Growth damage and silvery damage in chrysanthemum caused by *Frankliniella occidentalis* is related to leaf food quality

S.Y. Fung[1], I. Kuiper[2], C.M. van Dijke-Hermans[2] and E. van der Meijden[2]
[1]Chrysanthemum Breeders Asscociation Research B.V., Rijshornstr 205, 1435 HH Rijsenhout, P.O. Box 370, 1430 AJ Aalsmeer, The Netherlands; [2]Institute of Evolutionary and Ecological Sciences, Leiden University, Kaiserstr 5, P.O. Box 9516, 2300 RA Leiden, The Netherlands.
E-mail: YingFung@rulsfb.leidenuniv.nl

Abstract: Herbivory by *Frankliniella occidentalis* leads to two types of plant damage in chrysanthemum *Dendranthema grandiflora*: feeding on young plant tissue results in growth damage, while feeding on mature leaves leads to silvery damage. The difference in food quality of young and old leaves of 13 chrysanthemum genotypes was assessed in bioassays with thrips larvae, and larval survival was used to express chrysanthemum leaf food quality. Larval survival on leaf juice is correlated with both growth damage and silvery damage in the plant. It is concluded that there is a relationship between both growth damage and silvery damage in chrysanthemum and leaf food quality, which is affected by leaf chemical composition.

Key words: *Frankliniella occidentalis*, chrysanthemum, leaf age, growth damage, silvery damage, bioassay, phytochemistry.

Introduction

Phytophagous thrips can inhabit all non-woody aerial parts of plants where there is access to food (Kirk, 1997). Infestation of chrysanthemum, *Dendranthema grandiflora*, by Western flower thrips, *Frankliniella occidentalis*, causes two types of feeding damage. When young parts of the plant are affected by the insect, leaves and flowers can become distorted; this phenomenon is known as growth damage. Thrips feeding on mature leaves causes silvery damage due to the production of empty, air-filled clusters of plant cells.

Plant chemicals can be major factors influencing the interaction between plants and insects (Schoonhoven et al., 1998). The chemical composition of leaves changes during the development from young to mature leaves. Usually, young leaves are richer in nutrients than old leaves (Slansky, 1993), but they sometimes also contain more toxic compounds (Hoy et al., 1998). For example, the young leaves of *Cynoglossum officinale* contain 190 times more pyrrolizidine alkaloids than the old leaves (Van Dam et al., 1994). In *Senecio jacobaea* young leaves are richer in nitrogen and pyrrolizidine alkaloids than old leaves; in this plant, the generalist Lepidoptera herbivores *Spodoptera exigua* and *Mamestra brassicae* prefer feeding on older leaves than on younger ones (De Boer, 1999).

Leaf age can influence the feeding behavior and development of insects (Gall, 1987). Difference in host quality of young and old cucumber, *Cucumis sativus*, leaves for *F. occidentalis* is reflected in both reproduction and oviposition of the insect (de Kogel et al., 1997a and 1997b). Reproduction is higher on young leaves than on old leaves and female thrips prefer young leaves to the mature ones for oviposition. When chrysanthemum is infested by *Tetranychus urticae*, mite density is higher on young leaves than on old leaves (Kielkiewicz and van de Vrie, 1990). The performance of *Aphis gossypii* on chrysanthemum is also dependent on leaf age (Storer and van Emden, 1995).

The amount of growth and silver damage caused by *F. occidentalis* in a selection of chrysanthemum cultivars was found to be dependent on plant genotype and plant age (van Dijken et al., 1994 and 1996). Until now, the mechanism leading to growth damage in chrysanthemum is still not known (de Jager, 1995), which is highly unsatisfactory as this type of thrips feeding damage leads to a considerable loss of aesthetic and economic value of the crop. Knowledge of the mechanism(s) affecting thrips - host plant relationships is urgently needed when we want to breed more thrips resistant chrysanthemum varieties.

The study presented in this paper was set up to test the following hypothesis: we assume that young and old leaves of chrysanthemum differ in food quality for thrips, and that the amount of growth damage is related to food quality of young leaves, and of silvery damage to that of old leaves. In this study, food quality for thrips is measured by determining larval performance in bioassays with leaf juices, as developed by de Jager (1995a). These bioassays were originally set up to exclude non-chemical factors in testing the influence of plant genotype on thrips resistance in chrysanthemum.

Materials and methods

Insects

A laboratory strain of *F. occidentalis* was reared on flowers of the chrysanthemum cv. Reagan (T = 20 ºC, RH = 60%, photoperiod 12L/12D).

Plants

Three week-old rooted cuttings of 13 chrysanthemum genotypes, provided by the Chrysanthemum Breeding Association, were planted in 9 cm (diameter) pots with a 3:1 earth-vermiculite mixture. Plants were grown under controlled conditions (T = 20 °C, RH = 70%, photoperiod L16:D8) and received 75 ml nutrient solution (15:20:25 NPK, EC = 2 mS/cm) once a week. After one week, the photoperiod was changed to L8:D16. Young (= tip of the plant and upper four to five leaves) and old leaves (= other leaves excluding the four oldest) were harvested from 4 week-old (= long-photoperiod plants) and 6 week-old plants (= short-photoperiod plants), each series consisting of material from 15 plants of one genotype. These leaves were kept at -20ºC before processing. Leaf juice was obtained by shortly crushing the leaves in a Waring blendor at low temperature, and squeezing the leaves through Mira cloth. The juices were kept frozen at -20ºC until required.

Bioassay

After blending and filtering the leaf material through Mira cloth, a juice containing leaf sap and particles is obtained. In preliminary experiments the food quality of the juice and the clear sap, obtained by centrifugation of the juice, of old leaves of 6 genotypes was compared. These bioassays showed that feeding on the sap alone led to a high mortality of the thrips larvae and further tests were therefore carried out on leaf juices.

The relative growth and survival of 1st instar larvae of *F. occidentalis* were measured after feeding on leaf juices for 3 days (de Jager et al., 1995a). Relative growth is the quotient of the increase in length of the larvae after 3 days and the initial length on day 1. In this non-choice experiment, each thrips larva is put into a separate cell where it can feed on a specific leaf juice by puncturing a thin membrane of stretched parafilm covering the liquid diet. Each set of data consisted of measurements of 30 larvae. Leaf juices of 4-week and 6-week-old plants were used for this study. As control for the bioassays, an artificial diet developed by Sing (1983) was employed, containing 3.5% (w/v) casein, 3% (w/v) sucrose, 1% (w/v)Wesson's salt, 2% (w/v) VanderZant vitamin mixture, 0.05% (w/v) cholesterol and 0.25% (w/v) linoleic acid.

Determining thrips damage on plants

The amounts of growth damage and silvery damage were assessed in a choice experiment to mimic the situation in greenhouses where thrips could feed freely on available plants. Plants, 10 specimens per cultivar and treated as described above, were placed in a randomized design in a climate room (T = 20 °C, RH = 70%, photoperiod L16:D8). When plants were 4 weeks old, 10 adults of *F. occidentalis* per plant were released and the photoperiod was changed to L8:D16. The amount of plant damage was determined after 3 weeks, when plants were 7 week old. Growth damage was expressed as the number of distorted leaves (0.5 ∗ number of leaves with light degree of distortion + number of leaves with strong malformation) and silvery damage as the number of affected patches of 1 mm^2 on the entire plant. In previous experiments by de Jager et al. (1995b) growth damage was determined in 10-week-old plants and silvery damage in 13-week-old plants. As thrips feeding damage in young plants was found to be strongly correlated with that on old plants of the same genotype (de Jager et al., 1995b), the determination of feeding damage on intact plants and of bioassays with leaf juices in this study were determined in 7- and 6-week-old plants, respectively, instead of the older plants used by de Jager et al (1995b).

Statistical analyses
Analyses were performed with SPSS 8.0. Differences in the amounts of growth damage and silvery damage were analysed by Kruskal-Wallis test. Data of relative growth and survival were average values of 5 measurements from 5 different sets of plates, each measurement consisting of the mean of 6 observations of one plate. Differences in relative growth and survival in each series of bioassays were determined by Oneway ANOVA. Overall effects in all series of bioassays on relative growth and survival were analysed by univariate ANOVA using leaf age and photoperiod as fixed factors, and genotype as random factor. Correlations between sets of nonparametric data were analysed by Spearman correlation.

Results and Discussion

Effect of genotype, leaf age and plant age on chrysanthemum leaf food quality
For this study, a selection was made of 13 chrysanthemum genotypes from a recent breeding program. Similarly to the results of de Jager et al. (1995b) for older genotypes, we found that there was a strong difference in sensitivity towards growth damage (chi-square 92.5, df = 12, p = 0.000) and silvery damage (chi-square = 107.0, df = 12, p = 0.000) in chrysanthemum genotypes (fig. 1).

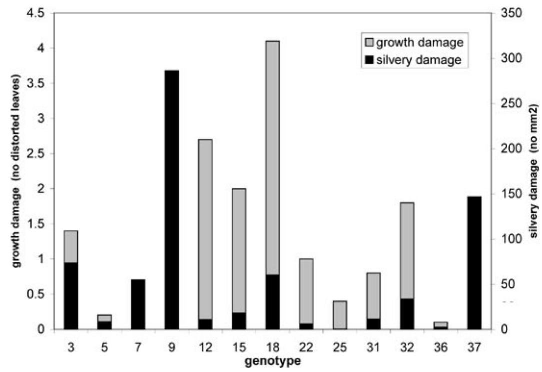

Fig. 1. Growth damage and silvery damage in 13 chrysanthemum genotypes

In greenhouses, chrysanthemum cuttings are first grown under a long photoperiod and then subsequently under a short photoperiod to induce flowering. Therefore plants from these two different stages were tested in this study. Bioassays were performed with juices of young and old leaves from plants of these two different photoperiods. Insect performance on leaf juices was expressed as relative growth and survival of first-instar larvae during 3 days. There is less difference in relative growth than survival in each series of bioassays. For relative growth, a significant difference was only detected in one series of bioassays, namely in the series with juices of young leaves from long-photoperiod plants (ANOVA, df = 12, F = 2.20, p = 0.02). Survival was significantly different for all 4 series of leaf juices; for juices of young leaves (ANOVA, df = 12, F = 5.11, p = 0.000) and of old leaves of long photoperiod-plants (ANOVA, df = 12, F = 8.06, p = 0.000), and for juices of young leaves (ANOVA, df = 12, F = 3.55, p = 0.001) and of old leaves of short photoperiod-plants (ANOVA, df = 12, F = 3.86, p = 0.000). The mean survival of thrips larvae on leaf juices from the 4 different series is depicted in fig. 2. Larval survival on leaf juices was low compared to that on the control, an artificial diet developed for insects by Singh (1983), which contained high amounts of casein as protein source (3.5 % w/v) and sucrose as carbohydrate source (3 % w/v).

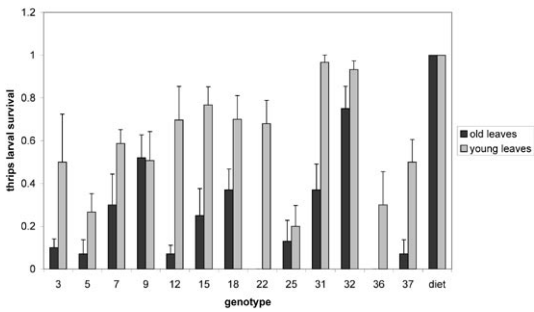

Fig. 2. A. survival on leaf juice long day plants

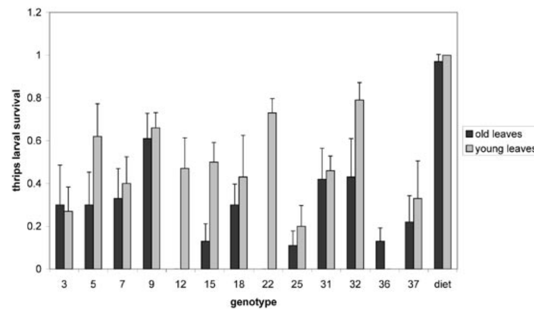

Fig 2B. Survival on leaf juice short day plants

Fig. 2. Larval survival of *F. occidentalis* fed with juices of old and young leaves from chrysanthemum genotypes from long photoperiod (A), and on juice of old and young leaves from chrysanthemum genotypes from short photoperiod (B) (mean and s.e. are given)

Factor		**Relative growth**		**Survival**	
	df	***F***	***p***	***F***	***p***
Genotype (A)	12	3.41	0.38	2.50	0.062
Leaf age (B)	1	0.76	0.40	26.41	0.000
Photoperiod (C)	1	5.68	0.03	1.80	0.20
Interaction					
AB	10	0.74	0.68	2.77	0.045
AC	11	0.73	0.70	1.65	0.20
BC	1	0.24	0.63	5.73	0.034

Table 1.Overall effects of genotype, leaf age and photoperiod on growth and survival in bioassays with *F. occidentalis*

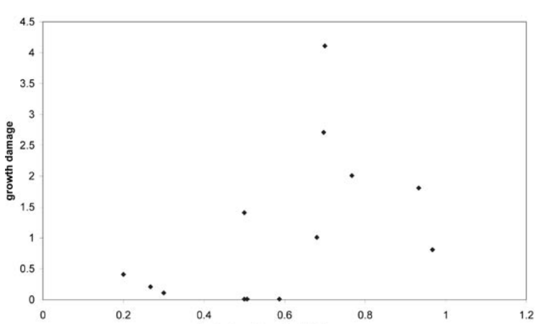

Fig. 3A. Correlation growth damage and thrips larval survival

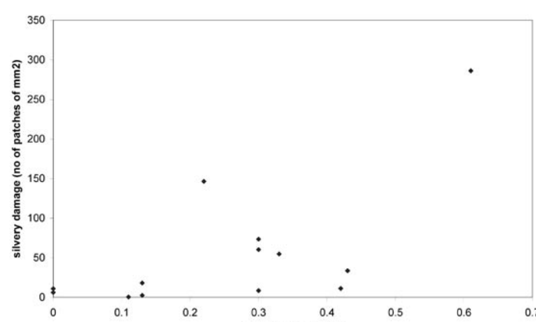

Fig. 3B. Correlation between silvery damage and thrips larval survival in bioassays

Fig. 3. Correlation between growth damage and larval survival of *F. occidentalis* in bioassays with juices of young chrysanthemum leaves of long-photoperiod plants (A) and between silvery damage and thrips larval survival in bioassays with juices of old chrysanthemum leaves of short-photoperiod plants (B)

Overall consideration of the factors genotype, leaf age and photoperiod (table 1) shows that relative growth is significantly affected by photoperiod, and survival by leaf age. There are significant interactions of genotype and photoperiod with leaf age for survival. In previous experiments with chrysanthemum no distinction was made between young and old leaves. From the results of our bioassays we conclude that these leaves do differ in food quality for thrips larvae.

Correlation between feeding damage and larval survival in bioassays with chrysanthemum leaf juices

According to our assumption, we expected that in the present set of 13 genotypes growth damage would be related to the food quality of young leaves. In this study, we expressed leaf food quality as larval survival measured in bioassays with leaf juices. Indeed, a significant positive correlation was found between growth damage and thrips larval survival, when the larvae fed on leaf juices of young leaves of long-photoperiod plants (Spearman correlation, $\rho = 0.56$, $p = 0.02$) (fig.3a).

The results also confirmed our assumption concerning a relationship between silvery damage and food quality of old leaves. There was indeed a positive correlation between silvery damage and the survival of thrips larvae when fed with juices of old leaves from short photoperiod-plants (Spearman correlation, $\rho = 0.62$, $p = 0.01$) (fig. 3b). Thus the amount of growth and silvery damage on the plant is indeed related to the food quality of young and old chrysanthemum leaves, respectively.

Conclusion

We conclude from our bioassays that there is a strong difference in food quality between leaf juices from the various chrysanthemum genotypes examined, and that young leaves seem to be a more suitable food source than mature leaves**.** The low survival of thrips larvae in these experiments indicates that the food quality of chrysanthemum leaves is in general too poor to sustain the development of this insect. Chrysanthemum leaves contain insufficient amounts of nutrients to support thrips larval development, and chrysanthemum flowers seem to be needed to achieve population growth of western flower thrips on this plant (Fung and van der Meijden, in prep.)

Since distortion is related to thrips damage of young plant tissue, and silvery damage to that of mature leaves, we expected that there would be a correlation between growth damage and food quality of young leaves, and between silvery damage and food quality of old leaves in chrysanthemum. The results of this study show that this assumption is correct. In the present set of 13 genotypes, which differ in their sensitivity towards growth damage and silvery damage, we found a significant correlation between growth damage and food quality of young leaves and between silvery damage and food quality of old leaves.

As larval performance in the bioassays is strongly determined by the chemical composition of the leaf juices, we can conclude that both growth and silvery damage in chrysanthemum are related to its leaf phytochemistry. In non-choice tests, feeding damage, expressed as silvery damage under these circumstances, has been linked with the leaf chemistry of chrysanthemum (de Jager et al., 1995a), but the mechanism leading to growth damage was still largely unexplained (de Jager, 1995). This study indicates that both growth damage and silvery damage in chrysanthemum are partly determined by its leaf phytochemistry. Future work will focus on the identification of the plant compounds leading to the occurrence of thrips feeding damage on chrysanthemum.

Acknowledgements

K. Pater assisted with the experiments. P.G.L. Klinkhamer and K. Vrieling are kindly acknowledged for their comments on statistical analyses. This study was financed by C.B.A. Research B.V. and carried out at Leiden University.

References

De Boer N. 1999. Pyrrolizidine alkaloid distribution in *Senecio jacobaea* rosettes minimises losses to generalist feeding. *Entomologia Experimentalis et Applicata* **91**, 169-173.

De Jager CM. 1995. *Mechanism of resistance to western flower thrips in chrysanthemum*. Thesis, University of Leiden.

De Jager CM, Butôt RPT, Klinkhamer PGL and van der Meijden E. 1995a. Chemical characteristics of chrysanthemum cause resistance to *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Economic Entomology* **88**, 1746-1753.

De Jager CM, Butôt RPT, Klinkhamer PGL, de Jong T and van der Meijden E. 1995b. Genetic variation in chrysanthemum for resistance to *Frankliniella occidentalis*. *Entomologia Experimentalis et Applicata* **77**, 277 – 287.

De Jager CM, Butôt RPT, van der Meijden E and Verpoorte R. 1996. The role of primary and secondary metabolites in chrysanthemum resistance to *Frankliniella occidentalis*. *Journal of Chemical Ecology* **22**, 1987-1999.

De Kogel WJ, Balkema-Boomstra A, van der Hoek M and Mollema C. 1997a. Resistance to Western flower thrips in greenhouse cumcumber: effect of leaf position and plant age on thrips reproduction. *Euphytica* **94**, 63-67.

De Kogel WJ, van der Hoek M and Mollema C. 1997b. Oviposition preference of Western flower thrips for cucumber leaves from different positions along the plant. *Entomologia Experimentalis et Applicata* **82**, 283-288.

Fung SY, Kuiper I, Butôt RPT, de Kogel WJ and van der Meijden E. 1999. Thrips and chrysanthemum: what is the influence of host plant substances? *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society*. **10**, 137-142.

Gall LF. 1987. Leaflet position influences caterpillar feeding and development. *Oikos* **49**, 172-176.

Hoy CW, Head GP and Hall FR. 1998. Spatial heterogenity and insect adaptation to toxins. *Annual Review of Entomology* **43**, 571-594.

Kielkiewicz M and van der Vrie M. 1990. Within-leaf differences in nutritive value and defence mechanism in chrysanthemum to the two-spotted spider mite (*Tetranychus urticae*). *Experimental and Applied Acarology* **10**, 33-43.

Kirk WDJ. 1997. Feeding. Pp. 119- 174, in: Lewis, T. (ed). *Thrips as crop pests*. CAB International, Oxon.

Schoonhoven LM, Jermy T and van Loon JJA. 1998. *Insect-Plant Biology*. Chapman & Hall, London.

Singh P. 1983. A general purpose laboratory diet mixture for rearing insects. *Insect Science Application* **4**, 357-362.

Slansky F. 1993. Nutritional Ecology: The fundamental quest for nutrients. Pp.29-91. in: Stamp NE and Casey TM (eds.), *Caterpillars*. Chapman & Hall, New York.

Storer JR and van Emden HF. 1995. Antibiosis and antixenosis of chrysanthemum cultivars to the aphid *Aphis gossypii*. *Entomologia Experimentalis et Applicata* **77**, 307-314.

Van Dam NM, Verpoorte R and van der Meijden E. 1994. Extreme differences in pyrrolizidine alkaloid levels between leaves of *Cynoglossum officinale* L. *Phytochemistry* **37**, 1013-1016.

Van Dijken FR, Dik MTA, Gebala B, de Jong J and Mollema C. 1994. Western flower thrips (Thysanoptera: Thripidae) effects on chrysanthemum cultivars: plant growth and leaf scarring in nonflowering plants. *Journal of Economic Entomology* **87**, 1312-1317.

Van Dijken FR, Koning N and Gebala B. 1996. Host plant resistance in transformable chrysanthemum cultivars to Frankliniella occidentalis? Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society **7**, 171-176.