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Within-leaf differences in nutritive value and defence mechanism in chrysanthemum to the two-spotted spider mite (*Tetranychus urticae*)

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ABSTRACT

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Some chrysanthemum leaf characteristics were evaluated to determine their importance as nutrition for, and as factors of resistance to, *Tetranychus urticae* Koch. These characteristics were: leaf age and chemical composition, content of phenolic substances, total nitrogen, and soluble protein.

Mite density was lower on young leaves than on mature ones. Young leaves appear to be protected against *T. urticae* by a higher concentration of mono- and polyphenols, although they contained higher levels of nutrients than mature ones. After mite infestation, the most valuable young leaves were also the best-protected leaves on their shoots. Content of phenolic compounds increased only in young chrysanthemum leaves after mite feeding.

INTRODUCTION

Two-spotted spider-mite (*Tetranychus urticae* Koch) feeding has a marked effect on the growth and physiology of several plant species (Storms, 1971; Sances et al., 1981; De Angelis et al., 1982, 1983; Kropczyńska and Tomczyk, 1984; Tomczyk and Kropczynska, 1984; Hildebrand et al., 1986). Also, the stage of plant development and its physiological activity affect the spider mites' fecundity and behaviour (Byrne et al., 1982; Mellors and Propts, 1983; Larson and Barry, 1984; Perring et al., 1982). The nutritive value of plant tissues to mites depends not only on the nutrient concentration of soluble sugars, proteins, amino acids, vitamins, macro- and microelements, but also on the

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level of secondary compounds such as phenolics, mono- and sesquiterpenes or alkaloids. Such secondary plant metabolites can reduce the food uptake by mites and thus may play a role in host-plant resistance to this pest. These substances are not uniformly distributed in the plant, and their content depends on the organ age, type of tissue, external conditions, etc.

This report is concerned with the study of some characteristics such as leaf age, and chemical composition to determine their importance as factors of resistance mechanisms in chrystanthemum cultivars. Fecundity and population development of *T. urticae* were studied on these plants and related to the parameters mentioned.

MATERIALS AND METHODS

Growth and infestation of the plants

Cuttings of three chrysanthemum cultivars (*Dendranthrema grandiflora* Tzvelev), Super Yellow, White Horim and Gelac, were planted in soil in plastic pots, and grown in a greenhouse at a mean temperature of 24°C during the day under natural light conditions. The plants were divided into two groups, control and infested. Four-week-old plants (30–50-cm high) were infested with 15 young female mites on each leaf.

Mite population development and damage rating

One, two, four and five weeks after infestation, the number of adult mites was counted on three leaves of 20 plants of each cultivar. Infested leaves were collected separately from the top (young leaves) and from the central part (mature leaves) of the plants.

Three weeks after being infested, the plants were rated visually for feeding damage, i.e. the number of chlorotic spots. Cultivars that did not show feeding injury were rated as resistant (R); cultivars with only slight injury were rated as moderately resistant (MR); and cultivars showing severe injury were rates as susceptible (S).

Oviposition

Three independent experiments, with five replicates each, were carried out to investigate the effects of chrysanthemum cultivars on oviposition. Full-grown leaves were placed on moist sponge and covered with moist paper tissue in which 2.5 cm-diameter holes had been cut. On such leaf discs of three chrysanthemum cultivars, ten mite females were placed. After three days the number of eggs laid was counted. Leaf discs from bean plants, the common laboratory host-plant, were used for comparison. All tests were conducted at a constant temperature of 15°C, RH of 70% and 16:8 L:D photoperiod.

Growth of infested plants

During five weeks of mite feeding, the length of the main stem of 25 control and 25 infested plants of each cultivar was measured. Fifteen leaves from the top and from the bottom of the plants were collected and the area of these leaves was measured with a Delta-T measurement system.

Sampling and chemical analysis

Non-infested and infested leaves were collected three weeks after mite feeding. Immediately after collecting, these leaves were either dried or frozen at $-30\,^{\circ}C$.

Nitrogen analysis. The content of organic nitrogen was determined by the micro-Kjeldahl method with Kjel-Foss Automatic 16210 Analyzer (A.S.N. Foss Electronic, Copenhagen) and expressed as percentage dry-matter.

Soluble-protein analysis. Soluble protein was extracted by grinding leaves with 4 ml 0.1*M* Tris-glycerine buffer, pH 8.3, per g of fresh leaf. The homogenate was centrifuged at 10 000×g for 15 min and the supernatant used directly for analysis. Bradford Reagent (2.5 ml) was added to each sample of supernatant (0.05 ml). Absorbancy was measured at 595 nm and the amount of protein was calculated on the basis of bovine serum albumine curve (Coomassie Briliant Blue G-250 and bovine serum albumine were obtained from Sigma Chemical Co., St. Louis, Missouri).

Phenol and polyphenol analysis. The contents of phenols and polyphenols were determined according to the procedure of Kritzman and Chet (1980). Leaves, 4–6 g fresh weight, were homogenized with 0.1*M* HCl and centrifuged at 1500×g for 30 min. The extract was mixed with an equal volume of propanol and NaCl was added. The determination of phenol content was carried out in a propanol fraction, whereas an aqueous fraction was used for polyphenol essays. The amount of phenols and polyphenols was calculated on the basis of a catechol and tannic standard curve, respectively.

Statistical analysis

For all chemical analyses five independent replicates were performed and the mean values are presented. Data were analyzed by Duncan's new multiple range test and Student's *t*-test.

RESULTS

Mite population development

Mite density differed significantly, and depended on the position of the leaf of the plant (Tables 1 and 2). The density of the two-spotted spider-mite

TABLE 1

Mean number of mite females per cm^2 of mature chrysanthemum leaves, and damage rating

Cultivar	Damage rating	Week of infestation			
		1	3	4	5
Super Yellow	R	0.22a	0.32a	0.58a	0.32a
White Horim	MR	0.30b	0.37a	0.59a	0.12b
Gelac	S	0.23a	0.31a	0.48a	0.47a

Values in the same column followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

TABLE 2

The mean number of mite females per cm^2 of young chrysanthemum leaves, and damage rating

Cultivar	Damage rating	Weeks of infestation			
		1	3	4	5
Super Yellow	R	0.01a	0.31a	0.40a	0.08a
White Horim	MR	0.04b	0.60b	0.38a	0.08a
Gelac	S	0.03b	0.52b	0.36a	0.15b

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

females was significantly higher on the mature leaves of White Horim than on Super Yellow or Gelac, one week after the infestation. No differences in female mite density on the mature leaves was observed after three or four weeks of infestation. The maximum density of the mite population was observed after four weeks.

Table 2 shows the mean mite density on the young chrysanthemum leaves. After three weeks of infestation the highest density was observed on White Horim, the lowest on Super Yellow. The mite population declined very sharply after five weeks and was 3–4 times lower compared with the density on mature leaves.

The mean numbers of females per cm^2 on mature leaves per week were 0.29, 0.28 and 0.30 for Super Yellow, White Horim and Gelac, respectively, whereas the respective values for young leaves were 0.16, 0.22 and 0.21 for Super Yellow, White Horim and Gelac.

From these data it is clear that, on the cultivars investigated a rather low density of *T. urticae* developed. The density did not increase above 0.6 females cm^{-2}; however, younger leaves were much less attractive.

Damage rating

Three weeks of mite feeding caused most severe damage on mature Gelac leaves as compared to White Horim and Super Yellow. The number of females, however, was not significantly higher on this cultivar (Table 1).

After three weeks of mite feeding on young leaves, the damage was heavier on Gelac than on White Horim, although their number of females was equal (Table 2). This indicates a higher tolerance to two-spotted spider mite in White Horim. After three weeks of mite infestation, neither mature nor young leaves of Super Yellow had visible symptoms of mite feeding.

Oviposition

Table 3 shows the mean number of eggs on the leaf discs of chrysanthemum and bean. After three and four days of mite feeding the lowest number of eggs was found on Super Yellow. The mean number of eggs on White Horim was higher than on Gelac; however, the Gelac leaf discs were heavily damaged.

The mean number of eggs on bean leaves was 2–5 times higher on the third oviposition day and 3–6 times higher on the fourth day as compared to all chrysanthemum cultivars, indicating a general lower suitability of chrysanthemum as a host plant for *T. urticae.*

These results show that the reproduction rate was affected by the chrysanthemum cultivar. Super Yellow was clearly less favorable as host plant than White Horim or Gelac.

Based on the damage rating of leaves from the results of the oviposition test, the degree of resistance in chrysanthemum cultivars can be classified as follows: Super Yellow, resistant (R); White Horim, moderately resistant or tolerant (MR); and Gelac, susceptible (S).

Plant growth

At the beginning of the experiment, Gelac plants were taller than Super Yellow or White Horim (Fig. 1). It is evident that, as the experiment pro-

TABLE 3

Average number of eggs laid on leaf discs of chrysanthemum and bean leaves

Cultivar/Plant	Oviposition day	
	3	4
Super Yellow	3.76a	6.0a
White Horim	10.5b	13.0ab
Gelac	7.8b	9.3b
Bean	19.7c	36.8c

Values in the same column followed by the same letter are not significantly different according to Duncan's new multiple range test ($P=0.05$).

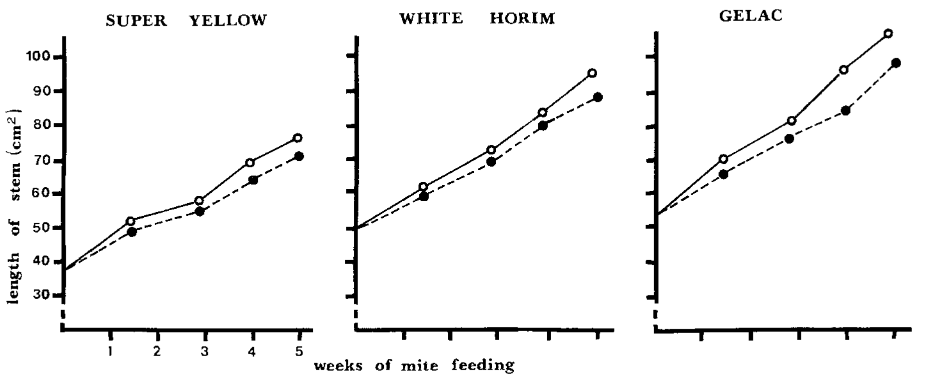

Fig. 1. Effect of *T. urticae* feeding on stem growth of cultivars: (—O—), control; (---●---), infested.

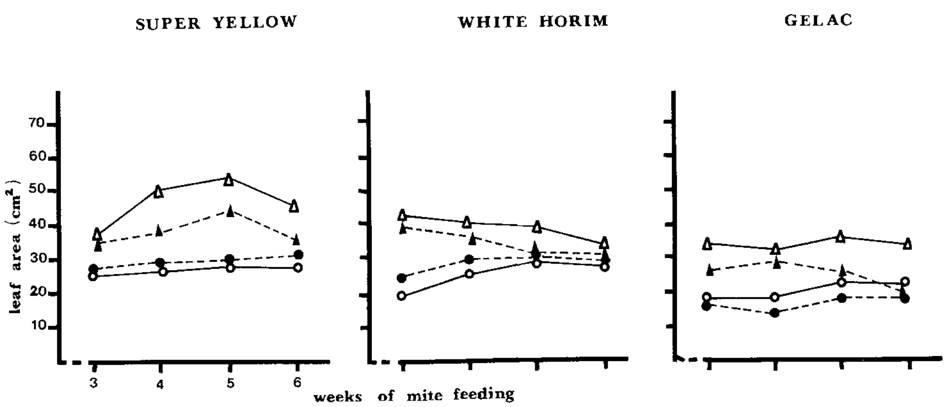

Fig. 2. Effect of *T. urticae* feeding on leaf area of chrysanthemum cultivars: (— △ —), and (--- ▲ ---), mature leaves; (—O—) and (---●---), young leaves; (——), control, (------), infested.

gressed, mite infestation influenced plant growth; all infested plants were shorter as compared with the untreated. The growth of Gelac was more seriously reduced than the other two cultivars.

As a result of mite feeding a reduction in mature leaf area in all cultivars was observed. The area of the younger leaves altered also during the mite infestation period, but increase or decrease depended on the cultivar (Fig. 2). The area of infested White Horim leaves was greater compared with the control plants. Contrary to these results, the area of Gelac leaves was reduced in comparison with the control plants. There were no statistically significant differences between control and infested leaf areas in the Super Yellow cultivar. In each case, the area of mature leaves was more seriously affected than that of younger leaves.

TABLE 4

Analysis of noninfested (a) and infested (b) chrysanthemum for some components in dry-weight of leaves

Chemical component	Young leaves						Mature leaves					
	Super Yellow		White Horim		Gelac		Super Yellow		White Horim		Gelac	
	a	b	a	b	a	b	a	b	a	b	a	b
Total nitrogen (% DW)	2.8	2.8	3.0	2.6	2.8	2.5	1.7	1.8	2.3	2.2	2.7	2.6
Soluble protein (mg g^{-1} DW)	6.2	4.0*	14.4	9.8*	15.4	12.8*	4.3	3.5*	4.4	3.1*	2.1	1.8*
Phenols ($\mu g\ g^{-1}$ DW)	4071	4489*	2793	3519*	3564	6224*	2269	1705*	2697	2311*	2886	2246*
Polyphenols ($\mu g\ g^{-1}$ DW)	2780	3057	1792	2378*	2356	4348*	1612	1066*	1615	588*	2193	1404*

*, differences statistically important at $P=0.05$.

Nitrogen content

Since nearly 90% of nitrogen in plants is present in the form of free amino acids and proteins, the Kjeldahl determination of the nitrogen content provides a good estimate of the maximum amount of nitrogen available for pest growth (Fenny, 1976). Young leaves of mite-free cultivars contained a higher amount of total N as compared to mature ones (Table 4). The content of total N in injured tissues of these leaves decreased by about 12%. Only infested leaves of Super Yellow maintained the same level of total N as the uninfested. No marked effect of mite feeding on the nitrogen level in the mature leaves was noticed.

Protein content

Uninfested young leaves of all chrysanthemum cultivars possessed a higher amount of soluble protein compared with mature ones (Table 4). The content of soluble protein has decreased in both young and mature leaves after mite feeding.

Phenol content

At the beginning of the experiment, the young leaves contained a higher amount of monophenols compared to the mature leaves. The highest level was found in young Super Yellow and the lowest in White Horim leaves (Table 4). The monophenol contents in the young leaves of all infested cultivars increased significantly. Mature leaves of all chrysanthemum cultivars possessed a similar level of monophenols, however, lower than the young ones. After three weeks of mite infestation a decrease in the monophenol content in mature leaves of all tested cultivars was found.

Polyphenol content

Mite-free Super Yellow young leaves contained a higher amount of polyphenols than White Horim and Gelac leaves. After mite feeding, a significant increase in the content of polyphenols was observed in all cultivars (Table 4). No differences in the content of polyphenols was found in mature leaves of Super Yellow and White Horim. The highest content of polyphenols was found in Gelac mature leaves (Table 4). Three weeks of mite infestation resulted in a marked decrease in phenol content in all three cultivars.

DISCUSSION

The results presented show that chrysanthemum leaves are a rather heterogeneous source of food for the two-spotted spider mite. It is also shown that young chrysanthemum leaves were not as suitable as mature ones as a source of food. Young leaves possessed a higher concentration of total nitrogen and soluble protein compared with mature leaves. This is in agreement with the results reported by Ikeda et al. (1977) and Cranshaw and Langenheim (1981), who indicate that terminal and basal leaves have different photosynthetic and storage capabilities. In our studies, the terminal (young) leaves contained a higher concentration of phenols and polyphenols than the basal (mature) leaves. These compounds may be considered to be defence factors against mites (Kiełkiewicz and van de Vrie, 1983; Larson and Berry, 1984). Thus, it is possible that such secondary metabolites deterred mite feeding on young leaves. A lower number of mites was found on those leaves although they were more valuable for the mites than the mature leaves. Consequently, the young leaves were less stressed and the observed damage was smaller as compared with mature leaves. However, after mite feeding, greater losses of protein and total nitrogen were found in young leaves than in mature ones. In these leaves, mite feeding created less favorable conditions for reproduction. Rhodes and Cates (1976) suggested that the terminal leaves of many plant species are defended by a higher level of toxins. Also Larson and Barry (1984) found that young, expanding lateral peppermint leaves had the highest monoterpene and phenolic contents. As leaf phenolic content increased, the number of eggs laid by the two-spotted spider mite significantly decreased.

In the chrysanthemum cultivars used in our experiments, significant differences in leaf size exist between young and mature leaves. The young leaves were smaller; Zuker (1982) found that smaller leaves have higher phenol content. High levels of phenolics may not be the only factor restricting mite feeding on young leaves. Tulisalo (1971, 1972) suggested that, on chrysanthemum cultivars, fecundity was restricted by the leaf structure and was independent of the level and variation in carbohydrates.

Other workers also have related leaf age to reactions in mites (cf. Rodriguez, 1960). Young citrus leaves were more favorable than mature leaves for development of the citrus red mite (*Metatetranychus citri*). The clover mite (*Bryobia praetiosa*) lived longer and laid almost ten times as many eggs when reared on 'new' apple foliage containing 2.14% nitrogen compared to 'old' leaves containing 1.04%. Oviposition of the avocado brown mite (*Oligonychus punicae*) was higher on mature leaves than on young ones (McMurtry, 1971).

Our data (Table 4) indicate that, after mite feeding, the level of phenolic compounds increased in young chrysanthemum leaves and decreased in mature ones. This suggests that the most valuable leaves are also the best-defended leaves. Our results are similar to those reported by Fenny (1970), who indicated that secondary chemicals such as tannins have a defence function against insects.

It is evident from the data presented that, after three weeks of mite feeding, increase in phenolics was substantially greater in low and moderately resistant cultivars than in resistant ones. Kiełkiewicz and van de Vrie (1983) indicated the role of phenolic compounds (dihydroxyphenols) in the resistance of strawberry leaves to *T. urticae*. The present results suggest that examination of leaves from different positions on the shoot after mite feeding-periods shorter than three weeks is necessary to clarify this topic.

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