Correlation between Epicuticular Wax Content in the Leaves of Early White Cabbage (*Brassica oleracea* L. var. *capitata*) and Damage Caused by *Thrips tabaci* Lindeman (Thysanoptera: Thripidae)

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In the period of 2001–2002 the susceptibility of five varieties of early white cabbage to onion thrips *(Thrips tabaci)* attack has been studied. The variety with the longest growth period (72 days) and the firmest head (19.5–21.5 kg/cm^2), i.e. Vestri, had the highest mean weight of the head (1220.0 g/head) and the highest net weight of the head (1050.6 g/head), while the mean index of damage (1.18) and the mean yield loss (13.9%) were the lowest in this variety. At higher plant density (16.6 plants/m^2) both parameters of the weight of the head were approximately less than half as compared to lower plant density (8.2 plants/m^2). Results of the mean net weight of the heads did not show significant differences between different plant densities if the yield was calculated per area unit (m^2). The mean content of epicuticular wax differs among varieties, although all varieties show similar relations in its content; the lowest mean content in the third and the fourth exterior leaves of the head, the highest mean content was within the first and the second, and the ninth and the tenth exterior leaves of the head. In spite of the apparent correlation between the content of epicuticular wax and the mean index of damage (with both plant densities its values were the highest between the third and the sixth exterior leaf of the head) caused by feeding of onion thrips hardly perceived negative correlation (Pearson coefficient of correlation between –0.338 and –0.436) were found between these two parameters using statistical analysis.

Keywords: *Thrips tabaci,* epicuticular wax content, cabbage, damage, antixenosis.

In Slovenia, western flower thrips (*Frankliniella occidentalis* [Pergande]) and onion thrips (*Thrips tabaci* Lindeman) are the economically most harmful species from the Thysanoptera order. While the former species was first recorded in Slovenia at the beggining of the 1990s (Janežič, 1993), we classified the latter as a native species because of its wide distribution (Trdan, 2003).

In spite of Slovenia's geoclimatic variability, western flower thrips is damaging only in greenhouses (Trdan et al., 2003). On the other hand, onion thrips can cause damage in the open, where in spite of its great polyphagous nature (Raspudić and Ivezić, 1999), the quality and quantity of the yield are decreased only in some of its hosts – onion (Trdan and Žnidarčič, 2002), leek, cabbage (Trdan and Žnidarčič, 2003), and in some ornamental plants.

In Slovenia, an increasing number of vegetable growers are choosing more environmentally friendly methods of cultivation, which allow the use of less poisonous pesticides or none at all. An important role of both methods of cultivation is also the choice of appropriate agrotechnical measures, which can significantly decrease the damage of some phytophagous insects. The variety choice (varieties differ among themselves in the length of the growth period) as well as the chemical and mechanical characteristics of the plants belong among most important agrotechnical measures. All these and many other factors influence the resistance of the plants to harmful organisms.

The mechanism, which includes morphological, physical or structural characteristics of the plants, by which the insect's biological processes are disturbed – for example mating, oviposition or feeding – is known as antixenosis. In the process of the plant's resistance to harmful insects, antixenosis acts as a barrier, which has a direct effect on the behaviour of the insect – on their choice of the host plant. The most important factors of antixenosis are the presence of trichomes (Naik et al., 2000), epicuticular waxes (Jenks and Ashworth, 1999) and chemicals in or on the plants (Bitenc and Milevoj, 2002), which act as repellents to the insect.

Epicuticular wax on the leaves protects plants against desiccation, infections of plant pathogens and the attack of pests. It is known that insects react differently to chemicals on the leaf surface (Panda and Khush, 1995). In addition, the wax can also physically hinder the movement of the insect on the leaf surface (Stork, 1980). The literature most often reports on the negative correlation between the number of insects and the epicuticular wax content on the leaves (Bergman et al., 1991; Bodnaryk, 1992; Eigenbrode et al., 1992), however the results of studies also show an inverse (i.e. positive) correlation between these two parameters (de Oliviera and Castellane, 1996). Other researchers do not dedicate much significance to these correlations (Tsumaki et al., 1989; Zeier and Wright, 1995).

Cabbage is the most widely grown vegetable in Slovenia and onion thrips (*Thrips tabaci* Lindeman) is one of the more important pests that decrease the quality of early white cabbage. The species is often difficult to detect because of its small size and hiding behaviour. By the time we detect the damage it has made it is already irreversible. Often even the use of insecticides is not sufficient to minimize the damage (Stoner and Shelton, 1988a). For these reason we wanted to establish the role of the epicuticular wax content in five varieties of early white cabbage in the context of its resistance to the attack of the onion thrips. Namely, in the previous research we established quite a difference in the susceptibility of thrips to attack of previously mentioned varieties (Trdan and Žnidarčič, 2003).

Material and Methods

Field experiment

An onion thrips' damage to early white cabbage (*Brassica oleracea* L. var. *capitata*) was observed during the 2001 and 2002 growing seasons, at the Experimental Field (46° 04' N, 14° 31' W, 300 m above sea level) of the Biotechnical Faculty in Ljubljana, Slovenia.

The soil was a heavy clay loam with 2.6 organic matter and a pH of 6.8. The climate at the site is a typical temperate continental climate.

The seedlings' breeding was described elsewhere (Trdan and Žnidarčič, 2003).

On both years the seedlings were transplanted to the field between May 01 and May 10, depending on the length of the growing period. The trials included three different plant densities (8.2, 10.8 and 16.6 plants m^{-2}), but only the plants from upper and lower plant densities from 2002 growing season were taken into account when epicuticular wax content was measured. The spacing was 30 cm between the rows and 20, 30 and 40 cm within the rows. The experimental unit (main plot) consisted of a raised bed 8.2 m long with three rows per bed. The plants in the outside rows and the end plants were guards, only the centre row was used for data collection.

The experiments were designed as factorial complete randomised blocks. In each of four blocks a combination of variety and plant density was replicated three times. There were varieties of early white cabbage that are grown commercially for fresh market in Slovenia: 'Vestri' (72 days, Royal Sluis), 'Parel' (61 days, Beyo Zaden), 'Delphi' (58 days, Royal Sluis), 'Destiny' (73 days, Beyo Zaden), and 'Hermes' (60 days, Royal Sluis).

The plants were single harvested (July 10, 2001, and July 08, 2002) when the majority of the heads reached maturity (initiation of the first head spliting). The percent of damaged leaf surface on the first 19 exterior leaves of the head caused by the feeding of onion thrips, the weight and the net weight of the heads were evaluated on eight heads per experimental unit immediately after harvest.

The frame leaves of the maturity cabbage were removed. The leaves were peeled off the head entirely free of frame leaves until four consecutive leaves showed no damage (Fail and Pénzes, 2001) and each leaf was visually inspected for the presence of thrips' damage. Evaluation of damage on the leaves was made using a slightly modified version of the Stoner and Shelton (1988b) method, because additionally the interval between 26 and 50% of damaged leaf surface was taken into consideration. The leaves were qualitatively assessed on a scale from 1 (no damage) up to 6 (severe damage) *(Table 1).* The mean harvest loss due to onion thrips' damage to the exterior leaves of the head was calculated as the difference between the mean weight and the mean net weight of the head. In percentage form this calculation was determined through the use of the following formula: (100–[MNW/MW]×100).

Table 1

A six-degree damage rating scale used for classification of damaged leaves caused by *Thrips tabaci* Lindeman according to % of damaged leaf surface

Scale (index of damage)	% of damaged leaf surface
1	0
2	< 1
3	1–10
4	11–25
5	26–50
6	> 50

The data on the weight of the head (without frame leaves), net weight of the head (without precedingly removed damaged leaves), and index of damage to the first 19 damaged exterior leaves of the head of 5 different early white cabbage varieties grown on two different plant densities (variety, plant density, no. of damaged exterior leaf of the head, block, and repetition was used as independent variables) were analysed using a general analysis of variance (ANOVA), and means were separated by Student–Newman–Keuls's multiple range test at $^{*}P<0.05$. Correlation between epicuticular wax content in the leaves of early white cabbage and damage caused by *Thrips tabaci* Lindeman was conducted by using SPSS 11.0 programme.

Laboratory assessment of the firmness of early white cabbage heads

Eight cabbage heads per replicate (an individual variety on fixed plant density) were used for evaluating their firmness. The firmness of the heads was measured with the digital penetrometer (Chattilon DFG-50, USA) that presses orthogonally into the cabbage head with a blade steel moving at constant speed of 0.95 mm s^{-1}. The head firmness is defined as penetration stress (kg cm^{-2}). In the individual head, this parameter was measured four times, each time from a different side of the cabbage head.

Laboratory assessment of epicuticular wax content in the leaves of early white cabbage

The procedure of O'Neal et al. (2002) was modified for measuring the surface area of early white cabbage leaves. An individual leaf area was measured with an Acer ScanPrisa 1240 UT desktop scanner. The first ten leaves from each of five cultivars and from both plant densities of cabbage cut of their base were scanned with MiraScan (version 3.43, images scanned at 150 dpi) software. In general, four replicates were used for each leaf. The individual leaf was placed on the scanner and the image was converted to greyscale. The digital image was saved as a TIFF file. The image was taken on the leaf then was performed using a graphics (Adobe Photoshop 6.0) to determine the pixel count. The leaf area was calculated by transferring pixels to standard units (centimetres). By using this technique we were able to express quantitative levels of epicuticular wax on the basis of leaf area.

The epicuticular wax is usually removed from the upper surface of the leaves by immersing the leaves in organic solvent (Bodnaryk, 1992; Pilon et. al, 1999). When scan was done the same leaves were sampled for extraction. Two by two leaves were dipped for approximately 1 min into 40 ml n-hexane. The two leaves were combined and extracted together because the values of wax were very low. During the extraction the leaf was gently agitated and moved around in the conical flask to optimise the physical contact between the plant tissue and the solvent. Subsequently, the hexane extract was transferred to pre-weighed Petri dishes. The extract was evaporated overnight in a fume hood to dryness at 30–40 °C. Analytical balance was used to measure weights of Petri dishes after the hexane portion was allowed to evaporate. The epicuticular wax was weighed and expressed as µg cm^{-2} leaf area. This procedure was repeated four times for each couple of leaves.

Results

Variety 'Vestri' – one of two varieties with the longest growth period – showed the highest mean weight of the heads as well as the highest mean net weight of the heads. Both values were statistically significantly different from adequate values with other four varieties. Mean yield losses due to removal of the damaged leaves was 13.9 to 26.1%. The highest yield loss was with variety 'Delphi', which has the shortest growth period and the lowest with variety 'Vestri'. The mean index of damage was the lowest with variety 'Vestri' and the highest with variety 'Parel' *(Table 2).*

Table 2

Mean weight and mean net weight of the heads, mean yield losses caused by damage of onion thrips (*Thrips tabaci* Lindeman) on exterior leaves of the heads, and mean index of damage of 19 exterior leaves of the head, in five different varieties of early white cabbage in 2002 (all three plant densities are taken into consideration)

Variety	Mean weight (g) of the heads (MW)	Mean net weight (g) of the heads (MNW)	Mean yield losses (100–[MNW/MW]× 100) in %	Mean index of damage on head's leaves
Delphi	1137.7 ab	840.0 b	26.1	1.49 b
Destiny	985.6 b	733.1 b	25.6	1.53 b
Hermes	1067.4 b	846.3 b	20.7	1.51 b
Parel	1088.4 b	843.0 b	22.5	1.87 c
Vestri	1220.0 a	1050.6 a	13.9	1.18 a

Data are analysed using a general analysis of variance (ANOVA). Means within a column followed by the same letter are not significantly different from each other based on Student–Newman–Keuls multiple range test (* $P < 0.05$).

Mean weight of the heads and mean net weight of the heads were approximately half smaller at higher plant density (30 × 20 cm) than they were at lower plant density (30 × 40 cm). Mean yield loss as well as mean index of damage showed higher values in the plants that were grown in a lower plant density *(Table 3).*

Table 3

Mean weight and mean net weight of the heads, mean yield losses caused by damage of onion thrips (*Thrips tabaci* Lindeman) on exterior leaves of the heads, and mean index of damage of 19 exterior leaves of the head, in two different plant densities of early white cabbage in 2002 (all five varieties are taken into consideration)

Density (plant m^{-2})	Mean weight (g) of the heads (MW)	Mean net weight (g) of the heads (MNW)	Mean yield losses (100–[MNW/MW]× 100) in %	Mean index of damage on head's leaves
16.6	696.9 b	578.9 b	17.9	1.33 a
8.2	1567.6 a	1183.9 a	24.5	1.73 b

Data are analysed using a general analysis of variance (ANOVA). Means within a column followed by the same letter are not significantly different from each other based on Student–Newman–Keuls multiple range test (* $P < 0.05$).

Varieties with the longest growth period (i.e. 'Vestri' and 'Destiny') had the firmest heads while the heads were substantially less firm with varieties with shorter growth period *(Fig. 1).* Yield, calculated per area unit (m^2), does not show any statistically significant differences between varieties, with the exception on variety 'Destiny', when different plant densities were compared *(Fig. 2).*

Fig. 1. Mean firmness of the heads of five varieties of early white cabbage at three different plant densities in 2002. Error bars represent the standard error of the means for each datum, analyzed separately with analysis of variance. Means based on three replicates of eight plants. Means within a column followed by the same letter are not significantly different from each other based on Student–Newman–Keuls multiple range test ($^*P<0.05$)

The mean index of damage was lower at higher plant density. The varieties 'Parel' – the variety with short growth period – showed the highest index of damage in both plant densities. On the other side, the lowest index of damage was ascertained with variety 'Vestri', which has longer growth period. The differences among mean damage indexes of different varieties are considerably smaller at higher plant density *(Figs 3* and *4).*

The content of the epicuticular wax in the leaves differs among five varieties tested, but with the most varieties the content is the lowest between the third and the sixth exterior leaf in the heads. The value of this potential parameter of plant resistance against phytophagous insects is the highest in the first two exterior leaves and grows from the sixth exterior leaf of the head towards inside of the head. The content of epicuticular wax was substantially higher in the exterior leaves of plants that were grown at higher plant density *(Figs 5–8).*

Pearson coefficient of correlation differs between different plant densities when epicuticular wax content in the leaves of early white cabbage and index of damage caused by *Thrips tabaci* Lindeman were compared. In a higher plant density (16.6 plants/m^2) the

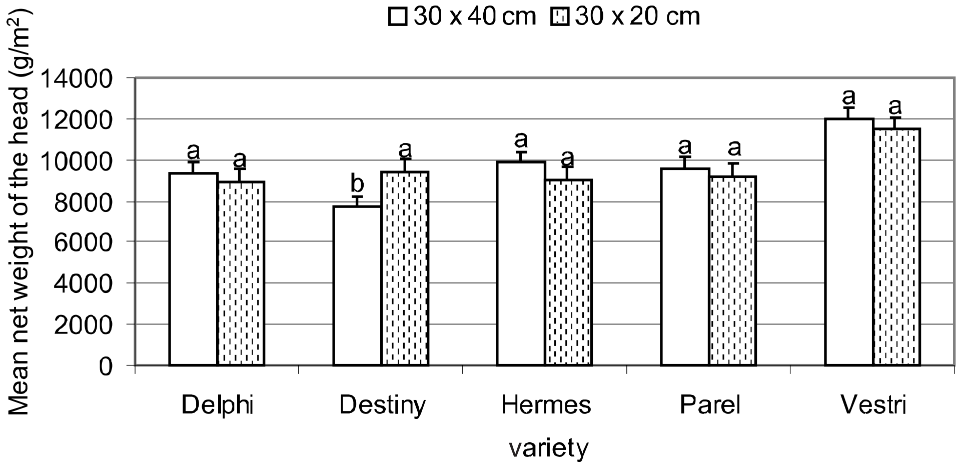

Fig. 2. Mean net weight of the heads (g/m^2) of five varieties of early white cabbage at three different plant densities in 2002. Error bars represent the standard error of the means for each datum, analyzed separately with analysis of variance. Means based on three replicates of eight plants. Means within a column followed by the same letter are not significantly different from each other based on Student–Newman–Keuls multiple range test (*P<0.05)

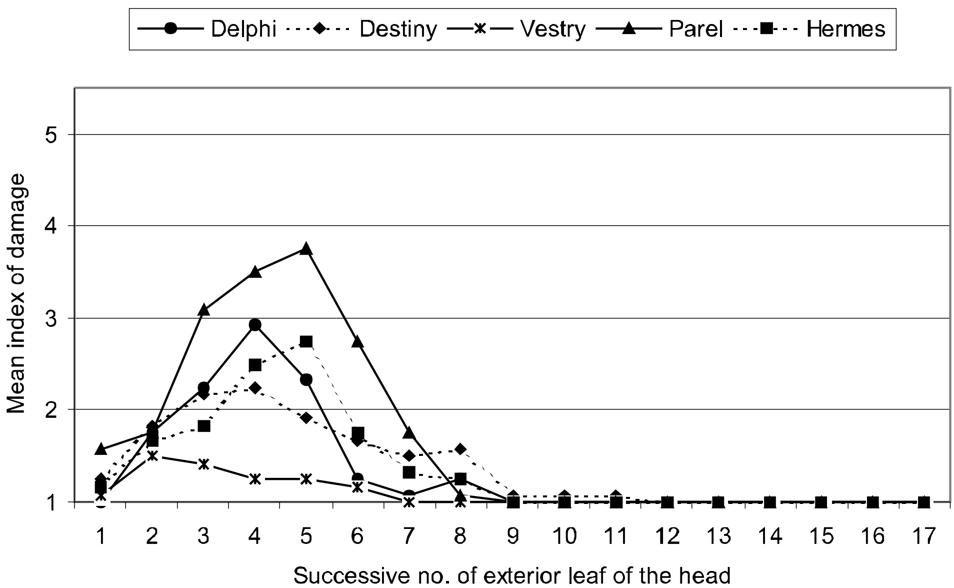

Fig. 3. Damage caused by the onion thrips (*Thrips tabaci* Lindeman) on five varieties of early white cabbage grown in a plant density of 30 × 20 cm (2002)

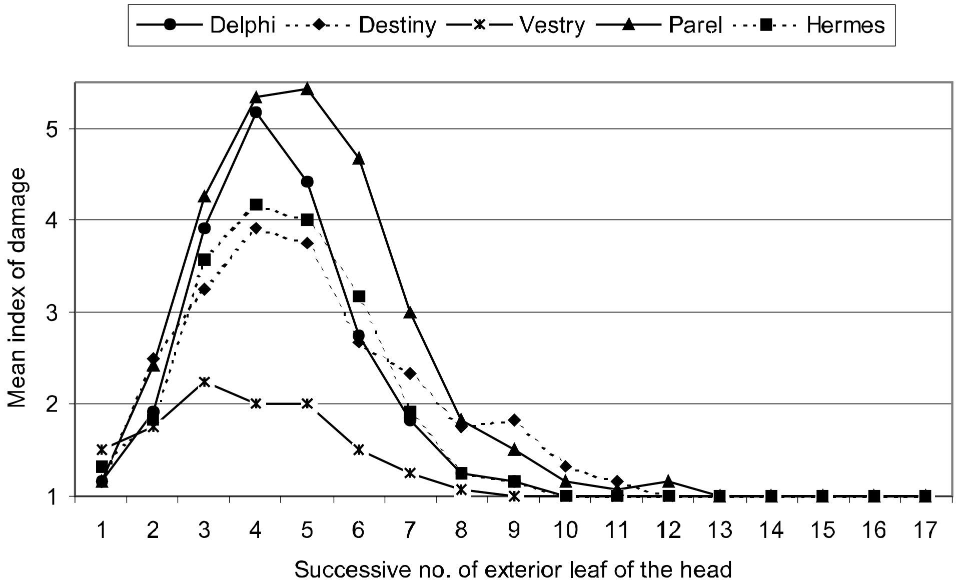

Fig. 4. Damage caused by the onion thrips *(Thrips tabaci* Lindeman) to five varieties of early white cabbage grown in a plant density of 30 × 40 cm (2002)

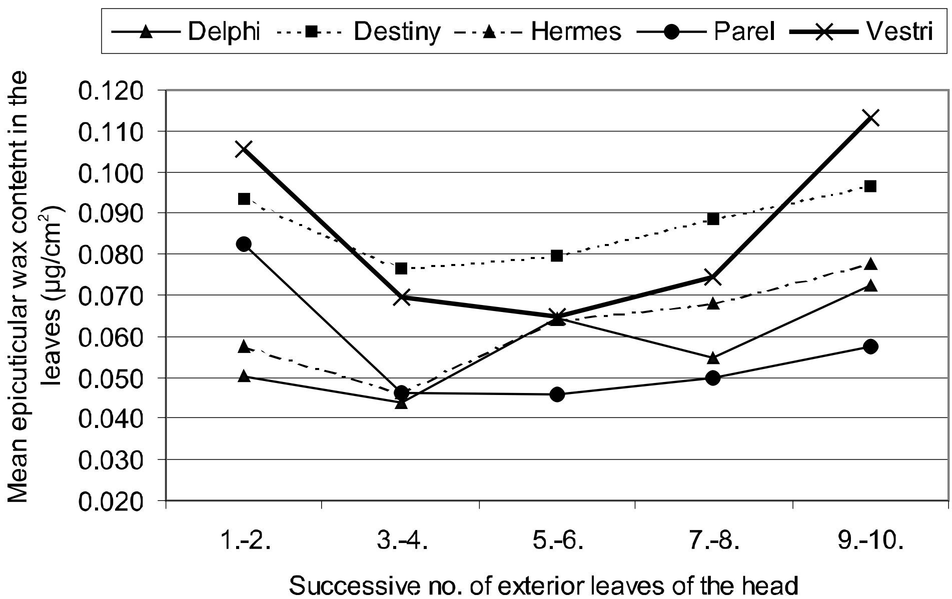

Fig. 5. Mean epicuticular wax content in the exterior leaves of early white cabbage heads in a plant density of 30 × 20 cm considering all five varieties

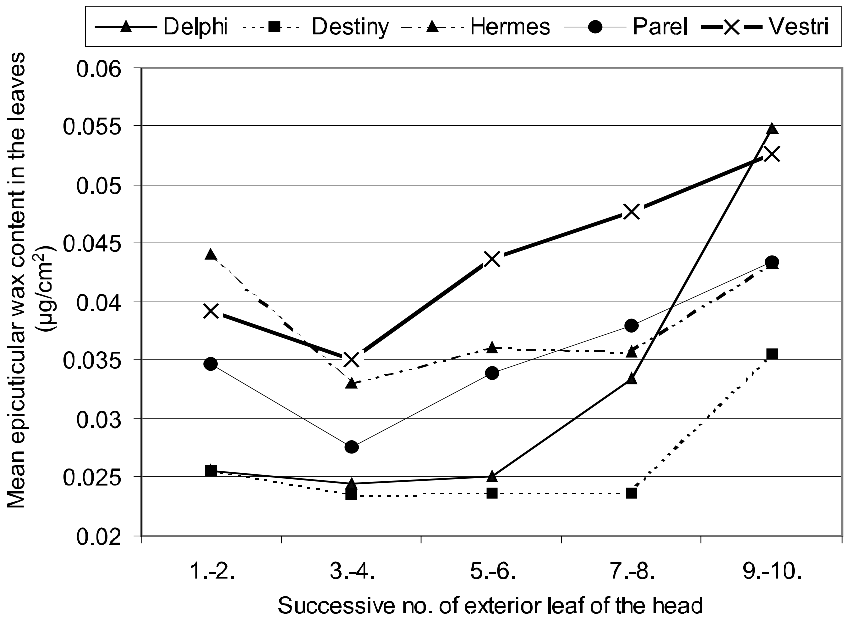

Fig. 6. Mean epicuticular wax content in the exterior leaves of early white cabbage heads in a plant density of 30 × 40 cm considering all five varieties

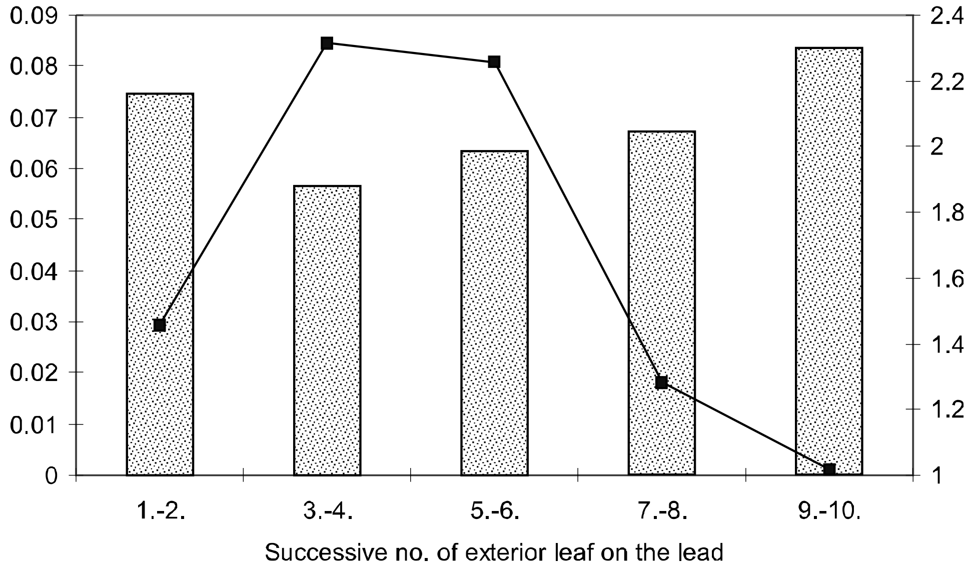

Fig. 7. Mean epicuticular wax content (primary y-axis [µg/cm^2]) and mean index of damage (secondary y-axis) of exterior leaves of early white cabbage grown in a plant density of 30 × 20 cm

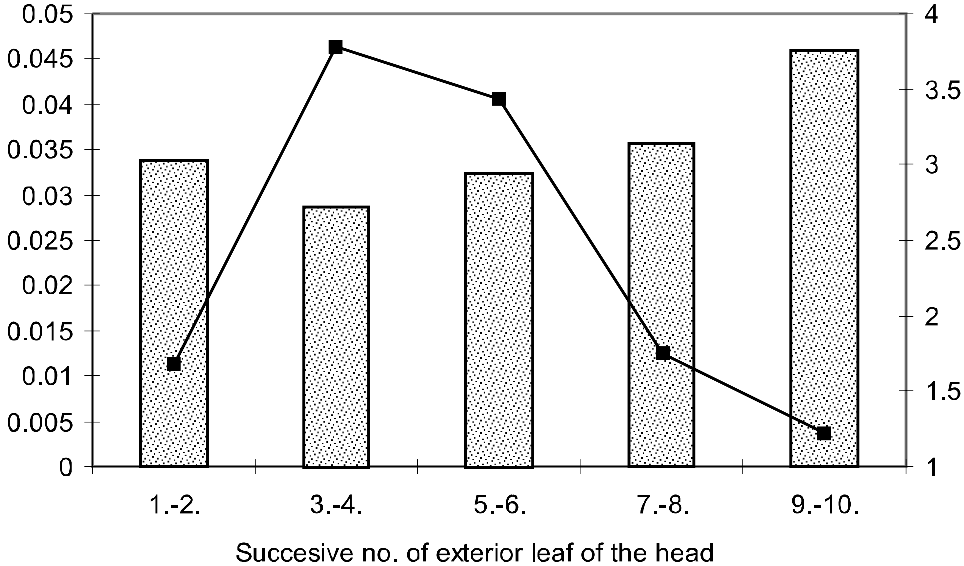

Fig. 8. Mean epicuticular wax content (primary y-axis [μg/cm²]) and mean index of damage (secondary y-axis) of exterior leaves of early white cabbage grown in a plant density of 30 × 40 cm

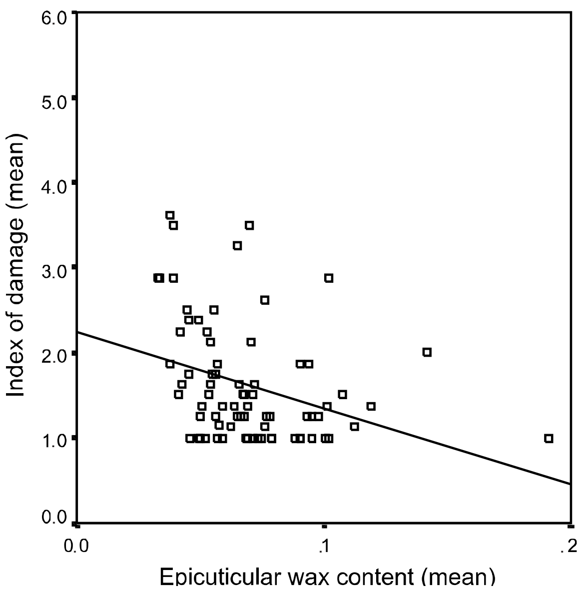

Fig. 9. The correlation between mean epicuticular wax content (μg/cm²) and mean index of damage on first ten exterior leaves of the heads grown in a plant density of 30 × 20 cm

mentioned coefficient was –0.436, while its value in a lower plant density (8.2 plants/m^2) was –0.338. That means that between epicuticular wax content and index of damage in the leaves of early white cabbage hardly perceived negative correlation occur. Regarding these results it can be stated that only 11 to 19% of damage on the early white cabbage leaves caused by feeding of *Thrips tabaci* Lindeman is dependent on the content of epicuticular wax *(Figs 9* and *10).*

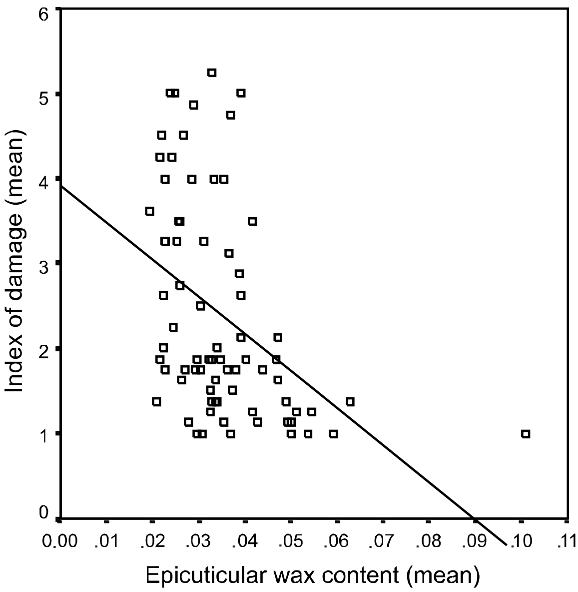

Fig. 10. The correlation between mean epicuticular wax content (µg/cm^2) and mean index of damage on first ten exterior leaves of the heads grown in a plant density of 30 × 40 cm

Discussion

By analyzing the results of the five varieties of early white cabbage grown in two different plant densities, we ascertain that plant density has an important influence on the mean yield of the heads. However, considering the mean yield of the heads calculated per area unit, the mean yield among different densities shows hardly any difference. The variety with the longest growth period and the firmest head (i.e. 'Vestri') achieved the highest yield, and was also least susceptible to onion thrips' (*Thrips tabaci* Lindeman) attack. Variety 'Destiny', which has a similar length of the growth period to the variety 'Vestri' does not show a statistically significant difference in the mean index of damage of the exterior leaves when compared to the two varieties with shorter growth periods (i.e. Hermes and Delphi). We conclude therefore that some other factors influence the resistance of this variety on this pest.

We consider the epicuticular wax content on the leaf surface as one of the more important parameters of the resistance to pests, even though the results of some studies do not support this hypothesis. The results of our research with hexan extraction of epicuticular wax show the smallest mean content in the third and the fourth exterior leaf of the head, while its content in the first ten exterior leaves is the highest, respectively, in the first and the second, and the ninth and the tenth exterior leaf. According to the charts *(Figs 7–8)* we anticipated highly negative correlation between mean wax content and the mean index of damage on the leaves, however we established hardly perceived negative correlation between the epicuticular wax content in the leaves and index of damage caused by feeding of onion thrips (Pearson coeficient of correlation from –0.436 in a lower plant density up to –0.338 in a higher plant density). That means that 11 up to 19% of damage on the early white cabbage leaves is dependend on their epicuticular wax content.

Therefore we conclude that epicuticular wax content in the exterior leaves of the head of early white cabbage is not one of the most important parameters in the resistance of this vegetable to onion thrips (*Thrips tabaci* Lindeman) attack. Together with other parameters (duration of the growth period and/or the coincidence of the sensitive developmental stage and mass occurrence of the pest) it certainly plays an important role in preventing the damage which these and other harmful insects create.

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