

## EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF *Thrips nigropilosus* Uzel (Thysanoptera: Thripidae) ON *Mentha × piperita* L. AND THE IMPACT OF PEST ON THE HOST PLANT

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### ABSTRACT

*Thrips nigropilosus* Uzel is a polyphagous species occurring mainly in temperate climates. Its life cycle depends on photoperiodic and temperature conditions. *T. nigropilosus* feeds on different plant species, but it is considered one of the most serious pests of pyrethrum plants causing serious economic problems. However, several additional agricultural host plants have been affected by *T. nigropilosus*, including spearmint, cucumber, and lettuce, indicating that this insect can significantly widen its habitats and occurs especially frequently in greenhouses. We report that *T. nigropilosus* massively attacked *Mentha × piperita* L. cultivated in greenhouses in central Poland and destroyed the entire mint crops within a short time. The study provided insight into the harmful effect of the thrips and showed that the length of the thrips developmental cycle was reduced with temperature increases from 18 to 26.6°C. The lower threshold temperatures were 13.7, 10.2, 5.0, and 10.1 for eggs, larvae, pupae, and total development, respectively, and the thermal constant for the same developmental stages was 65.9, 90, 132.5, and 284.9-degree days. Both parameters were estimated by linear regression analysis. During our experiment, *T. nigropilosus* developed by thelytokous parthenogenesis. The morphological and anatomical changes in damaged plants were associated with the fact that the insect began feeding on the lower lamina surface close to the leaf midribs, but no damage to vascular bundles and glandular cells was observed.

**Key words:** chrysanthemum thrips, feeding, life cycle, *Mentha × piperita*, morphology, mint anatomy

### INTRODUCTION

*Thrips nigropilosus* Uzel (common name: chrysanthemum or pyrethrum thrips) has European origin, but now it is widespread around the world [Nakahara 1994, Mound 2010]. It was first described from Bohemia in 1895 [Uzel 1895]. *T. nigropilosus* is a poly-

phagous species occurring mainly in temperate climates. It feeds more frequently on leaves than on flowers of different plant species; however, it prefers plants from the *Asteraceae* family. So far, there have been no reports of its harmful effects in areas, in which thrips

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naturally occur, but there are reports showing its deleterious influence in areas, where it was accidentally introduced in agriculture [Bullock 1963, Sengonca et al. 2006, Nelson et al. 2011, Parsons and Munkvold 2010, Reitz et al. 2011]. *T. nigropilosus* is considered one of the most serious pests of pyrethrum (*Chrysanthemum* sp.) causing serious economic problems [Lewis 1973, Mound 2010, Sartiami and Mound 2013, Wandahawa et al. 1996]. It was shown that *T. nigropilosus* fed more intensively on mature than young leaves of pyrethrum, significantly reducing the photosynthetic capacity and plant vigor and finally lowering productivity [Bullock 1965]. The first report about its detrimental influence on agriculture was made in Kenya, showing mass occurrence of thrips on *Tanacetum cinerariifolium* (Trevir) [Glynne and Sylvester 1966]. Also, Ganaha-Kikumura et al. [2012] have found that *T. nigropilosus* is a major pest of *Chrysanthemum morifolium* Ramat. in Okinawa. Chrysanthemum thrips has also been reported as a pest of *C. morifolium* in the USA and the UK [Stannard 1968, Mound et al. 1976]. However, it was reported that several additional agricultural host plants were affected by chrysanthemum thrips, including spearmint, cucumber, and lettuce, indicating that this insect can significantly widen its habitats and occur frequently in glasshouses [Sakimura 1939, Morison 1957]. In line with this finding, an interesting observation was made in New Zealand. This thrips species was found on the underside of tomentose leaves of *Cirsium vulgare* (Savi.) Ten. and *Verbascum thapsus* L. [Walker and Michaux 1989], underscoring the fact that thrips have potential to expand its environmental niche; however, the cause of such behavior has never been explored.

The life cycle of *T. nigropilosus* depends on photoperiodic and temperature conditions. The species can develop macropterous or brachypterous forms [Nakao 1994a]. Two reproductive modes are known in this species: arrhenotoky with sexual reproduction, where diploid females and haploid males coexist, and thelytoky with asexual reproduction, where only diploid females are present in the population [Nakao and Yabu 1998, Li et al. 2014]. Moreover, as in other insects, temperature has a significant influence on the length of the thrips life cycle [Anyango 1992, Murai 2000, Stacey and Fellowes 2002, Régnière et al. 2012, Padmavathi et al. 2013]. It has also been found that

the sex ratio in thrips is influenced by temperature and is 1 : 1 (males : females) at moderate temperature. In higher temperature areas, there are fewer males, and the male : female ratio is 1 : 10 [Morison 1957]. Additionally, the highest mean fecundity was noted at 30°C, with approx. 42 eggs per female, and the so-called intrinsic rate of a natural increase was the highest at 30°C [Ganaha-Kikumura and Kijima 2016]. Thus, higher temperature, that stimulates female development and fecundity, has been proposed to cause parthenogenetic reproduction, which may quickly increase the number of thrips and enhance their harmful influence on agriculture, as shown on a pyrethrum field. At the same time, Anyango [1992] found in his experiment that the mortality of larval stages significantly increased at 30°C compared with 20°C, when it was the lowest.

This is particularly important given the tendencies towards a rise in the mean annual temperatures observed currently in the European climate [Bergant et al. 2005, Christensen and Christensen 2007, Tchórzewska et al. 2017].

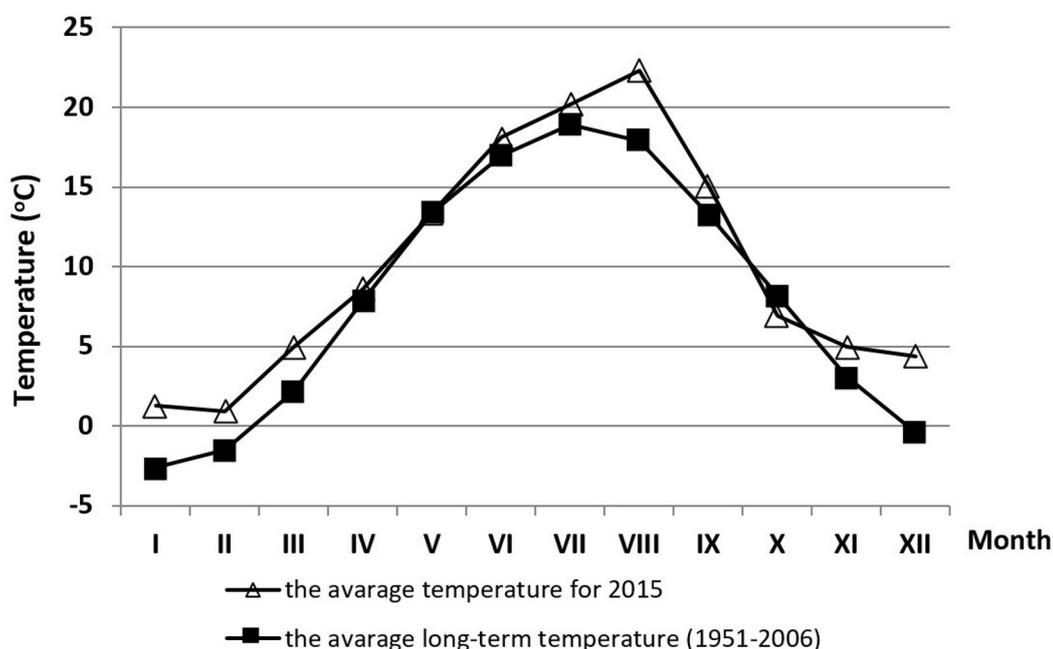
Thus, in the light of many reports, thrips belong to a class of pests that can quickly adapt to changing environmental conditions; especially its temperature-dependent parthenogenetic reproduction may cause severe agricultural problems.

In central Poland, the abnormally high temperature in the summer of 2015 exerted a strong influence on the behavior of insects; especially, it caused extinction of the green peach aphid (*Myzus persicae* Sulzer) and the hawthorn-mint aphid (*Ovatus crataegarius* (Walker)), i.e. the pests of mint occurring in glasshouse farms, leaving this uninhabited niche (Lubiarz M. pers. inf.). Immediately, *T. nigropilosus* massively infested *M. × piperita* cultivated in greenhouses, causing large agricultural losses. In the examined greenhouses, on average 35000 pots per month were usually produced, with marginal destruction. However, in the summer of 2015, the recorded losses exceeded 20000 pots in total, causing severe economic loss (producers pers. inf.).

Interestingly, the mean monthly air temperature in June, July, and August 2015 was by 1.1°C, 1.3°C, and 4.4°C higher, respectively, than the temperature noted during the last 55 years (Fig. 1, Tab. 1). These data show a steady rise in the mean air temperatures

**Table 1.** Meteorological data of monthly average temperatures in 2015 year and multiyear values (1951–2006) from the meteorological station in Legionowo near Warsaw (Institute of Meteorology and Water Management – National Research Institute, Warsaw 2016)

Month	Temperature (°C)	
	mean monthly 2015	mean monthly 1951–2006
I	1.3	–2.6
II	0.9	–1.5
III	5.0	2.2
IV	8.6	7.9
V	13.4	13.4
VI	18.1	17.0
VII	20.2	18.9
VIII	22.3	17.9
IX	15.1	13.3
X	6.9	8.2
XI	5.0	3.1
XII	4.4	–0.4



**Fig. 1.** Meteorological data for 2015 and the multi-year average (1951–2006) temperature (°C). Institute of Meteorology and Water Management – National Research Institute (IMGW), Podleśna 61, 01-673 Warsaw

in Poland as well as in Europe [Marsz 2005, Bitá and Gerats 2013]. Probably, these temperature fluctuations have contributed not only to the extinction of aphids, i.e. common mint pests, but also to the massive infestation of a new host *M. × piperita* by *T. nigropilosus*, which resulted in a destruction of significant part of mint crops in greenhouse cultivations.

*M. × piperita* (peppermint) is a hybrid plant between *Mentha aquatica* L. and *Mentha spicata* L. belonging to the *Labiatae* family [Murray et al. 1972]. Essential oils and other components contained in peppermint leaves have been used for centuries as a folk remedy and in alternative medical therapy. Besides, they are used in many cosmetics and food products [Iscan et al. 2002, McKay and Blumberg 2006, Singh et al. 2015]. Furthermore, essential oils present in peppermint leaves have insecticide activity [Kumar et al. 2011a] and are used as a protectant against mosquito *Aedes aegypti* [Kumar et al. 2011b] and a fumigant against storage pests *Tribolium castaneum* Herbst and *Callosobruchus maculatus* Fabricius [Ansari et al. 2000, Khani and Asghari 2012] and *Musca domestica* L. [Hanan 2013]. Thus, peppermint is a very important agricultural plant and all threats are a significant concern for mint growers. It should be underlined that the mass occurrence of *T. nigropilosus* and its harmful influence on *M. × piperita* in Europe has never been reported so far.

In this paper, we have studied the impact of temperature on the length of the life cycle of *T. nigropilosus* reared on *M. × piperita*, and we have conducted the first studies of anatomical and morphological changes in mint leaves induced by this insect feeding, providing an explanation for the harmful effect of the thrips.

## MATERIALS AND METHODS

**Rearing of thrips.** The specimens of *T. nigropilosus* originated from populations developed on *Mentha × piperita* crops cultivated in glasshouses in the vicinity of Warsaw (Poland, 52°11'21"N 21°04'53"E; 115.7 m a.s.l.) in 2015. The cultivation conditions in greenhouses allow mint to grow in a mild environment in summer (June–August) at a temperature about 22–25°C. In 2015, the average temperature in August was higher and reached 30°C and 20°C in

greenhouses during the day and night, respectively (producers pers. inf.).

Both the infested and non-infested plants were selected in the greenhouse and transported to a laboratory at the Department of Zoology, MCSU in Lublin for further research. Initially, plants with and without thrips were separately reared in phytotron chambers (KK 1200 TOP+FIT, produced by POL-EKO APARATURA) in laboratory conditions at 22°C, 16 h light : 8 h dark (LD 16 : 8) in 60% humidified atmosphere. For the main experiments, leaves of similar age and sizes were cut randomly from middle parts of mint plants free of thrips and their eggs, and were placed severally into plastic boxes (100 mm diameter and 50 mm height) with a few layers of wet paper towel at the bottom. This paper was a good substitute of a ground, where pupae develop in natural conditions. One newly developed *T. nigropilosus* female was put into every box. It was removed after a few days, when the number of laid eggs did not increase. The boxes were stored in climatic chambers (KK 115 TOP +, produced by POL-EKO APARATURA) at different temperatures: 18°C, 22°C, 26°C in LD 16 : 8 conditions and at 30°C during the day and 20°C at night with LD 16 : 8 (average daily temp. 26.66°C; 30/20°C in the following text and tables). In the latter case, the extreme conditions prevailing in the production glasshouses in 2015 were repeated. All experiments were performed in 20 replicates for every temperature. The observations were conducted every morning between 8.00 and 10.00 am under an Olympus SZX10 stereomicroscope at 10× and 20× magnification. If necessary, the paper towel was moistened with distilled water. Every day, the subsequent developmental stages (first and second larval instars, propupae, pupae, and finally adults) were calculated. If necessary, new fresh leaves free of thrips eggs and larvae were added every 10 days as a food for the larval stages. The breeding of insects lasted to the emergence of the next generation of females, and the experiment was continued for 20 females, which were transferred onto fresh leaves and reared in the same conditions in order to observe the morphological forms and reproduction type in the subsequent generations. Photographs of the life stages of *T. nigropilosus* were taken with a scanning microscope VEGA3 TESCAN (SEM HV: 30.00 kV) and eggs were photographed with Olympus ZX 40.

### Statistical analyses

A one-way ANOVA test was used to determine the statistical significance of the number of females from the second generation, the length of the total *T. nigropilosus* life cycle, and the length of the developmental stages depending on different temperatures; a P value less than 0.05 was considered statistically significant. The homogeneity of variance was confirmed with Levene's test [Brown and Forsythe 1974]. Post-Anova (Scheffe's test) was used, where the 'F' value showed significant differences at  $p < 0.05$  [Downe and Heath 1970]. The regression line was calculated ( $1/D = a + bT$ ) to estimate the relationship between the temperature (T) and the rate of development (1/D) of *T. nigropilosus*. The lower threshold temperatures for the eggs, larvae, and pupae were calculated when  $1/D = 0$ . Thermal constants, i.e. the day-degrees required to complete the life cycle, were determined using  $k = 1/b$  [Campbell et al. 1974, Padmavathi et al. 2013].

### Plant material

The research plants *Mentha × piperita* (peppermint) were obtained from the greenhouse mentioned above and cultivated in a phytotron chamber (KK 1200 TOP+FIT, produced by POL-EKO APARATURA) at the Department of Zoology, MCSU in Lublin. Peppermint leaves (of the same age) were placed in boxes with one specimen of adult thrips, and the thrips feeding symptoms were observed after 1, 2, and 5 days; healthy leaves were the control material. Macroscopic images of the leaves were taken with a Nikon D300 photographic camera equipped with a 60 mm AF MICRO NIKKOR lens.

**Morphological and anatomical analysis of mint leaves.** For anatomical analyses, the material with and without feeding symptoms was collected from 20 leaves of the plant mentioned above. Leaf fragments were fixed in AA fixative (100% acetic acid, 95% ethanol at 1 : 3, v : v), dehydrated in a series of ethanol concentrations of 30, 50, 70, 90, 96, and 100%, and embedded in paraffin wax with conventional methods [Gerlach 1972]. The 7- $\mu$ m-thick sections were cut on a rotation microtome MICROM HM340. Next, the sections were stained with safranin, which stains lignified fragments red, and with light green, which gives green staining to the cytoplasm and cellulose cell walls. The

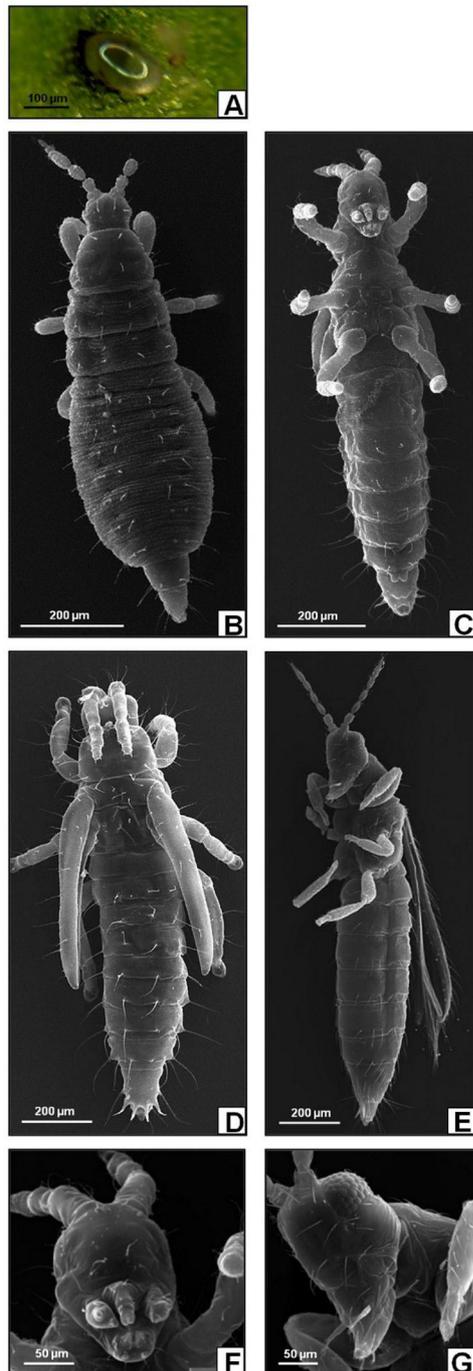
observations were carried out using a Nikon eclipse Ni light microscope (LM). Photographic documentation was made with a digital camera and NIS-Elements BP software.

Fragments of both healthy controls and leaves with foraging symptoms were collected for the analysis of the organ surface using a scanning electron microscope (SEM). The fragments of leaves were fixed in a 5 : 5 : 90 (v : v : v) mixture of glacial acetic acid : formalin (40%) : ethanol (70%) and dehydrated in a graded acetone series (40%, 70%, 80%, 100%). Subsequently, the whole investigated material was frozen in liquid nitrogen. The samples were analyzed under a scanning electron microscope (LEO1430VP) with an accelerating potential of 15 kV.

To visualize the cuticle on the epidermis of the mint leaves, hand-made cross-sections of healthy leaves were prepared. They were placed in a 0.02% auramine 0 solution in TRIS buffer for several minutes [Heslop-Harrison and Heslop-Harrison 1980]. After rinsing with distilled water, the slides were analyzed under a fluorescence microscope Nikon eclipse Ni-u (FM) at an excitation wavelength of 330–380 nm and an emission wavelength over 480 nm (UV). Photographic documentation was made with a digital camera and NIS-Elements BP software.

### RESULTS

**Life cycle of *T. nigropilosus*.** The observations of the life cycle of *T. nigropilosus* reared on *M. × piperita* plants were carried out in controlled conditions at constant temperatures of 18, 22, and, 26°C, and 30/20°C day/night (26.66 av. temp.). The initial analysis of the females' behavior after colonization of the mint leaves revealed that the insects began laying eggs on the upper surface of the leaf, evading glandular trichomes (Fig. 2A). Depending on the temperature, the first larval instar hatched 3.8 (mean value) days after oviposition at 30/20°C, and the hatching was slower at the lower temperature, i.e. after 11.7 (mean value) days at 18°C. The differences were statistically significant (Tab. 2). The first larva had a light white color of the body and this stage of development lasted the shortest time in the thrips life cycle. Molting and emergence of the second larval instar (Tab. 2, Fig. 2B) was accomplished after 1.3 days at 30/20°C and 4.1 (mean value)



**Fig. 2.** Developmental stages of *Thrips nigropilosus*: A – egg on the leaf; B – second instar of larva, dorsal side; C – propupa, ventral side; D – pupa, dorsal side; E – female, ventro-lateral side; F – head with a mouth cone of a propupa, ventral side; G – head with a mouth cone of a female, lateral side

days at 18°C. Hatching the first-instar larvae migrated to the lower side of the lamina, where they fed together with successively emerging second-instar larvae. The second larval instar lasted 3.1 days at 30/20°C, and 5.5 (mean value) days at 18°C (statistically significant differences) (Tab. 3). Body of the second instar of larva was yellow, with a green color of the gut, and the intestines were visible through the body. The larvae together with the female (until the moment of removal thereof after oviposition) fed on the bottom side of the leaf, causing severe damage. After 3.1 and 5.5 days at 30/20°C and 18°C, respectively (statistically significant differences), the larvae pupated under the dry and destroyed leaves and in some cases propupae and pupae (Fig. 2C, D) formed clusters of a few individuals between layers of paper towel at the bottom of the cages. Both pupal stages did not eat and move, and their bodies were whitish. Compared with adults and larvae, the parts of their mouth cone were reduced (Fig. 2F, G). The dorsal side of the 9<sup>th</sup> abdominal segment in both stages was equipped with long thorns (Fig. 2C, D). The length of both pupal stages (propupa and pupa) lasted 5.3 and 9.9 (mean value) days at 30/20°C and 18°C, respectively (statistically significant differences) (Tab. 2). After that time, females of the second generation appeared (Fig. 2E).

Detailed analysis of the *T. nigropilosus* life cycle demonstrated that the total length of the development time from egg to adult significantly decreased with the increasing temperature and lasted 31.2, 26.3, 20.2, and 13.5 (mean value) days at 18°C, 22°C, 26°C, and 30/20°C, respectively (statistically significant differences) (Tab. 2). The greatest number of second-generation females achieved sexual maturity at a temperature of 26°C (mean 18.1 ± 5.28); this number was substantially lower at 22°C (mean 13.6 ± 4.7; Scheffe's test:  $P = 0.0253$ ) and at 18°C (mean 11.8 ± 2.17; Scheffe's test:  $P = 0.0009$ ). The mean number of second-generation females developing at 30/20°C was 16.0 ± 4.3, but the difference between this number and that of mature females that developed at 26 and 22°C was not statistically significant (Scheffe's test:  $P = 0.439$  and  $P = 0.325$ , respectively) (Tab. 3). All numerical data are presented in Tables 2 and 3.

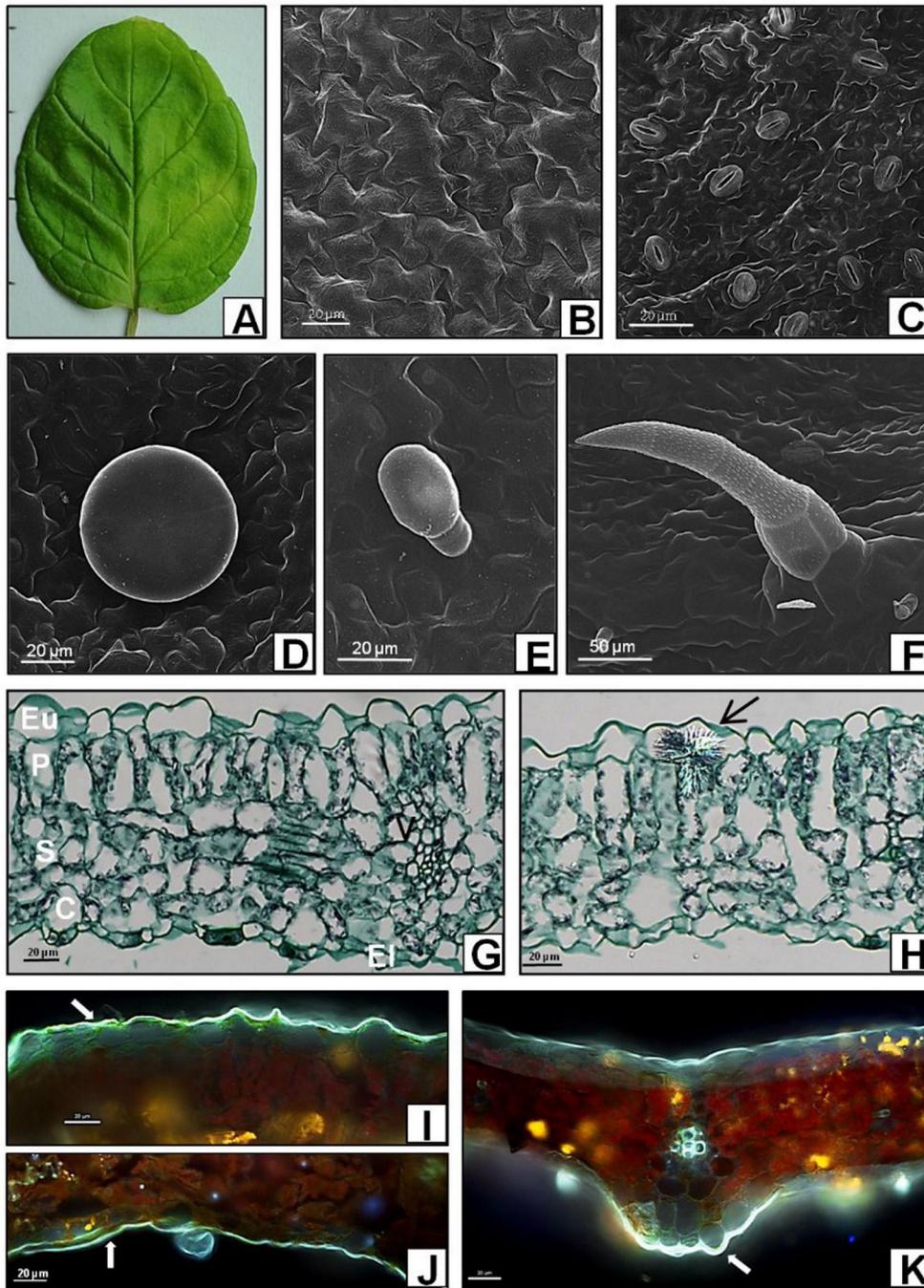
Comparison of the length of the developmental stages of *T. nigropilosus* at four different temperatures facilitates calculation of lower threshold temperature

**Table 2.** Duration of the developmental stages of *T. nigropilosus* reared in different temperature conditions (mean ± SD) and number of adults of the second generation

Temperature (°C)	Stage					Total	Number of adults
	egg	first-instar larva	second-instar larva	propupa	pupa		
18	11,7 ±2,04	4,1 ±0,75	5,5 ±0,87	5,9 ±0,97	4,0 ±0,71	31,2 ±2,04	11,8 ±2,17
22	9,4 ±0,92	3,7 ±0,77	5,1 ±0,80	3,3 ±0,91	5,0 ±0,84	26,3 ±1,33	13,6 ±4,67
26	6,5 ±0,87	2,6 ±0,87	4,2 ±0,66	2,6 ±0,49	4,3 ±0,69	20,2 ±1,35	18,1 ±5,28
30/20	3,8 ±0,81	1,3 ±0,47	3,1 ±1,03	2,2 ±0,53	3,1 ±0,60	13,5 ±1,46	16,0 ±4,32

**Table 3.** Statistical significance of the developmental duration of *T. nigropilosus* and number of adults at different temperatures (constant 18, 22, 26°C and variable 30/20°C, D/N): one-way ANOVA test (F) and post-hoc Scheffe's test (\*\*\*)  $p < 0,00001$ ; \*\*  $0,001 > p > 0,00001$ ; \*  $0,05 > p > 0,001$ ; ns – non significant)

Stage	One-way ANOVA F	Scheffe's test			
		18	22	26	30/20
Egg	147,68 ***	18	***	***	***
		22	***	***	***
		26	***	***	***
		30/20	***	***	***
First-instar larva	49,42 ***	18	ns	***	***
		22	ns	**	***
		26	***	**	**
		30/20	***	***	**
Second-instar larva	26,63 ***	18	ns	*	***
		22	ns	ns	***
		26	*	ns	*
		30/20	***	***	*
Propupa	23,21 ***	18	*	***	***
		22	*	*	**
		26	***	*	ns
		30/20	***	**	ns
Pupa	38,99 ***	18	*	***	***
		22	*	ns	***
		26	***	ns	**
		30/20	***	***	**
Total	408,04 ***	18	***	***	***
		22	***	***	***
		26	***	***	***
		30/20	***	***	***
Number of adults	7,21 **	18	ns	**	*
		22	ns	*	ns
		26	**	*	ns
		30/20	*	ns	ns



**Fig. 3.** *Mentha × piperita* leaf (control): A – macroscopic view; B – epidermis on the upper side of the leaf blade (SEM); C – epidermis on the lower side of the leaf blade (SEM); D–F – trichomes (SEM); G–K – cross-section through the leaf: G – upper epidermis (Eu), palisade mesophyll (P), spongy mesophyll (S), vascular bundle (V), air chambers (C), bottom epidermis (Eb); H – calcium oxalate crystal (arrow) (LM); I – cuticle layer on the upper epidermis; J – cuticle layer on the lower epidermis; K – cuticle on the leaf midrib (FM, arrows)

**Table 4.** Regression equations, lower thresholds, and thermal constants for *T. nigropilosus* reared on *Mentha × piperita*

Stage	Regression equation	R <sup>2</sup>	P	Lower threshold (°C)	Thermal constant (k) (day-degrees)
Egg	$y = -0,208 + 0,015x$	0,752	<0,00001	13,7	65,9
Larvae	$y = -0,113 + 0,011x$	0,625	<0,00001	10,2	90,0
Pupae	$y = -0,038 + 0,008x$	0,647	<0,00001	5,0	132,5
Total	$y = -0,036 + 0,004x$	0,841	<0,00001	10,1	284,9

and thermal constant (day-degrees) values. The lower threshold temperatures were 13.7, 10.2, 5.0, and 10.1 C for eggs, larvae, pupae, and total development, respectively, and the thermal constant for the same developmental stages was 65.9, 90, 132.5, and 284.9 degree days. We observed a negative relationship between both these data for the eggs, larvae, pupae, and egg-to-adult period (Tab. 4).

*T. nigropilosus* feeding on the mint plants in laboratory conditions reproduced *via* thelytokous parthenogenesis. Interestingly, macropterous females dominated at all the examined temperatures. However, in the second generation of females reared at 30/20°C, some specimens with curled wings were observed, and there were no apterous forms.

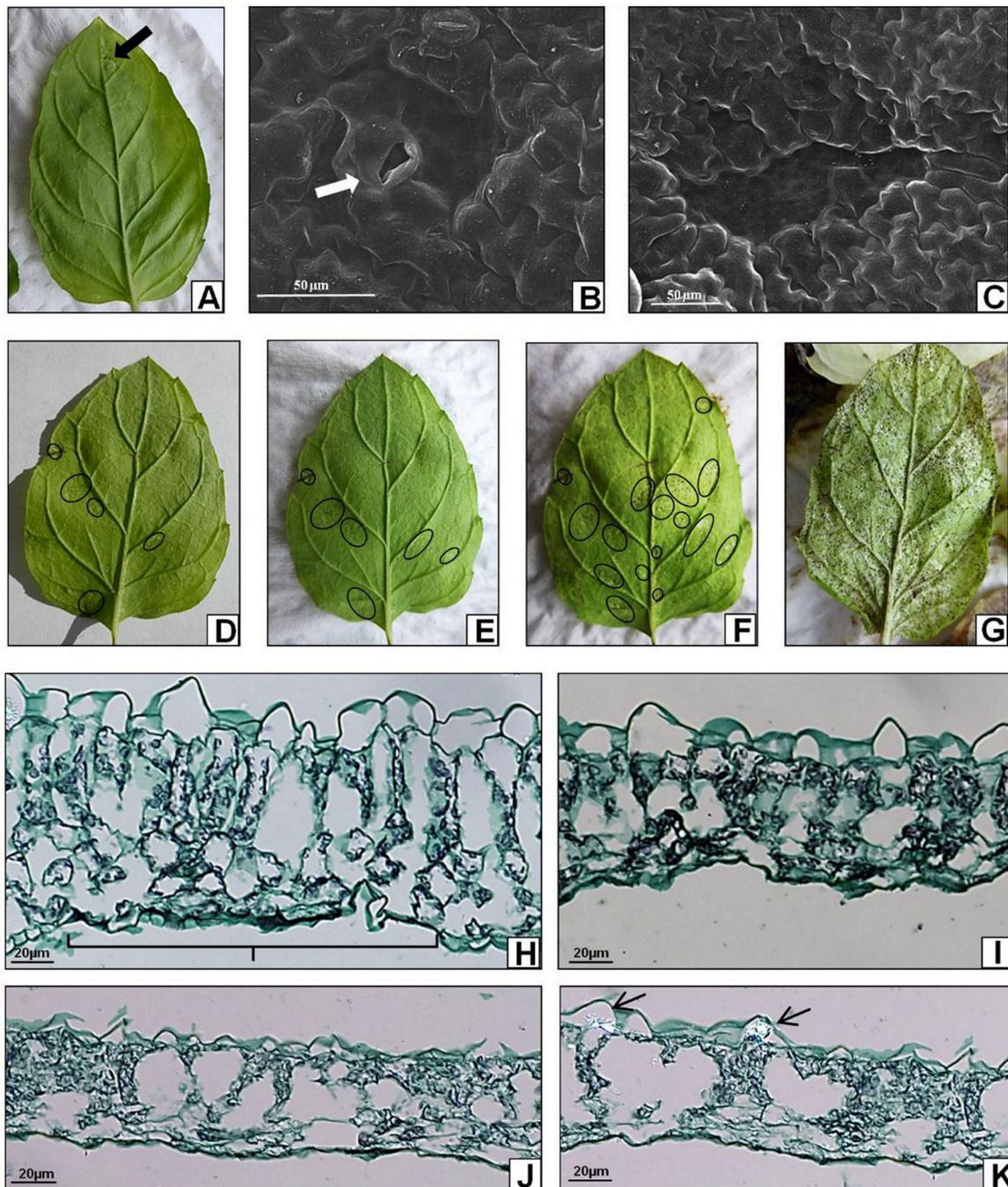
#### **Morphology and anatomy of *M. × piperita* leaves.**

First, the morphology and anatomy of *M. × piperita* assimilative leaves without foraging symptoms, i.e. the control material, were analyzed. The leaf blade of the analyzed plants has an oval shape with a rounded base, a sharply and unevenly serrate margin, and a pointed apex. It has pinnate venation; the veins on the upper side of the blade are concave and those on the lower part are convex (Fig. 3A). The analysis of the morphology of mint leaf epidermis consisted in SEM observations. Epidermal cells viewed from above have a wavy outline and closely adhere to each other, which is typical for this tissue (Fig. 3B). The lower side of the lamina bears numerous diacytic stomata (Fig. 3C). There are three types of trichomes, i.e. epidermal formations on both the upper and lower epidermis of the mint leaves. One type is represented by multicellular *Labiatae*-type glandular trichomes composed of a stalk and a head and located in characteristic leaf blade cavities (Fig. 3D). Additionally, there are capitate trichomes with a unicellular head

(Fig. 3E) and sharply pointed mechanical trichomes (Fig. 3F). Cross-sections of the mint leaves demonstrated the anatomical structure of these organs. They are bifacial and have single-layered epidermis (Fig. 3G – Eu and Eb) with a single row of palisade parenchyma composed of elongated, closely adherent cells (Fig. 3G – P). The spongy parenchyma forms several (4–6) layers of oval, loosely arranged cells (Fig. 3G – S). Vascular bundles are located at the border between the palisade and spongy parenchyma (Fig. 3G – V). The large air chambers (Fig. 3G – C) in the spongy parenchyma are located under numerous stomata. In the upper epidermis cells, which are substantially larger than the cells of the lower epidermis, there are many calcium oxalate crystals, sometimes filling the entire epidermal cell (Fig. 3H, arrow). To visualize the cuticular layer covering the entire aboveground shoot of the plant, cross-section leaf fragments were stained with the auramine fluorochrome. Under the fluorescence microscope, the cuticle emitted blue-range light, which facilitated quantitative analyses of this layer. These observations showed the same thickness of the cuticle on both the upper and lower epidermis on the mint leaves together with all types of trichomes (Fig. 3I, J). Only the midrib was covered with a substantially thicker cuticle layer (Fig. 3K).

#### **Influence of *T. nigropilosus* feeding on the morphology and anatomy of *M. × piperita* leaves.**

The analysis of morphological and anatomical changes induced by chrysanthemum thrips feeding was performed using mint leaves from laboratory cultivation. Leaves with visible traces of foraging, i.e. leaf blade necrosis and thrips excrements, were collected (Fig. 4A, arrow). The females were found to feed mainly on the lower lamina. SEM images of the surface of leaves on which *T. nigropilosus* began feeding revealed punc-



**Fig. 4.** A – *Mentha × piperita* leaf with traces of *T. nigropilosus* feeding (arrow); B–C – fragments of epidermis on the lower side of an infested leaf blade, B – piercing site (arrow); D–G – lower side of the leaf blade on consecutive rearing days: D – after 1 day, E – after 2 days, F – after 5 days (circles indicate an area destroyed by feeding); G – leaf after 10 rearing days; H–K – cross-section through the leaf: H – after 1 rearing day (the parenthesis indicates damaged epidermis), I – after 2 days, J–K – after 5 rearing days, K – the least damaged upper epidermis cells with calcium oxalate crystals (arrows)

ture sites (Fig. 4B, arrow) and damage to the epidermis layer of the mint leaves (Fig. 4C). After one day of the culture, several necrotic sites with a small surface area were visible (Fig. 4D – circles). The lesions noted after 2 days were larger and new feeding sites appeared (Fig. 4E – circles), which was a result of not only female foraging but also migration of the larvae. After 5 days, twice as many leaf fragments destroyed by thrips feeding and a larger area of previously recorded necrotic lesions were observed, in comparison with the second day (Fig. 4F). Based on the five-day observation of the thrips culture, it can be noted that the insects foraged mainly on the lower leaf blade but not on the veins and trichomes. Moreover, the feeding sites were located near the large vascular bundles. After 10 days, when the females and the second-instar larvae were feeding on the leaf blade, numerous fecal drops and silver spots produced by puncture of the epidermis cells, uptake of their content, and penetration of air were observed. The entire surface of the leaf blade was destroyed, which led to death of the mint leaves (Fig. 4G).

The analysis of the anatomical changes in leaves induced by *T. nigropilosus* feeding on the subsequent days of the culture showed damage to the lower leaf epidermis layer only after the first day (Fig. 4H – parenthesis). After the next 2 days, the spongy parenchyma layer died (Fig. 4I). After 5 days of thrips foraging, the damage covered not only the lower layer and spongy parenchyma but also the palisade parenchyma and upper epidermis (Fig. 4J). Upper epidermis cells containing calcium oxalate crystals retained their shape for the longest time (Fig. 4K, arrows).

## DISCUSSION

Climate variables, such as air temperature and precipitation, are the major abiotic factors influencing the growth and reproduction of plants and animals [Bergant et al. 2006]. The frequent fluctuations observed currently in the climatic conditions greatly determine the environment by limitation of the development of native species on the one hand and promotion of expansion of new plant and animal species on the other. Consequently, only species that can adapt quickly and efficiently to climate change can survive. Large-area crops, in particular monoculture herbal plantations, are extremely sensitive

to climate fluctuations. Culinary herbs, such as *Allium schoenoprasum* L., *Mentha × piperita*, *Ocimum basilicum* L., and *Rosmarinus* sp. L., are cultivated in such systems in Poland.

Until recently, the major threat to mint plantations has been posed by fungal pathogens: *Puccinia menthae* Pers., *Erysiphe biocellata* Ehrenb., *Alternaria* sp., and *Verticillium* sp. [Juronis and Snieškienė 2004, Szczeponek and Mazur 2006], and insect pests e.g. moths *Spilosoma obliqua* (Walker), *Agrotis flammatra* Fabr., *Orphanostigma abruptalis* (Walker), and *Helicoverpa armigera* (Hübner) [Verma 2006, Rita and Animesh 2011]. *Eupteryx atropunctata* Goere (*Hemiptera*) and *Longitarsus lycopi* Found (*Coleoptera*), have been regarded as the most serious pests of peppermint found in Lithuania [Juronis and Snieškienė 2004]. In Brazil, two species of thrips *Caliothrips phaseoli* (Hood) and *Dinurothrips hookeri* (Hood) have been noted on peppermint in glasshouses; infestation of the latter led to losses in crop production [Lima et al. 2013]. For many years, many insects, including thrips, have been found on *Mentha × piperita* plants in glasshouses near Warsaw. However, aphid species, e.g. *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Aulacorthum solani* Kaltentbach, and *Ovatus mentharius* (van der Goot) have been their main pests [Lubiarsz et al. 2013]. The massive appearance of *T. nigropilosus* on *M. × piperita* in greenhouse cultivation in central Poland, which has led to large crop loss, is an unknown phenomenon undescribed in world literature to date. For this reason, the present study involved the observation of the developmental cycle of thrips reared on *M. × piperita* as a host plant in different temperature conditions. Additionally, morphological and anatomical changes in the mint leaf blade infested by thrips were analyzed. The analyses indicated that thrips underwent a full developmental cycle on the mint and elevated temperature accelerated its development. In comparison with a recent comprehensive analysis of the impact of temperature on thrips development on chrysanthemum leaves performed by Ganaha-Kikumura and Kijima [2016], the development of the insect on the mint leaves at a temperature of 22°C was slower, i.e. it took as many as 26 days, compared with the 17-day developmental cycle on the chrysanthemum. In particular, the second stage of the thrips life cycle, i.e. development of the sec-

ond-instar larva, propupa, and pupa was slowed down, significantly contributing to the extension of the total life cycle. Similarly, the full developmental cycle of *T. nigropilosus* reared on the peppermint at a temperature of 26°C lasted 20 days, in comparison with 14 days reported in the chrysanthemum cultivation. Such substantial differences, mainly the extension of the developmental cycle in the peppermint cultivation, indicate that the plant does not offer *T. nigropilosus* optimal growth conditions at moderate temperature ranges, which may explain the absence of massive mint infestation by thrips in greenhouse conditions at a temperature not exceeding 26°C. However, surprising results were obtained at temperatures of 30/20°C, at which the thrips developmental cycle was substantially shortened to 14 days, which is similar to the development time of this insect on chrysanthemum, i.e. 11 days. Interestingly, the egg development period was considerably shortened at this temperature in the peppermint cultivation, whereas the duration of first-instar larva development was the same as in the case of chrysanthemum, i.e. nearly one day. These results unambiguously confirm earlier findings suggesting that the temperature of 30°C may promote parthenogenetic reproduction of the thrips, as shown on a pyrethrum field [Anyango 1992], regardless of the dietary preferences of the insect. However, the developmental periods of some stages were altered, and the development of the propupa and pupa on the peppermint was delayed in comparison with the length of the development period on the chrysanthemum, which indicates that feeding on mint has a negative influence mainly on the final stages of thrips development. A similar phenomenon was observed by Nakao [1994b], who demonstrated that the duration of the developmental cycle could be extended depending on the species of the infested plant, e.g. thrips developed efficiently on chrysanthemum, pumpkin, or eggplant, whereas the developmental cycle of the insect was substantially reduced on broad beans. Therefore, although peppermint is not its optimal environment, the thrips was able to develop on this plant at a high temperature; more importantly, its developmental cycle was sufficiently shortened to induce parthenogenetic reproduction. This resulted in an increase in the number of generations and, consequently, in huge losses in herb yields in central Poland in 2015. Additionally, our research showed a negative

relationship between the values of the lower threshold temperature (°C) and thermal constant (day-degrees), which was reported also for other pests, e.g. *Thrips tabaci*, *T. major*, and *Frankliniella occidentalis* [Stacey and Fellowes 2002]. Interestingly, we have established the minimal value of the *T. nigropilosus* threshold temperature for pupal stages. The relatively low threshold temperature allows pupae to continue their development in early spring in temperate climate.

Some investigations show that the type of thrips diet and its chemical composition have an impact on the fecundity, hatching rate, and developmental time [Steinbach et al. 2012, Pobożniak and Koschier 2013]. Our analyses have indicated that peppermint is extremely sensitive to infestations by thrips, which are phytophages, as a majority of thrips species from the *Terebrantia* suborder. *T. nigropilosus* ingests the contents of plant cells with a piercing-sucking apparatus, leaving silvery spots on the leaves. Leaves damaged in this way wilt gradually due to intensive feeding by larvae and adult stages. Furthermore, by mechanical damage to plants, thrips may facilitate penetration of bacteria, viruses, and fungi into plant tissues and may thus additionally reduce yields [Juronis and Snieškienė 2004, Szczeponek and Mazur 2006]. The presented analyses of peppermint leaves infested by *T. nigropilosus* revealed that the insect began feeding on the lower lamina surface close to the leaf midribs, which ensured easier access to substances transported by the vascular system. In the present study, no damage to vascular bundles was observed, which was probably associated with the fact that the outer walls of epidermal cells at these sites are covered by a thicker cuticle layer than on other cells of the peppermint leaf epidermis. A similar manner of cacao thrips feeding was observed on cashew leaves [Lewis 1973, after: Fenneah 1963]. In our work, it was observed that the thrips did not feed on glandular cells in the mint leaves due to their content of essential oils. Interestingly, the presence of these oils did not deter the insect completely, although herbal plants, such as mint, are often used as repellents and insecticides. It can be assumed that this is associated with the concentration of essential oils: as described in the literature, only after application of a 1% concentration of peppermint oil, oviposition deterrence in *Thrips tabaci* Lindeman was observed [Koschier and Sedy 2003]. Based on these

observations, it can be noted that *T. nigropilosus* fed on the peppermint in strictly defined areas of the lamina near the vascular bundles. The anatomical changes in the peppermint leaves indicated that the damage to the epidermis caused by *T. nigropilosus* led to gradual death of adjacent cells and destruction of the epidermis on the upper side of the leaf blade. Epidermal cells containing calcium oxalate druses filling the entire interior of these cells were the only undestroyed cells. It can therefore be assumed that this mode of feeding is particularly dangerous to leaves, as cell damage in the immediate vicinity of vascular bundles can limit nutrient transport within the leaf and can lead to its rapid death.

## CONCLUSIONS

In summary, the presented analyses indicate that the high adaptability of thrips to changing environmental conditions is associated with the close correlation of the life cycle of this insect with air temperature. High temperature substantially reduces the duration of the thrips developmental cycle, thereby facilitating its massive development on peppermint, i.e. a plant species that is not preferred by the insect. The data obtained not only allow exploration of the biology of *T. nigropilosus* in terms of its high adaptability but also provide peppermint growers with important information that can be applied in large-scale cultivation. Therefore, lowering the temperature can be an ecological method for *T. nigropilosus* control on *M. × piperita*.

## Authors' contributions

HK – performed the entomological analyses; DT – conducted plant anatomy and morphology analyses; MK – statistical analyses and thrips rearing; KW – SEM analysis; ML – provided materials. HK and DT – conceived the study, analyzed and interpreted data, wrote the manuscript.

## Compliance with Ethical Standards

All authors declare that they have no conflict of interest.

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