant material without destruction or injury of the individual plant would be highly advantageous, because the F1 generation would consist of a single plant from which new cuttings could be taken later. The non-destructive Dualex Scientific sensor (FORCE-A, Orsay, France) measures the relative flavonol content in nearly all plant organs and works well for leaf measurements (Cerovic et al., 2012). Currently, it is not clear whether resistant chrysanthemum plants exhibit a higher or lower total relative flavonol content in leaves, and whether the flavonol content can act as an easy determinant method of cultivar-specific differences in resistance for breeding purposes.

In this study, we characterized 77 chrysanthemum cultivars according to their resistance levels based on silver damage by *F. occidentalis* on leaves during the generative phase. This should guarantee the earliest possible time for the determination of resistance (non-preference) against *F. occidentalis*. To assess the influence of flowers on the choice of hosts and to emphasize resistance discrimination, we also counted adult thrips and larvae in flowers, and assessed whether the color or shape of flowers plays a role in thrips development. To easily and quickly determine a resistance discrimination method, we used the Dualex Scientific 4 sensor and analyzed the relative flavonol content, because we hypothesized there would be a higher leaf flavonol content in resistant cultivars. The experiment was conducted with cultivars mixed in blocks to simulate a design similar to that of the breeder, i.e., when new cultivars are selected for their flower appearance (Spaargaren & van Geest, 2018).

Materials and methods

Plant material, growth conditions, and cultivar screening setup

In total, 77 chrysanthemum cultivars were used for the resistance screening, including seven garden, 40 cut, and 30 pot chrysanthemums (Table S1). Plants had six flower shapes (Figure S1). Plants were grown in individual pots (14 cm diameter) with Fruhstorfer soil type P (Hawita Group, Vechta, Germany) and then placed in plastic tunnel tents (29 ± 10 °C, 70 ± 10% r.h., measured with

Tinytag plus data loggers; Gemini, Chichester, UK) for quick rooting. The screening took place in two greenhouse compartments (9.0 × 7.7 m), each divided into five blocks (1.7 × ca. 3.3 m). Three-week-old plants were pruned, removed from the tunnels, and placed into the respective block of the greenhouse compartments. The photoperiod was changed to short-day conditions (L8: D16) to induce flower formation. Supplemental light was added when the sunlight intensity dropped below 30 klx. In the first 4–6 weeks, the plants were fertilized twice a week with 0.2% Wuxal Top N and twice a week with 0.3% Wuxal Super (both Aglukon Spezialdünger, Düsseldorf, Germany) during weeks 7–12.

Each block in a greenhouse compartment contained a single plant per cultivar (n = 10 for each cultivar). Plants were arranged in a randomized complete block design. The screening was replicated twice, once in spring (weeks 13–25) and once in summer (weeks 30–41). In both seasons, silver damage was assessed 3× (Figure 1). In the first assessment (vegetative plant phase), all plants were assessed and placed back to the corresponding block. Because of differences in the reaction time, i.e., time until flowering, two assessments took place in the generative plant phase: plants with a reaction time of 7 weeks were evaluated during the second assessment and those with a reaction time of 8–9 weeks were evaluated during the third assessment. Finally, all flowers were evaluated for the presence of thrips at the end of the experiment (Figure 1).

The process of resistance level determination and counting of thrips was quite labor intensive. In spring, damage and number of thrips was evaluated for all 77 cultivars. Although the trial was set up in the summer in the same way as in the spring, that is, including all 77 cultivars, the evaluation of damage and counting of thrips was completed in detail only for nine selected cultivars and a reference cultivar (Figure 1). These nine cultivars ranged from susceptible to resistant and were selected for follow-up breeding and further experiments. Cultivar 1 served as reference cultivar, because the mean silver damage of this cultivar was the closest to the overall mean. Cultivars 2–5 were selected because of low silver damage levels in spring, and cultivars 6 and 8–10 because of high silver damage (all daisy-type inflorescences; Figure S1). Cultivar 7 (pom-pom-shaped inflorescences; Figure S1) was selected because of very high silver damage in spring. These cultivars were labeled from 1 (reference) to 10. The remaining cultivars were labelled from 11 to 77 (for data for all cultivars in the spring, see https://data.uni-hannover.de/dataset/chrysanthemum-resistance-to-western-flower-thrips). All plants were 4 weeks old when thrips were released.

Rearing and release of insects

The rearing of *F. occidentalis* took place in a climate chamber (L16:D8, 25 ± 1 °C, 50 ± 5% r.h.). They were reared in four custom-made acrylic glass cages with three windows covered with thrips gauze for ventilation. Two cages contained bean plants, *Phaseolus vulgaris* L. 'Speedy' (Fabaceae) (Hild Samen, Marbach am Neckar, Germany), and two cages contained chrysanthemum plants 'Pemba Purple' (Brandkamp, Isselburg-Anholt, Germany), which were later switched to the more suitable 'Aviso' (Deliflor Chrysanthemums, Maasdijk, The Netherlands), both non-flowering. Because the thrips population in this *P. vulgaris* laboratory was established years ago, the gene pool was replenished by adding *F. occidentalis* individuals collected from *F. occidentalis* rearings on *P. vulgaris* (Julius Kühn Institute, Braunschweig, Germany) and on roses (North Rhine-Westphalia Chamber of Agriculture, Straelen, Germany) to all cages. During the spring period, the thrips population had time to stabilize within 14 days. During the summer period, the rearing was not refreshed. Weekly, two new chrysanthemum plants were added to each chrysanthemum rearing cage and four new *P. vulgaris* plants were added to each *P. vulgaris* rearing cage.

To obtain enough thrips for experimental release, a 1:1 mixture of thrips from the chrysanthemum and *P. vulgaris* cages was used. Adult thrips were randomly collected from the four cages with a sex ratio of 3:1 (female-to-male).

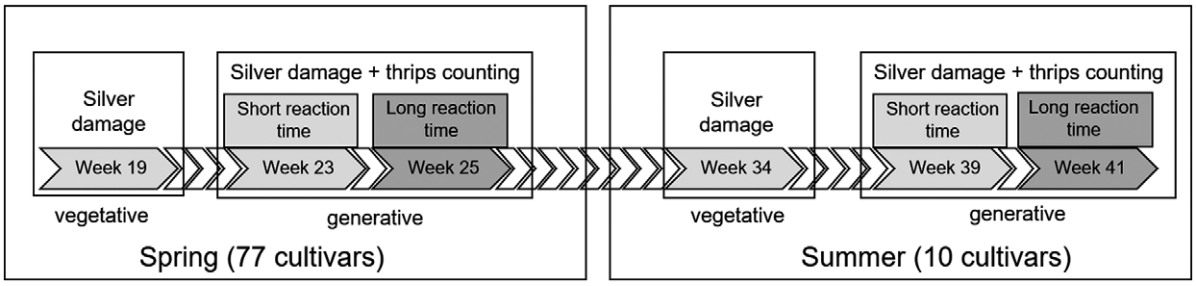

Figure 1 Schematic diagram of the time sequence of the chrysanthemum resistance screening against *Frankliniella occidentalis* in spring (weeks 13–25) and summer (weeks 30–41). Short reaction time (time until flowering) equals 7 weeks, long reaction time equals 8–9 weeks.

Therefore, 2–4 thrips were carefully picked up with a fine brush and transferred into an Eppendorf tube (Eppendorf, Hamburg, Germany) prepared with moist cotton wool and a piece of *P. vulgaris* leaf. The tubes were kept in the laboratory at room temperature for 20–24 h. On the following day, 26 (spring) or 40 thrips (summer) per block were released as the initial population between plants, totaling 260 in spring and 400 in summer.

Quantification of silver damage and counts of *Frankliniella occidentalis* in flowers

To assess plant resistance levels at the earliest point, we evaluated thrips feeding damage, i.e., silver damage. Therefore, the first removal of the main shoot of each plant for evaluation was done after 8 weeks in spring and 7 weeks in summer, and the second and third removals were done after 12 and 14 weeks, respectively, for both spring and summer. For documentation and subsequent visual quantification of silver damage, all leaves from the shoots were scanned with a Perfection V39 flatbed scanner at 300 dpi (Epson, Suwa, Japan).

For damage quantification, each leaf was initially evaluated on both the upper (adaxial) and lower side (abaxial) of the leaf. Because leaves showed no or minor damage on the abaxial side, the subsequent statistical analysis was based solely on the adaxial side. The damaged area was estimated as a percentage of the total leaf area on the adaxial side. From all percentage values, the mean value per shoot was calculated. Hence, the overall resistance level was calculated based on 10 shoots, which is 10 plants per cultivar. To determine whether the 1–2-week differences in time to flowering had effects on estimated silver damage, mean values were standardized per week, and statistics were calculated. Results with standardized values showed no differences compared to the original values, and therefore, further analysis was based on the original values.

Finally, all flowers of the cut shoots were counted and collected in a white isolation box with a tight lid to prevent escape of thrips. Boxes were stored in a dry and warm space. After 1 week, all lids were perforated with a needle to prevent condensation of water. After another 3 weeks, dried flowers were tapped to the bottom of the box and dead thrips (adult females, adult males, and larvae) of all flowers per shoot were counted using a stereomicroscope. Again, to evaluate whether the 1–2-week differences in time to flowering affected the population density of thrips in flowers, the mean values were standardized per week, and statistics using linear mixed model were calculated. Results with standardized values showed no differences compared to the original values, and therefore further analysis was based on the original values.

Analyses of flavonol content in chrysanthemum leaves

To analyze differences in relative overall epidermal flavonol contents, non-destructive measurements were taken with the hand-held sensor Dualex Scientific 4 (FORCE-A). The sensor measures leaf epidermal flavonols at 375 nm using the chlorophyll fluorescence screening method (Bilger et al., 1997; Cerovic et al., 2002; Agati et al., 2005). The relative flavonol content was calculated using the decadic logarithm of the ultraviolet (UV) excitation ratio of far-red chlorophyll fluorescence (flavonol absorbance). This value is proportional to the flavonol content of the leaves (Cerovic et al., 2002, 2012; Pfündel et al., 2007).

Measurements were always taken in the lower middle part of the main shoot and from the abaxial side of the leaf, avoiding the inclusion of the middle vein. Therefore, leaves in position six (pot chrysanthemums) or seven (cut chrysanthemums) from the plant base were labelled to ensure measurements of the same leaf during the experimental period. Each leaf was measured 3× and the mean value was calculated (n = 3; 3× three measurements per cultivar). Measurements were taken during all three assessments (Figure 1).

Statistical analysis

All data were analyzed using R v.4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) and RStudio v.1.3.959 (RStudio, Boston, MA, USA). For the analysis of silver damage in spring and summer, the mean damage of all 77 cultivars was calculated for the vegetative and generative period. Thereafter, the overall mean of all cultivars was calculated, with cultivar 1, being closest to the mean, serving as a reference cultivar. This provides information on how much silver damage appears on the various cultivars compared to that of the mean.

For the statistical analyses, percentages were $\log(x + 1)$-transformed and a linear mixed model (GLM) by REML t-test using Satterthwaite's method ($\alpha = 0.05$) was applied. The analysis of silver damage was calculated with all 77 cultivars in spring and with 10 selected cultivars in summer. GLM test was also used to analyze the differences in number of adults or larvae in shape and color of flowers between cultivars (as a reference, spider-type flowers, and yellow color were used according to the literature, De Jager et al., 1995; Blumthal et al., 2005) and for differences in flavonol contents. In all calculations, the greenhouse compartments and the blocks were included as random effects [basic formula: log damage ~ cultivar + (1|compartment: block)].

Spearman's correlation was used for the correlation between: (1) the total number of thrips (adults + larvae) and the number of flowers in both the selected cultivars and all cultivars, (2) the total number of thrips and silver

damage on leaves of all cultivars, (3) the silver damage on leaves and relative flavonol contents in leaves of all cultivars, and (4) the relative flavonol contents in leaves and the total number of thrips in the selected cultivars.

After the assessment of silver damage, cultivars with similar resistance levels were characterized. Plants were clustered based on the estimated marginal means value (EMMs) for the silver damage (package 'emmeans'; Lenth et al., 2019). Hierarchical clustering (method: Euclidean distance and average) was performed using silver damage EMMs in the generative phase (spring). In general, four groups were considered: resistant (rr), moderately resistant (r), susceptible (s), and very susceptible (ss).

Results

Silver damage on leaves in the vegetative and generative phase of plants

Considering all cultivars used in the screening, the nine selected cultivars sustained silver damage in percent on the adaxial leaf surface ranging from zero to more than twice that of reference cultivar 1. In general, the mean silver damage of the 10 cultivars in the vegetative phase during spring (mean ± SD = 0.51 ± 1.27; n = 98) was close to the mean of the vegetative phase during summer (0.59 ± 0.83; n = 98). During the generative phase, mean silver damage of the 10 cultivars in the spring period was higher (2.88 ± 3.92; n = 98) than that in the summer season (1.45 ± 2.7; n = 87).

During the spring period, cultivars 2, 4, 5, and 6 exhibited significantly less silver damage in the vegetative stage compared to that of reference cultivar 1 (Figure 2). There was no significant difference for cultivar 3. For cultivar 6, no damage was detected, whereas cultivars 2 and 4 had damage up to 44× less and cultivar 5 ca. 13× less. In contrast, cultivars 7 and 8 had significantly higher levels of silver damage on leaves, which was ca. 5× higher than that of reference cultivar 1 in the vegetative phase (Figure 2). In general, silver damage increased from the vegetative to the generative phase as expected, and most cultivars showed a similar reaction pattern compared to that of the vegetative phase. In detail, cultivars 2–5 were all less damaged (2–57×) in the generative phase than the reference cultivar 1. Only cultivar 8, which was more damaged during the vegetative phase, was less damaged than the reference cultivar in the generative phase in spring (Figure 3).

During the summer period, no cultivar showed significantly higher damage than the reference cultivar 1 during the vegetative phase, but cultivars 2, 4, 5, and 6 showed 4–10× lower damage (Figure 2). In the generative phase, cultivars 2, 3, 4, and 5 showed 6–19× less damage compared to reference cultivar 1 in summer (Figure 3). The

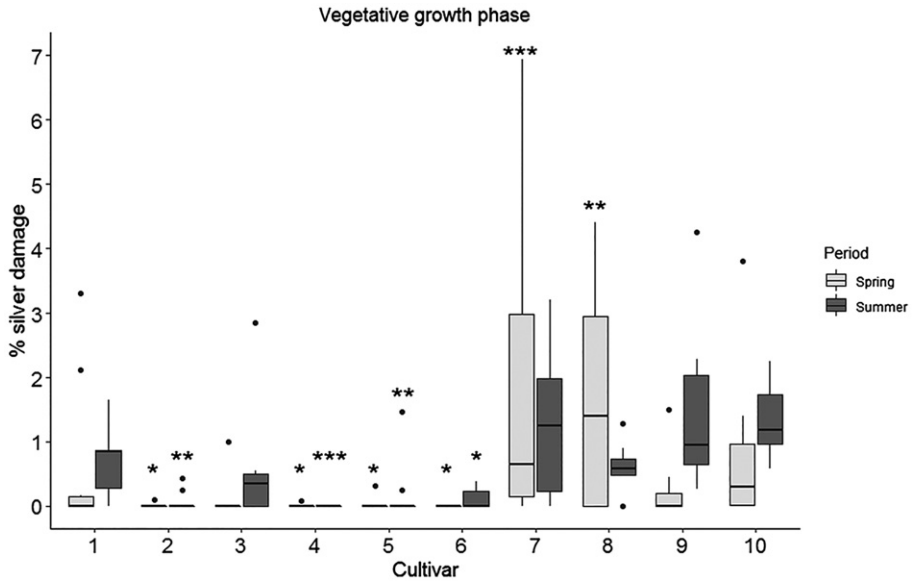

Figure 2 Silver damage (%, n = 10) per chrysanthemum cultivar per shoot caused by *Frankliniella occidentalis* on leaves in the vegetative growth phase, in spring (light grey) and summer (dark grey) at 4 weeks after thrips release. The reference cultivar was cultivar 1. The upper and lower boxes indicate the first and third quartiles, the thick line within shows the median value. The whiskers indicate 1.5× the interquartile range. The dots indicate outliers. Asterisks indicate significant differences with the reference cultivar within spring or summer (GLM and t-test using Satterthwaite's method: *0.01<P<0.05, **0.001<P<0.01, ***P<0.001).

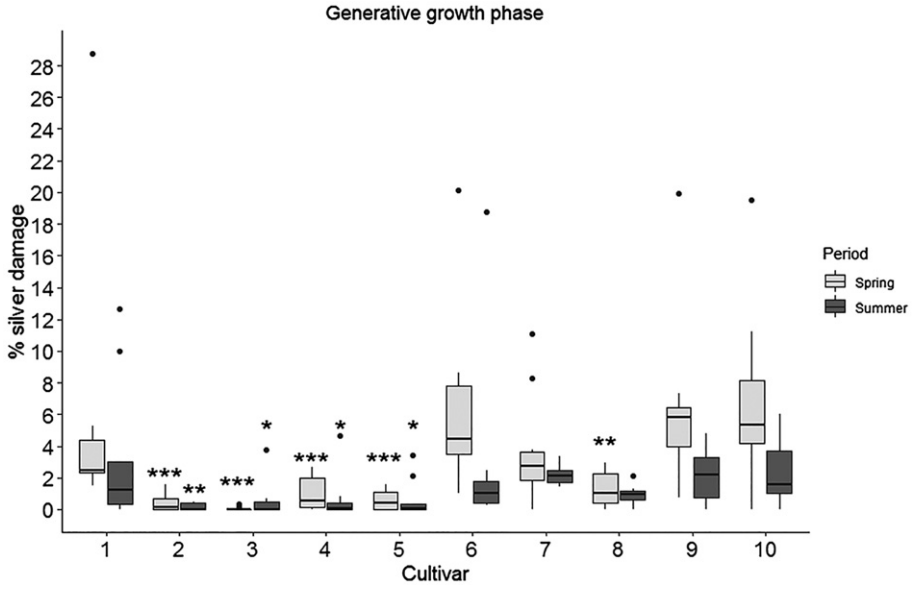

Figure 3 Silver damage (%, n = 10) per chrysanthemum cultivar per shoot caused by *Frankliniella occidentalis* on leaves in the generative growth phase, in spring (light grey) and summer (dark grey), at 8–10 (spring) and 9–11 (summer) weeks after thrips release. The reference cultivar was cultivar 1. The upper and lower boxes indicate the first and third quartiles, the thick line within shows the median value. The whiskers indicate 1.5× the interquartile range. The dots indicate outliers. Asterisks indicate significant differences with the reference cultivar within spring or summer (GLM and t-test using Satterthwaite's method: *0.01<P<0.05, **P<0.01).

results showed that susceptibility was consistent across both seasons: cultivars with low damage in spring also showed low damage in summer. Furthermore, nearly all cultivars resistant in the vegetative phase were also classified as resistant in the generative phase, except for cultivars 6, and 8: cultivar 6 showed more (both periods) and cultivar 8 lower damage (spring) in the generative phase compared to the vegetative phase (Figures 2 and 3).

Based on the silver damage results in the generative phase in spring, the cultivars were clustered into four groups. This was done by using the EMMs of silver damage for each cultivar and hierarchical cluster analysis. Groups were labelled as resistant (rr: EMM −1.456 to −0.911), moderately resistant (r: EMM −0.780 to −0.123), susceptible (s: EMM −0.069 to 0.382), and highly susceptible (ss: EMM 0.487–0.938). Cultivar 2 was categorized as rr, cultivars 3, 4, 5, and 8 as r, cultivar 7 as s, and cultivar 1 (reference), 6, 9, and 10 as ss. An overview of all 77 cultivars and their calculated resistance levels is accessible through the data availability link (https://data.uni-hannover.de/dataset/chrysanthemum-resistance-to-western-flower-thrips).

Attractiveness of flowers to *Frankliniella occidentalis*

Overall, considering the nine selected cultivars and the reference cultivar, the mean number of adult *F. occidentalis* (females + males) found in chrysanthemum flowers per shoot in spring was lower (mean ± SD = 73 ± 80; n = 99) than in summer (195 ± 122; n = 84). Conversely, the mean number of thrips larvae in flowers per shoot in summer was lower (146 ± 130; n = 84) than in spring (276 ± 229; n = 99). In spring, the mean number of adult thrips ranged from 30 ± 31 to 229 ± 58 individuals in the flowers per shoot. Flowers of cultivar 9 had 4× more adult thrips and cultivar 10 had 2× fewer adult thrips than did the reference cultivar 1 (Figure 4). For larvae, no significant differences were detected. The number of larvae ranged from 148 ± 106 (cultivar 4) to 586 ± 386 (cultivar 7) individuals (Figure 5). In summer, the number of adults ranged from 49 ± 44 (cultivar 7) to 288 ± 159 (cultivar 10) individuals in flowers per shoot. Flowers of cultivar 3 and 4 showed 3–4× fewer adult thrips, but no other cultivar significantly differed regarding the abundance of adult thrips in flowers per shoot with that of the reference cultivar 1 (Figure 4). In summer, the number of larvae ranged from 39 ± 49 (cultivar 3) to 265 ± 226 (cultivar 4) thrips larvae in flowers per shoot. Cultivar 3 had significantly fewer thrips larvae in flowers than did reference cultivar 1 (Figure 5).

To assess the impact of flower color and shape, we analyzed all 77 cultivars in spring to determine differences in the number of adults or larvae. Male thrips had lower

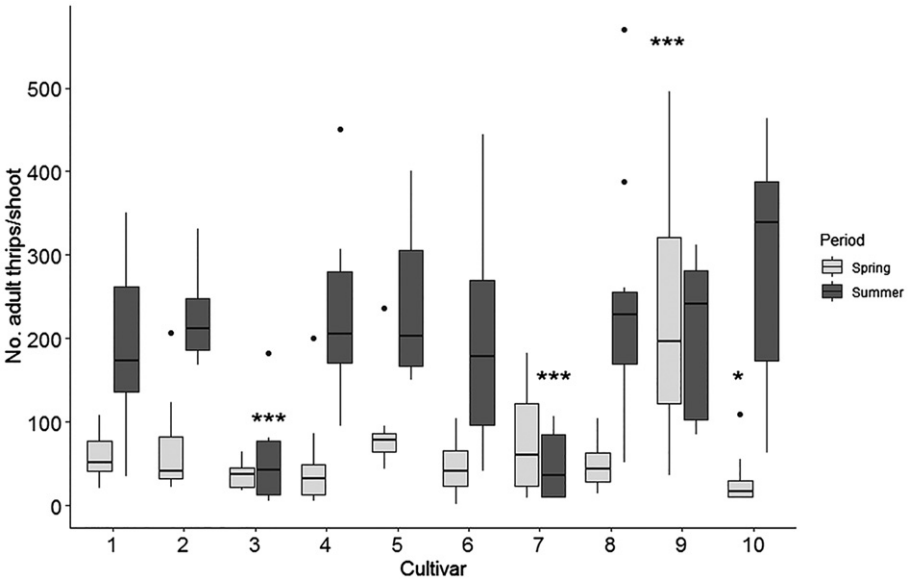

Figure 4 Number of adult *Frankliniella occidentalis* in chrysanthemum flowers per shoot per cultivar (n = 6–10) in spring (light grey) and summer (dark grey). The reference cultivar was cultivar 1. The upper and lower boxes indicate the first and third quartiles, the thick line within shows the median value. The whiskers indicate 1.5× the interquartile range. The dots indicate outliers. Asterisks indicate significant differences with the reference cultivar within spring or summer (GLM and t-test using Satterthwaite's method: *0.01<P<0.05, **0.001<P<0.01, ***P<0.001).

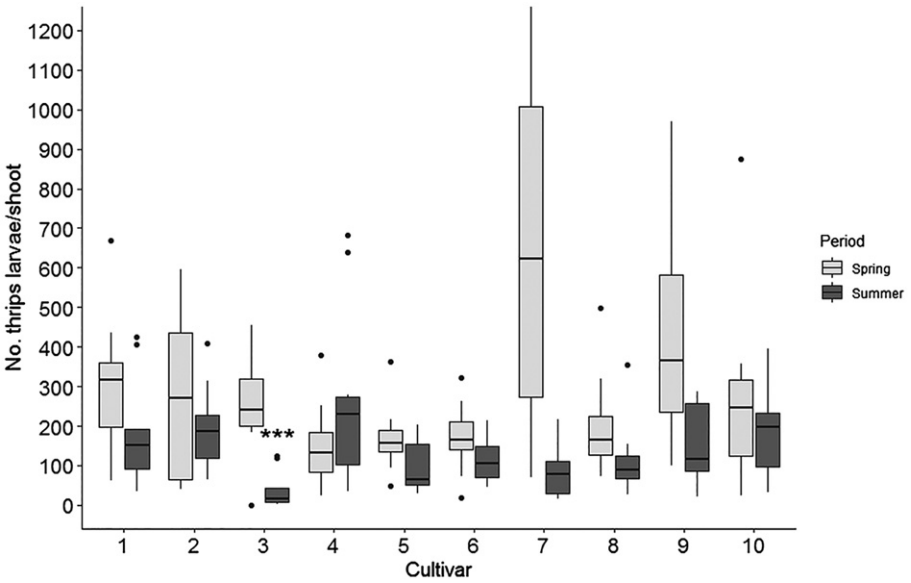

Figure 5 Number of *Frankliniella occidentalis* larvae in chrysanthemum flowers per shoot per cultivar (n = 6–10) in spring (light grey) and summer (dark grey). The reference cultivar was cultivar 1. The upper and lower boxes indicate the first and third quartiles, the thick line within shows the median value. The whiskers indicate 1.5× the interquartile range. The dots indicate outliers. Asterisks indicates a significant difference with the reference cultivar (GLM and t-test using Satterthwaite's method: ***P<0.001).

abundance in apricot (t = −2.34, d.f. = 741.991, P = 0.019; n = 10), orange (t = −2.801, d.f. = 741.991, P = 0.005; n = 70), pink (t = −6.040, d.f. = 742.003, P = 0.0001; n = 178), red (t = −4.762, d.f. = 742.015, P<0.001; n = 69), violet (t = −3.942, d.f. = 741.992, P<0.001; n = 20), and white (t = −2.588, d.f. = 742.001,

P<0.001; n = 129) flowers compared to that in yellow flowers (reference color, n = 30). In contrast, female thrips were found at a higher abundance in green (t = 2.401, d.f. = 742.042, P = 0.02; n = 39) and pinkish flowers (t = 3.026, d.f. = 741.989, P = 0.002; n = 20) and at a lower abundance in red flowers (t = −2.74, d.f. = 742.014, P = 0.006; n = 69) compared to that in yellow flowers (n = 30). For larvae, we detected no differences in abundance on different flower colors. Concerning flower shape, female thrips showed lower average abundance in multiflora (22 ± 19, t = −2.244, d.f. = 748.99, P = 0.025; n = 70) than in the reference spider-type (41 ± 33; n = 10), whereas males showed no preference at all (P>0.1 for all). In contrast, larvae showed 2× higher abundance in anemone-type (524 ± 418, t = 4.036, d.f. = 748.99, P = 0.001; n = 30) than in reference spider-type flowers (175 ± 248).

Relative flavonol content in chrysanthemum leaves

In spring, all 77 cultivars exhibited a lower relative flavonol content (mean ± SD = 0.433 ± 0.128; n = 231) in the vegetative phase than in the generative phase (0.542 ± 0.174; n = 228). This was not confirmed during the summer season for the 10 selected cultivars (vegetative: 0.591 ± 0.227; n = 47; generative: 0.460 ± 0.169; n = 44).

In the spring period in the vegetative phase, cultivars 3, 6, 7, and 8 showed significantly lower relative flavonol contents compared to that of the reference cultivar 1. In the generative phase, we again determined significantly lower levels of flavonols in cultivars 6, 7, and 8, but not in cultivar 3 (Figure 6 Spring).

In the summer period, only cultivar 9 had a significantly lower flavonol content in the vegetative phase. In the generative phase, cultivars 3 and 7 had significantly lower

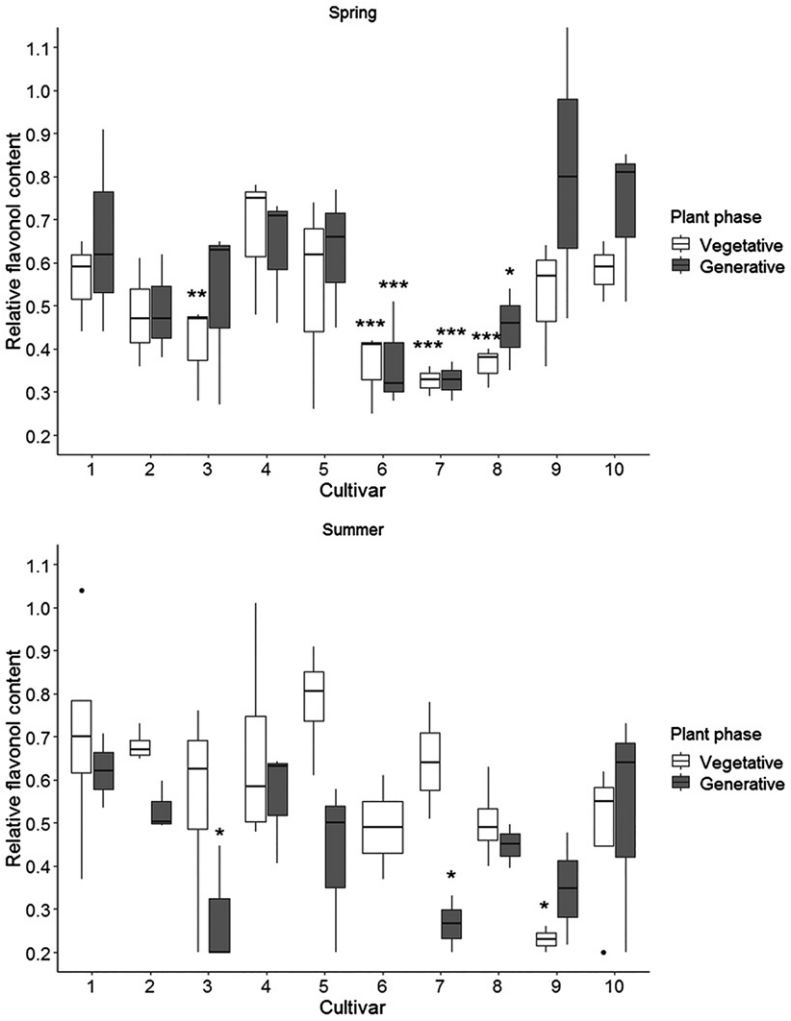

Figure 6 Relative flavonol content (n = 6–10) in chrysanthemum leaves in (A) spring and (B) summer in both the vegetative (4 weeks post-release of *Frankliniella occidentalis*) and generative plant phase (8–10 weeks post-release of *F. occidentalis*). The reference cultivar was cultivar 1. The upper and lower boxes indicate the first and third quartiles, the thick line within shows the median value. The whiskers indicate 1.5× the interquartile range. The dots indicate outliers. Asterisks indicate significant differences with the reference cultivar (GLM and t-test using Satterthwaite's method: *0.01<P<0.05, **0.001<P<0.01, ***P<0.001).

levels of flavonols. Flavonol content could not be determined for cultivar 6 in the generative phase in summer, because the plants in the respective blocks wilted early. For the other cultivars, plants wilted partially (Figure 6 Summer).

Correlations of the relative flavonol content, silver damage, number of thrips, and number of flowers

We detected a significant positive correlation between the number of flowers and the total number of thrips (adults + larvae) in the selected cultivars (spring rho = 0.274, P = 0.006; n = 100; summer rho = 0.424, P<0.001; n = 85). Numbers of flowers of all 77 cultivars in spring were positively correlated with the number of thrips (spring rho = 0.303, P<0.001; n = 760). The correlation between total number of thrips in flowers with silver damage on the leaves of all cultivars was significant for spring (rho = 0.0822, P = 0.02; n = 757), but not summer (rho = 0.0006, P = 0.99; n = 142). There was no significant correlation between relative flavonol contents of all cultivars and silver damage on leaves (spring vegetative rho = −0.014, P = 0.83; n = 231, generative rho = 0.047, P = 0.48; n = 231; summer vegetative rho = −0.143, P = 0.34; n = 47, generative rho = 0.033, P = 0.83; n = 44). Additionally, there was no significant correlation between the number of thrips and the relative flavonol contents in leaves of the selected cultivars in spring (rho = −0.076, P = 0.25; n = 227) and summer (rho = 0.294, P = 0.061; n = 42).

Discussion

Differences in leaf silver damage among physiological phases of chrysanthemum

Several authors evaluated silver damage on the whole plant/whole leaf surface (de Jager et al., 1995; Kumar et al., 1995; Fung et al., 2002; Leiss et al., 2009b; Kos et al., 2014). However, assessing silver damage on the adaxial and abaxial sides of leaves in spring in the current study showed that thrips mainly fed on the adaxial side, which was previously described for chrysanthemum by van Dijken (1992) and for *Capsicum* by Visschers et al. (2019). This is contrary to the findings from Fiene et al. (2013), wherein *F. occidentalis* fed preferentially on the abaxial side of cotton (*Gossypium hirsutum* L.) leaves. Based on our initial results, silver damage was evaluated solely on the adaxial side of leaves.

We observed large differences in silver damage among all chrysanthemum cultivars in our assessment. The most resistant (non-preferred) cultivar had 191× less damage in spring and 154× less damage in summer than did the most susceptible (preferred) one in these seasons, respectively. In both seasons, only a single multiflora cultivar showed no damage. This is consistent with previous publications reporting a wide range of thrips damage (de Jager et al., 1995; Leiss et al., 2009b). In our study, susceptibility was consistent across both seasons; cultivars with low damage in spring also showed low damage during the summer period. Furthermore, nearly all cultivars characterized as resistant in the vegetative phase were also characterized as resistant in the generative phase, except for cultivars 6, where damage increased and 8, where damage decreased. The lower damage was likely attributed to a change in secondary metabolites with development from the vegetative to generative phase in plants, as described for the leaves of soybeans (Song et al., 2014). This could also explain the lower damage in cultivar 8 in the generative phase in spring. However, in summer, as with the other cultivars, we detected more damage in the generative phase on cultivar 8. There was higher damage in cultivar 6, which changed its susceptibility in the opposite direction and became more susceptible in the generative phase. De Jager et al. (1993) noted the strong influence of flowers on thrips abundance, which might have played a role in the attractiveness of cultivar 6 in its generative phase.

Seven of the selected cultivars had a reaction time of 8 weeks, and three cultivars had a reaction time of 7 weeks. This did not seem to influence resistance determination. As an example, cultivar 7 was characterized as susceptible, although it flowered after 7 weeks. Similarly, cultivars 2, 4, and 5 were characterized as resistant, although they flowered after 8 weeks. It should be noted that the nine cultivars were selected out of 77 cultivars and that there could have been an influence among one another, e.g., because of the flower scents, which was not considered in-depth here.

Attractiveness of flowers to *Frankliniella occidentalis*

Adult thrips can fly; thus, they can change plants and flowers after feeding or oviposition, contrary to thrips larvae, which are mobile on the plant but do not necessarily move between plants. This larval immobility may explain our results. Between the 10 selected cultivars, we observed no differences in the number of larvae as opposed to the number of adult thrips in flowers. In more detail, female and male thrips were significantly more numerous in cultivar 9 and males in cultivar 10 in spring, whereas both were significantly less abundant in cultivars 3 and 7 in summer. We also observed higher larval numbers in cultivars 7, 9, and 10 than in the other cultivars. De Jager et al. (1995) showed that female *F. occidentalis* from different generations selected the same chrysanthemum cultivars for oviposition. Thus, two generations of larvae caused silver

damage until the first flowers occurred. From then on, thrips stopped damaging leaves, because flowers were preferred for oviposition and feeding. As a result, the third generation of larvae was expected to feed mainly in flowers. Cultivars 9 and 10 were characterized as susceptible according to silver damage. Hence, more females of the first generations appeared to have visited and deposited eggs on these cultivars. Larvae of the following generation (L2) aggregated and migrated into the flowers (Kiers et al., 2000) or later emerging females laid their eggs mainly in flower tissue. As already described, hatched larvae remained in the flowers, explaining the higher number of *F. occidentalis* in cultivars 9 and 10. Cultivar 3 exhibited a trend toward fewer adults in spring, whereas cultivars 3 and 7 had significantly fewer adults in summer compared to the reference. Of these two, only cultivar 3 was characterized as resistant considering silver damage and the oviposition activity on these cultivars appeared to be reduced.

The flower type of chrysanthemum is described as an important factor in thrips development (de Jager et al., 1995). Broadbent et al. (2003) reported that 55.5 ± 2.7% (mean ± SE) of thrips remained on chrysanthemum plants with a complex floral structure. In daisy-type flowers, *F. occidentalis* larvae, prepupae, and pupae were able to stay in the disc florets of the inflorescence (Broadbent et al., 2003). Spider-type chrysanthemum flowers have no disc florets, and thus it is difficult for adult thrips and larvae to feed on pollen (de Jager et al., 1995; Buitenhuis & Shipp, 2008). Cultivar 7 was characterized as susceptible only in the vegetative phase. This was the only selected cultivar with a pompon-type flower shape. The shape might be of no interest for the nutrition of adult thrips, but may be attractive for the smaller-sized larvae and pupae, because of the ability to reach the pollen or hide. In spring, we observed a very high number of larvae in the flowers of cultivar 7, which confirms the former hypothesis. However, considering all 77 cultivars, larvae were significantly more frequent in anemone-type flowers, which is a floral structure with disk florets formed by five fused petals forming a raised and rounded center surrounded by longer and colored petals (Spaargaren & van Geest, 2018). Disk florets in the center of the flower might function as a protective zone and could also be a suitable food source for larvae. It should be remembered that flower scents might have an important effect on decision making by female thrips as to where they lay their eggs (Annand, 1926; Kirk, 1985a; Koschier et al., 2000; Ren et al., 2020). Additionally, some plants or their flowers wilted and might have changed their scent. We assume that this happened at the end of the experiment and therefore had no influence on the behavior of the females over time. However, we did not analyze floral volatiles in this study at any time.

Temperature is an important factor in the developmental time of thrips (Lublinkhof & Foster, 1977; Gaum et al., 1994; Cloyd, 2009). The average temperature in our greenhouse compartments in spring and summer were nearly the same (spring: mean ± SE = 23.3 ± 5.1 °C; summer: 23.4 ± 5.0 °C). However, in spring, the temperature increased in the second half of the experimental period, with an average of 22.8 ± 5.6 °C in weeks 13–19 to 25.5 ± 6.7 °C in weeks 19–25. The most favorable temperatures for *F. occidentalis* are in the range of 25–30 °C. Thus, development from egg to adult is possible in 9–13 days. The lower the temperature (minimum threshold of 8–10 °C), the longer the development (Lublinkhof & Foster, 1977; Gaum et al., 1994; McDonald et al., 1998; Cloyd, 2009). Hence, the spring thrips population in our experiment was able to rapidly increase because of the warm temperatures in the greenhouse compartments. This shortened their lifecycle and development time and increased the number of thrips. Hence, the damage was mainly caused by larvae (see 'Attractiveness of flowers to *F. occidentalis*'). In the first 4–6 weeks of summer, the temperature was also high (mean 25.5 ± 5.8 °C). However, after this period, we recorded a drop in temperature (mean 21.7 ± 3.5 °C in weeks 35–41), which slowed population development (Figure S2).

Some studies have shown that the color of flowers plays a minor role in host selection by thrips (van Dijken et al., 1993; de Jager et al., 1995). In contrast, Blumthal et al. (2005) and Cloyd (2009) reported the high importance of color for *F. occidentalis*, especially yellow. Yaku et al. (2007) described red as an important color for *Franklinella schultzei* (Trybom), which may be explained by their red-flowering primary host *Malvaviscus arboreus* Cav. Unfortunately, the importance of color to *F. occidentalis* has been mainly studied by using colored traps or different hues instead of living flowers (Walker, 1974; Gillespie & Vernon, 1990; Vernon & Gillespie, 1990). In our study, we detected significant differences in the number of adult thrips in flowers based on color. Contrary to Matteson et al. (1992), we found fewer male thrips in violet and white flowers. Additionally, pink and orange flowers were also less attractive to males. However, for red flowers, there was a lower number of adult thrips of both sexes. Vernon & Gillespie (1990) suggested that the polyphagous *F. occidentalis* prefers allogamous host plants, which usually do not come in red, orange, green, and UV white. In our experiment, the abundance of males on these colors was low. More female thrips were found in green flowers in

spring, which contradicts the experimental findings by Matteson et al. (1992), as well as the assumption of Vernon & Gillespie (1990). However, some studies have described a strong attractiveness of green to aphids as a signal to the insect for landing on a potential host plant (Moericke, 1955; Hardie, 1989; Döring, 2014; Stukenberg & Poehling, 2019). The behavior of thrips to green flowers might also be explained by this 'settling behavior' of insects. Western flower thrips might not only respond to the flower colors, but most likely also respond to the vegetative, and thus, corresponding green parts of a plant (Stukenberg et al., 2020).

Finally, it must be mentioned that cultivars with severe silver damage on leaves did not automatically exhibit a high abundance of thrips in flowers. Maharijaya et al. (2011) reported significant differences in the duration of the developmental phases of *F. occidentalis* in pepper, although the differences were not correlated with resistance level. The authors mentioned that this result could have been expected because the level of resistance was assessed based on leaf injury (antixenosis) and not on the duration of the developmental phases (antibiosis). This could be the case in our study as well. We found that plants with more flowers had higher total numbers of thrips. However, the analysis of the number of adult thrips and larvae in flowers per cultivar revealed no correlation for further discrimination of cultivars as resistant or susceptible. This could also be caused by the missing analysis of flower scent, because scent plays an important role in host location and attraction to host plants for flower thrips (Annand, 1926; Kirk, 1985a; Ren et al., 2020).

Flavonol absorbance in 6th or 7th leaf position showed no clear differences

Different flavonols have been described as growth inhibitors against several insect species, e.g., *Pieris brassicae* (L.), *Helicoverpa armigera* Hübner, and *Spodoptera frugiperda* (J.E. Smith) on artificial diets or *Arabidopsis* plants (War et al., 2013; Onkokesung et al., 2014; Liu et al., 2015; Silva et al., 2016). It has also been previously described that thrips-resistant *Senecio* hybrids contained higher amounts of the pyrrolizidine alkaloids jacobine and jaconine, especially in younger leaves (Leiss et al., 2009a). In thrips-resistant chrysanthemums, a higher amount of phenylpropanoids, chlorogenic acid and feruloyl acid, were described. Both are known for their inhibitory effect on herbivores and pathogens (Leiss et al., 2009b). Hence, phenols can act as resistance factors (Kos et al., 2014). In our resistance screening, we were also interested in differences in flavonol content in the tested cultivars. To avoid a time-consuming and complex analysis of flavonols for resistance, we used the hand-held Dualex Scientific 4 sensor, which displays ease of use and good accuracy, for flavonol measurements (Cerovic et al., 2002; Goulas et al., 2004; Cerovic et al., 2012). With these fast measurements, we investigated an easy method for the discrimination between resistant and susceptible chrysanthemum cultivars.

Overall, the analysis of epidermal flavonol contents in our nine selected cultivars indicated only a few significant differences. Compared to the reference cultivar 1, cultivars 6, 7, 8, and 9 showed a significantly lower flavonol content in the vegetative phase, and cultivars 6, 7, and 9 were subsequently classified as susceptible, whereas cultivar 8 was moderately resistant. However, the relative flavonol content of the reference cultivar 1 was very high. Cultivar 1 was selected as the reference cultivar because of its mean silver damage and characterization as susceptible. Moreover, cultivar 3 was characterized as resistant and showed significantly lower flavonol content in the vegetative phase in spring and in the generative phase in summer. This was unexpected, but the significant differences could be explained by the already high flavonol content in the susceptible reference cultivar 1.

Our results indicated there was no correlation between leaf damage and relative flavonol content. Additionally, there was no correlation between the total number of thrips and the relative flavonol content in leaves. We measured flavonol content on the abaxial side because higher damage was expected on the adaxial side, as described by van Dijken (1992). A correct measurement on the abaxial side by the Dualex sensor could have been impaired by silver damage (air-filled cells). Because we measured flavonol content on the abaxial and determined silver damage on the adaxial side of the leaf, a correlation between both might not be meaningful.

Moreover, a flavonoid accumulation in combination with increased peroxidase activity in leaves of *Pisum sativum* L. after *Acyrthosiphon pisum* (Harris & M.) infestation was described by Morkunas et al. (2016). Additionally, alteration of precursors of flavonoids and alkaloids in *Glycine max* (L.) Merr. after *Aulacorthum solani* (Kaltenbach) infestation and an increased flavonoid level in sorghum leaves after *Rhopalosiphum maidis* (Fitch) infestation have been previously described (Sato et al., 2013; Anjali et al., 2017). A comparison between damaged and undamaged chrysanthemum plants could clarify the importance of flavonols. In our experimental design, we measured the flavonol content in chrysanthemum leaves only after the release of thrips. A comparison with measurements before the release of thrips was not feasible because we measured relative flavonol contents at leaf level 1 (oldest leaf of the plant; data not shown) for few cultivars and did not measure the contents in young leaves, i.e., leaf level 6 or 7,

before thrips release. Comparing data of different leaf levels for a distinction of status quo (constitutive resistance) in the resistant or susceptible cultivars would be wrong, because there might be differences in flavonol content in the different leaf levels. This means that we could not determine if flavonol content increased, decreased, or remained constant after damage by thrips at leaf levels 6 and 7. Additionally, we did not determine whether resistant cultivars showed constitutively higher or lower flavonol contents. Different studies have noted constitutive resistance properties formed by flavonoids for a resistant soybean before being fed on by *Anticarsia gemmatalis* Hübner, or in *Arachis kempff-mercadoi* Krapov. et al. against *Spodoptera litura* Fabricius (Mallikarjuna et al., 2004; Gómez et al., 2018). High concentrations of flavonoids in fruits are often related to a low incidence of pathogens; hence, they play a role in post-harvest resistance (Treutter, 2005). As mentioned before, a better distinction between resistant and susceptible chrysanthemum cultivars based on constitutive flavonol content still needs to be conducted. Another important point that should be noted is that measurements of leaves were taken in position 6 for pot and 7 for cut chrysanthemums from the base only. The middle part of the plant probably does not represent the most suitable part of the plant in terms of in vivo resistance measurements. De Kogel et al. (1997) described a preference of *F. occidentalis* for younger leaves in cucumbers (*Cucumis sativus* L.). However, Kos et al. (2014) described higher growth damage in young chrysanthemum leaves after thrips feeding, and silver damage occurred mainly on the old leaves. Van Haperen et al. (2019) showed that the youngest fully opened leaves of a resistant *Capsicum* accession were significantly more resistant to *F. occidentalis* larvae than older ones. An analysis of flavonol content in chrysanthemum leaves at different levels, as well as an analysis before and after thrips infestation, would provide more detailed information regarding the role of flavonols in resistance against *F. occidentalis*. The suitability of the Dualex Scientific 4 sensor for easy and rapid measurements must be investigated in more detail before a clear recommendation can be provided.

Conclusion

In this greenhouse experiment, we found a large variability in resistance against Western flower thrips among 77 chrysanthemum cultivars, although we analyzed only 10 chrysanthemum cultivars in detail. For future research on chrysanthemum, the ontogenetic phase (vegetative, bud, or generative phase) of the plant should be considered, or at least it should be stated exactly in which phase measurements are made. Resistant cultivars in the vegetative phase were not automatically resistant in the generative phase as well, although resistance was similar in most cases. The mechanisms underlying these ontogenetic changes in resistance remain unclear.

Flower color appeared to play a more important role for adult *F. occidentalis* than for larvae, which might be explained by adult flight activity and foraging preferences. Flower shape had a significant influence only on thrips larvae. However, plants with many thrips in the flowers did not necessarily have high silver damage and hence did not confirm the resistance levels according to silver damage.

Integration of the Dualex Scientific 4 hand sensor revealed no correlation between the measured relative flavonol content and silver damage on leaves in the middle part of the plants. Some susceptible cultivars contained significantly lower flavonol contents. By measuring different leaf positions (old, middle, and young from the base) and comparing their relative flavonol content, clearer discrimination of resistant and susceptible cultivars might be possible in the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Various shapes of chrysanthemum flowers: (A) daisy-type, (B) anemone, (C) pompom, (D) spider, (E) filled, and (F) garden mums.

Figure S2. Mean (± SE) temperature (°C) per week over time during the spring (weeks 13–25, black line) and summer (weeks 30–41, grey line) period in the resistance screening.

Table S1. Number of chrysanthemum cultivars used in the resistance screening against *Frankliniella occidentalis*. Listed are: corresponding plant shape (c = cut, p = pot, m = garden mum); mean (± SD) number of leaves in vegetative and generative phase in spring and summer; leaf size (1 = shorter than 4 cm, 2 = 4–6 cm, 3 = longer than 6 cm with petiole); reaction time until flowering; flower color; flower shape; and mean (± SD) number of flowers per main shoot in generative phase in spring and summer.