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Does Application of Pyrethroid Insecticides Induce Morphological Variations of *Oedothorax apicatus* Blackwall, 1850 (Araneae: Linyphiidae)?

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Abstract: Pesticides can have a lethal or sublethal effect on spiders and are able to influence their locomotion, activity, web building, reproduction and abundance. Effects of pesticides on spider morphological variations have been poorly investigated. In this study, we explored the influence of pyrethroid insecticides (Fastac®, Talstar® and Trebon®) on variations of linear measurements (body length, carapace and abdomen length, carapace and abdomen width) and carapace shape in *Oedothorax apicatus* (Blackwall, 1850) from conventional, integrated and organic oilseed rape fields. Multiple applications of various pyrethroid insecticides on the conventional field over a longer period of time influenced significantly the morphological variability in female specimens only. These females had longer bodies with longer and wider carapaces and abdomens in comparison with females from the organic and integrated fields. A wider posterior part of the carapace and less protruded frontal part were detected in female spiders from the integrated and conventional fields. We presumed that these results may be attributed to faster growth, which might be the consequence of a pyrethroid hormetic effect on female individuals. In the case of male spiders, significant morphological differences between the experimental fields were not observed, probably due to their higher mobility.

Key words: agriculture, pesticides, oilseed rape, spiders

Introduction

Agrochemicals and agricultural management practices can have a significant impact on biodiversity of primary and secondary decomposers (collembolans, mites, nematodes, molluscs, insects, isopods) and predators (spiders, beetles) in agroecosystems (Dinter & Poehling 1995, Desneux et al. 2007, Giglio et al. 2017). Spiders are among the most abundant and significant groups of invertebrate predators in terrestrial agroecosystems (Marc et al. 1999, Nyfeller & Sunderland 2003, Manenti et al. 2015, Perkins et al. 2018). They have a major beneficial role in all temperate agricultural habitats as regulators of insect pests and bioindicators (Nyfeller & Sunderland 2003, Ossamy et al. 2016, Yang et al. 2016). Studies of the ecological importance of spiders have been conducted mainly in the USA, Canada and some European countries (Luczak 1979, Marc et al. 1999, Wise et al. 1999, Samu & Szinetár 2002, Nyfeller & Sunderland 2003, Pearce & Venier 2006, Samu et al. 2011, Kozlov et al. 2015). The group of small, ground-dwelling, web-building spiders from the family

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Linyphiidae prevails among the arachnofauna in European crop fields, with several species such as *Oedothorax apicatus* (Blackwall, 1850), *Erigone atra* Blackwall, 1833, *Erigone dentipalpis* (Wider, 1834) and *Tenuiphantes tenuis* (Blackwall, 1852) usually dominating in the numerical sense (Blick et al. 2000, Nyfeller & Sunderland 2003). Differences in crop types, microclimate conditions and prey availability can affect diversity and community structure of the spider fauna (Nyfeller & Sunderland 2003, Manenti et al. 2015).

Studies on the impact of pesticide treatments on the agrobiont spider fauna have mostly been focused on their lethal/sublethal effect (Everts et al. 1991, Mullié & Everts 1991, Dinter & Poehling 1995, Pekár 2002, 2012, Fernandes et al. 2016) or influence on the spiders' locomotion (Baatrup & Bayley 1993, Pekár & Haddad 2005, Pekár & Beneš 2008), predatory activity (Deng et al. 2007, Pekár 2013, Korenko et al. 2016), web building (Benamú et al. 2010) and reproduction (Dinter et al. 1998, Tietjen 2006, Deng et al. 2008). Some research has been focused on the relationship between the use of insecticides and overall spider abundance (Bogya & Markó 1999, Thorbek & Bilde 2004, Diehl et al. 2013).

Until now, the effects of pesticides and environmental conditions on morphological variations have been analysed in other predator groups of Coleoptera (Holland & Luff 2000, Maryanski et al. 2002, Magagula 2003, Magura et al. 2006, Giglio et al. 2017) but rarely in spiders (Deng et al. 2008, Tahir et al. 2010, Michalko et al. 2016). The aim of the present research was to investigate the influence of pyrethroid insecticides and different agricultural practices on the variation of certain morphological characteristics in specimens of *O. apicatus* as one of the most common agrobiont spiders inhabiting crop fields in large parts of Europe (Nyfeller & Sunderland 2003). This study will help to better understand the effect of pyrethroid insecticides such as Fastac® (active ingredient: alpha-cypermethrin), Talstar® (active ingredient: bifenthrin) and Trebon® (active ingridient: etophenprox) on body features (body length, carapace and abdomen length, carapace and abdomen width, carapace shape) in *O. apicatus* as an important natural predator in agroecosystems.

Materials and Methods

In this study, we analysed the spider species *O. apicatus* adults obtained from experimental fields located in Ahlum, Germany. We compared specimens from three differently managed (conventional, integrated and organic) oilseed rape *Brassica napus* L. (OSR) fields (E 10°34' N 52°10') collected from Barber pitfall traps (ecoTech®). Insecticides were applied in October of 2010 (Fastac® 0.1 l/ha), March 2011 (Talstar® 0.2 l/ha) and April 2011 (Trebon® 0.2 l/ha) on the conventional field and in April 2011 (Trebon® 0.2 l/ha) on the integrated field (Fig. 1). The number of analysed individuals in most analyses was 56 (30 males and 26 females) from the conventional field and 60 (30 individuals per sex) from each of the other two types of fields (integrated and organic). However, abdomen width was analysed on

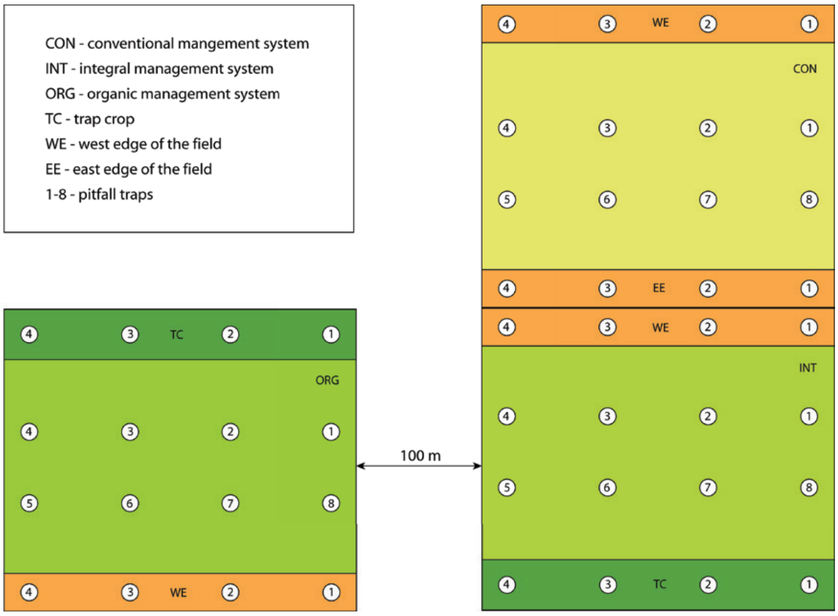

Fig. 1. Sampling points on three differently managed (conventional, integrated and organic) oilseed rape *Brassica napus* L. fields

49 spiders (30 males and 19 females) from the conventional field, 56 spiders (28 males and 28 females) from the organic field and 60 spiders (30 individuals per sex) from the integrated field. Specimens were collected from October 2010 to July 2011.

ImageJ software was used to measure several morphological traits: body length, length and width of the carapace and abdomen (Abràmoff et al. 2004). Fans with six lines were positioned on the carapace and abdomen in MakeFan software (Sheets 2003). The width of both structures was measured on the fourth fan line. Differences of linear measurements (body length, length and width of the carapace and abdomen) among spiders from the three different fields were tested using one-way ANOVA followed the Tukey HSD post-hoc test for unequal N values. We computed correlation coefficients between linear measurements using correlation analysis and performed regression analysis with abdomen and carapace width as dependent variables and body and carapace length as predictor variables. Abdomen width was the dependent variable except in the case of regression analysis of the relationship between carapace length and abdomen length. Statistical analysis and construction of graphs were performed in Statistica 7 software (StatSoft Inc. 1997) and R software version 3.4.2 (R Development Core Team 2017).

Apart from linear measurements, we also studied the carapace shape using geometric morphometrics, which is widely employed in animal studies (Jojić et al. 2012, Wojcieszek & Simmons 2012, Lazić et al. 2015, Changbunjong et al. 2016, Sasakawa 2016). Here we positioned 24 landmarks on the places where legs, pedipalps or chelicerae emerged from the carapace and two landmarks on the ruler (Fig. 2) in TpsDig software (Rohlf 2008). Variation in carapace shape among individuals from the conventional, organic and integrated fields was examined by canonical variate analysis (Canonical Variate Analysis) in the MorphoJ software (Klingenberg 2011).

Results

We observed persistence of sexual dimorphism in all analysed traits of spiders from the conventional (all traits: $P < 0.0001$) and integrated (body length: $P < 0.0001$; carapace length: $P = 0.0001$; carapace width: $P = 0.0469$; abdomen length: $P < 0.0001$; abdomen width: $P < 0.0001$ and carapace shape: $P < 0.0001$) fields. Moreover, males and females from the organic field differed statistically in body length ($P < 0.0001$), abdomen length ($P < 0.0001$) and width ($P < 0.0001$) and carapace shape ($P < 0.0001$).

Fig. 2. Position of 24 landmarks on the carapace of *O. apicatus* and two landmarks on the ruler.

Using the Tukey HSD post-hoc test for unequal N values, we obtained that females from the conventional field had significantly different body length, carapace length and width and abdomen length and width in comparison with females from the organic (all traits: $P < 0.0001$) and integrated (all traits: $P < 0.0001$) fields. Females from the conventional field had significantly longer bodies (Fig. 3B), carapaces (Fig. 3D) and abdomens (Fig. 3F) and wider carapaces (Fig. 3H) and abdomens (Fig. 3I), in comparison with females from the organic and integrated fields. Also, females from the organic field had significantly longer carapaces than females from the integrated field ($P = 0.0459$; Fig. 3D). No significant differences in body (Fig. 3A), carapace and abdomen length (Fig. 3C,E) or in carapace and abdomen width (Fig. 3G,J), were observed among males from fields of different management type (for the conventional and organic fields: $P = 0.4000$, $P = 0.0861$, $P = 0.4776$, $P = 0.4462$ and $P = 1.0000$, respectively; for the conventional and integrated fields: $P = 0.6504$, $P = 0.2114$, $P = 0.9357$, $P = 0.3243$ and $P = 0.7576$, respectively and for the organic and integrated fields: $P = 0.9989$, $P = 0.9988$, $P = 0.9600$, $P = 0.9999$ and $P = 0.8140$, respectively).

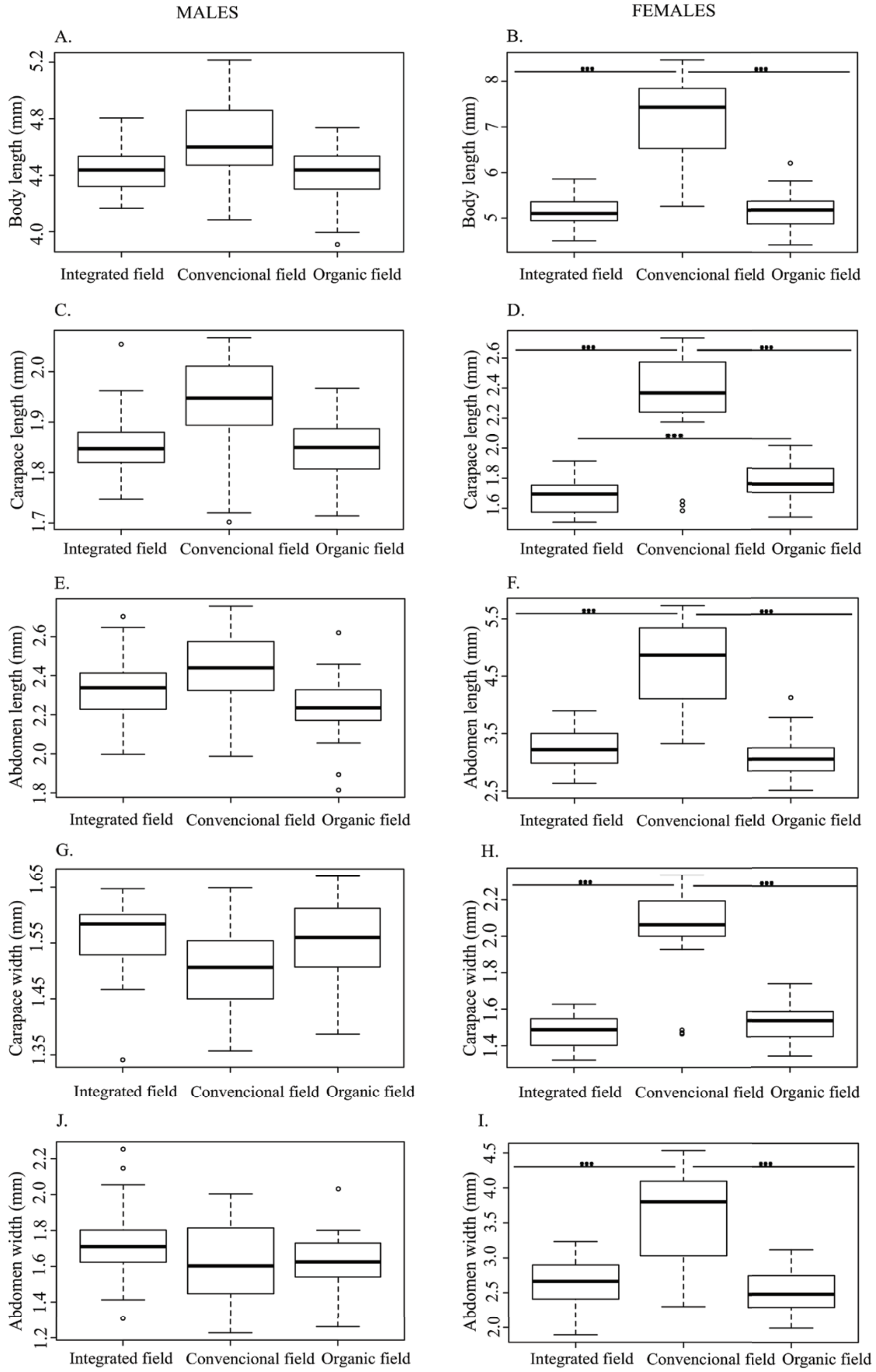

Fig. 3. Morphological variation of body length (A and B), carapace length (C and D), abdomen length (E and F), carapace width (G and H) and abdomen width (J and I) in males and females from the integrated, conventional and organic fields. The median with the first and the third quartiles is shown (in boxes), together with the range of variation and outliers. On the subfigures, significant differences are labelled using asterisks (*** $P<0.0001$).

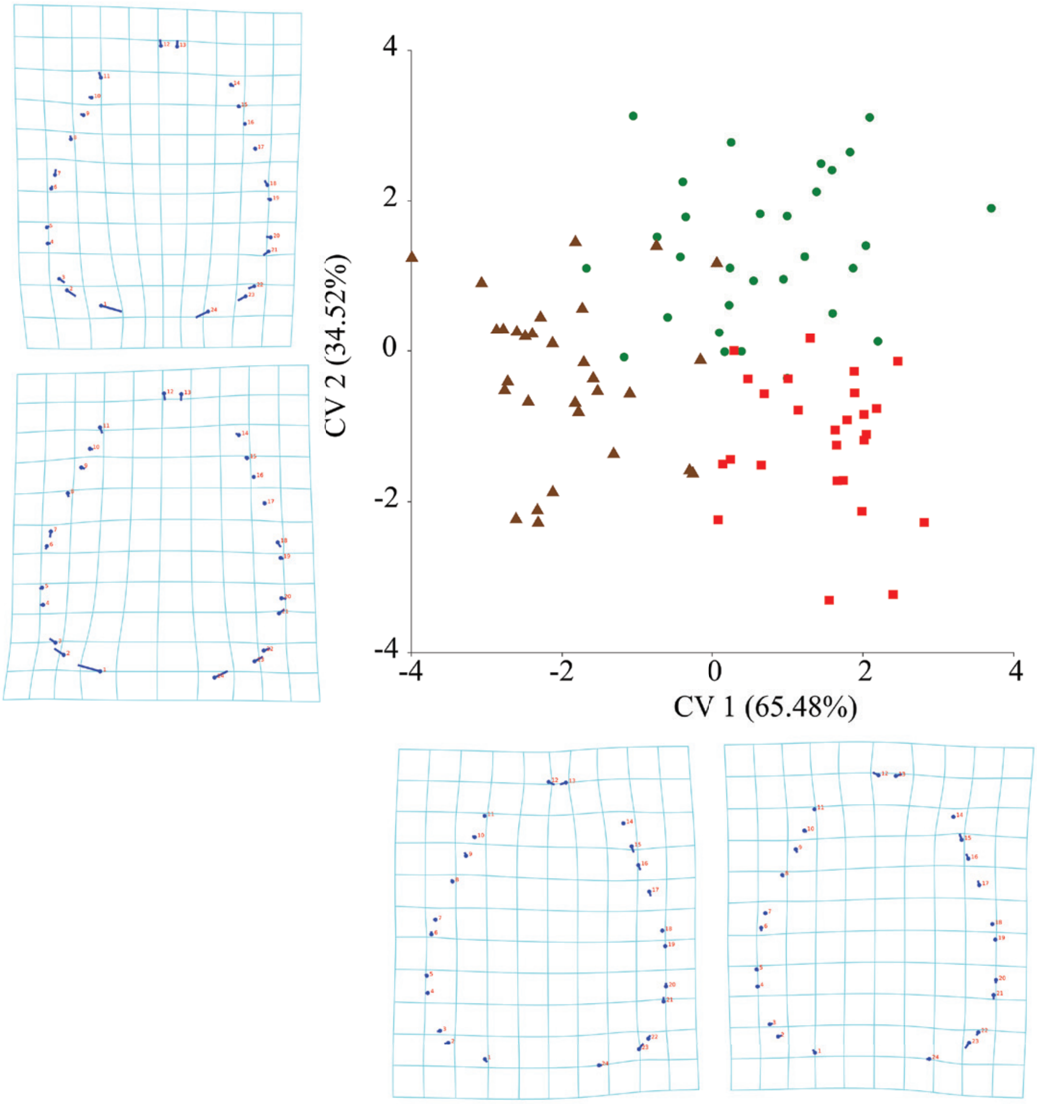

Fig. 4. Differences of carapace shape among females from the conventional (red squares), organic (green circles) and integrated (brown triangles) fields obtained by Canonical Variate Analysis (CVA).

Females from the organic field and those from the integrated and conventional fields differed significantly in carapace shape (P = 0.0207 and P = 0.0296, respectively). We found that the females from the conventional and integrated fields had a wider posterior part of the carapace and were less cephalically protruding on the CV 2 axis compared to those from the organic field (Fig. 4). Carapace shape differences of females from conventional and integrated fields are not found (P = 0.1763). No significant differences of carapace shape were obtained among males from the conventional field and those from the integrated and organic fields (P = 0.1594 and P = 0.0928, respectively) or among males from the organic and integrated fields (P = 0.1434) (Fig. 5).

Body length was significantly correlated with carapace length, abdomen length, carapace width and abdomen width in females from all of the analysed fields (conventional field: r = 0.6309, P = 0.0010, r = 0.9609, P < 0.0001, r = 0.8079, P < 0.0001 and r = 0.9321, P < 0.0001, respectively; organic field: r = 0.4180, P = 0.0220, r = 0.9021, P < 0.0001, r = 0.6656, P < 0.0001 and r = 0.7608, P < 0.0001, respectively; integrated field: r = 0.6063, P < 0.0001, r = 0.8825, P < 0.0001, r = 0.8699, P < 0.0001 and r = 0.7885, P < 0.0001, respectively).

In males, body length was significantly correlated with carapace length (conventional field: r = 0.6659, P < 0.0001; organic field: r = 0.4617, P = 0.0100; integrated field: r = 0.5263, P = 0.0030) and with abdomen length (conventional field: r = 0.6923, P < 0.0001; organic field: r = 0.8547, P < 0.0001; integrated field: r = 0.8122, P < 0.0001).

Significant correlations between body length

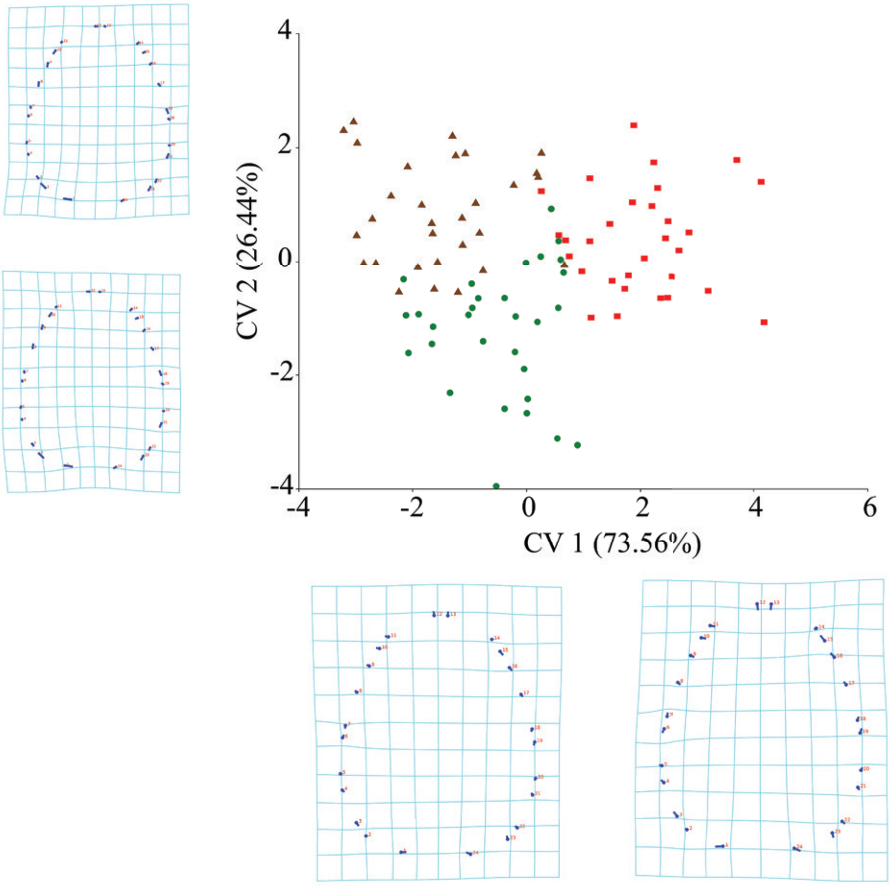

Fig. 5. Differences of carapace shape among males from the conventional (red squares), organic (green circles) and integrated (brown triangles) fields obtained by Canonical Variate Analysis (CVA).

and abdomen width were detected in males from the conventional ($r = 0.6908$, $P < 0.0001$) and organic ($r = 0.6627$, $P < 0.0001$) fields. Also, body length and carapace width correlated significantly in males from the organic ($r = 0.3656$, $P = 0.0470$) and integrated ($r = 0.5049$, $P = 0.0040$) fields.

Body length was positively correlated with carapace length, abdomen length, carapace width and abdomen width in females from the conventional ($r^2 = 0.3980$, $P = 0.0005$; $r^2 = 0.8144$, $P < 0.0001$; $r^2 = 0.6528$, $P < 0.0001$; and $r^2 = 0.8688$, $P < 0.0001$; respectively), organic ($r^2 = 0.1748$, $P = 0.0215$; $r^2 = 0.8138$, $P < 0.0001$; $r^2 = 0.4431$, $P < 0.0001$; and $r^2 = 0.5788$, $P < 0.0001$; respectively) and integrated ($r^2 = 0.3676$, $P = 0.0004$; $r^2 = 0.7788$, $P < 0.0001$; $r^2 = 0.7568$, $P < 0.0001$; and $r^2 = 0.6217$, $P < 0.0001$; respectively) fields (Figs. 6A-6D).

Our results indicated that body length is positively correlated with carapace and abdomen length in males from the conventional ($r^2 = 0.4434$, $P < 0.0001$ and $r^2 = 0.4793$, $P < 0.0001$; respectively), organic ($r^2 = 0.2132$, $P = 0.0102$ and $r^2 = 0.7306$, $P < 0.0001$; respectively) and integrated ($r^2 = 0.2770$, $P = 0.0028$ and $r^2 = 0.6596$, $P < 0.0001$; respectively) fields (Figs.6A and 6B). We showed that males from the organic and integrated fields with longer bodies had a wider carapace ($r^2 = 0.1337$, $P = 0.0469$ and $r^2 = 0.2549$, $P = 0.0044$, respectively; Fig. 6C). Males with longer bodies had a wider abdomen on the conventional ($r^2 = 0.4772$, $P < 0.0001$) and organic ($r^2 = 0.4392$, $P = 0.0001$) fields (Fig. 6D).

Discussion

Using traditional and geometric morphometrics, we observed that agrochemical treatments indeed affected morphology of *O. apicatus* females, contrary to males, which were mostly unaffected. We showed that females from the studied conventional OSR field had higher values of linear measurements and a wider posterior part of the carapace in comparison with females from the organic field.

In the present study, female specimens were larger than males on all experimental OSR fields (conventional, integrated and organic alike), which is usually a common trait for dwarf spiders of the genus *Oedothorax* (see Maes et al. 2004). Our results showed that the type of agricultural management

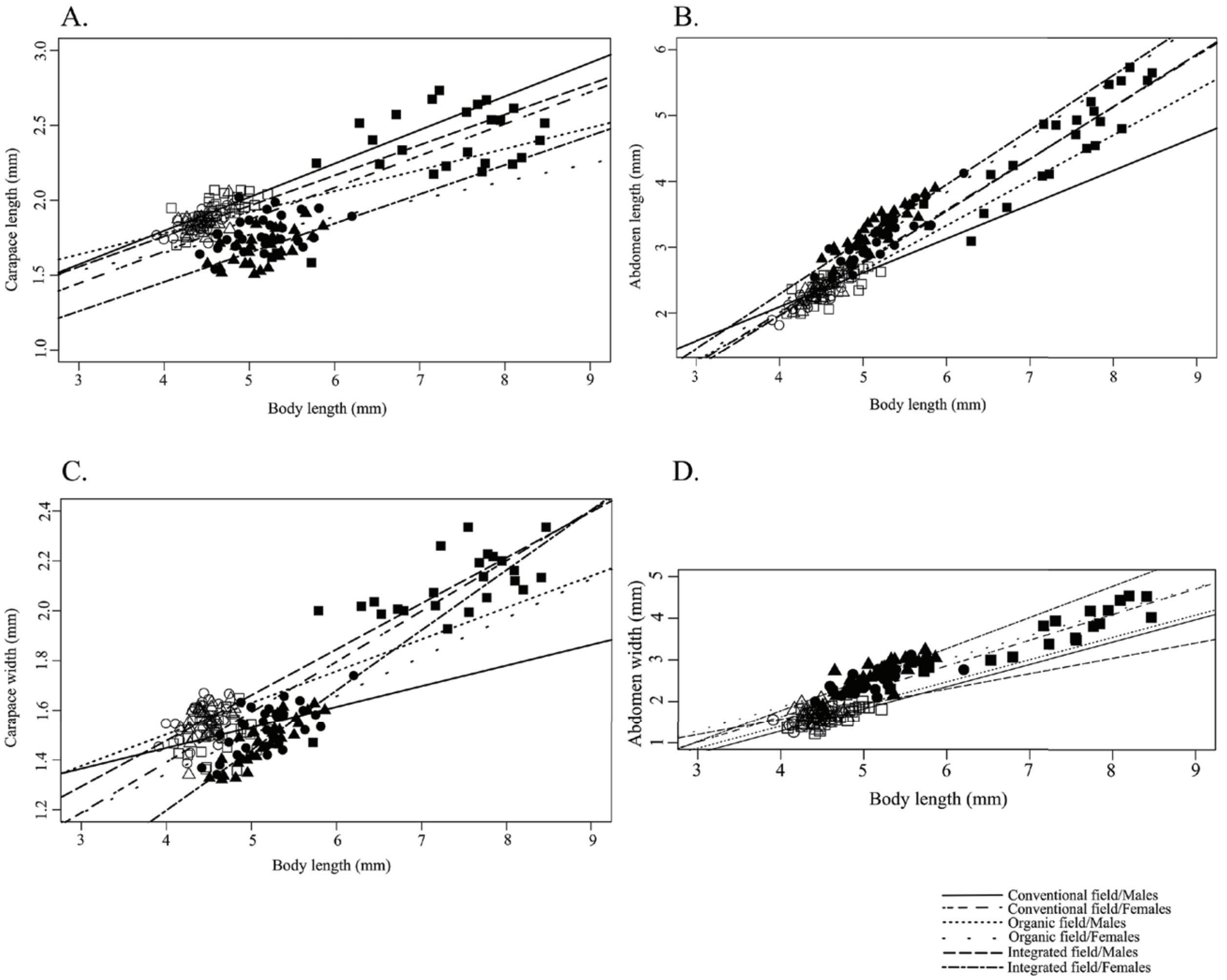

Fig. 6. Relationship between body length (mm) and carapace length (mm) (A), abdomen length (mm) (B), carapace width (mm) (C) and abdomen width (mm) (D), separately for males and females from the conventional, organic and integrated fields.

practice influenced body length in females of *O. apicatus*. Significant differences of overall body length were observed between females collected from the conventional OSR field, whose carapaces and abdomens were wider and longer and females from the integrated and organic fields. However, no significant differences were observed in the body length, carapace length and width and abdomen length and width of males from the conventional, integrated and organic fields.

These results indicate that treatment of the conventional OSR field with Fastac®, Talstar® and Trebon® at the end of October of 2010, end of March of 2011 and beginning of April of 2011, when *O. apicatus* starts breeding (Thorbek et al. 2004), did not negatively affect total body size of female *O. apicatus* individuals. Moreover, significantly higher values of morphological traits of individuals from the conventional field compared to specimens from the integrated and organic fields could indicate increased foraging ability leading to higher body growth (Prenter et al. 1999). Our results of correlation analysis showed that body length was positively correlated with length and width of carapace and abdomen in females from the conventional field, thereby supporting this hypothesis.

In spiders, body size and other morphological characteristics (carapace length and width) which have been used to describe growth (Schaefer 1987) are closely related to the feeding rate (Vollrath 1987, Marc et al. 1999, Uetz et al. 2002, Lomborg & Toft 2009, Toft 2013). It has been found that a low dose of insecticides can stimulate predation in *Hylyphantes graminicola* (Sundevall, 1830) (Linyphiidae) (Deng et al. 2007), *Pardosa pseudoannulata* (Böesenberg & Strand) and *P. amentata* (Clerck, 1757) (Lycosidae) (Toft & Jensen 1998, Wang et al. 2006), which killed more prey organisms but did not consume them. Buprofezin also induced a higher growth rate and larger body size in *Pirata piratoides* (Schenkel) (Lycosidae) (Deng et al. 2008) due to improvement in the efficiency of searching for prey (Pekár 2013). Low doses of pesticides can have a beneficial effect and improve performance, despite being toxic at higher levels, a phenomenon known as hormesis (Pekár 2013). We only can assume that the larger size of female individuals of *O. apicatus* on the conventional OSR field can be explained by this. Until now, the existence of hormesis has been proved in mites (Liu et al. 1998, Bowi et al. 2001, Zhang et al. 2012) but not in spiders. Interestingly, those studies showed that pyrethroids can produce increased oviposition and population growth in mites, while they had a negative effect on different physiological processes and movement (Everts et al. 1991), as well as on prey capture and egg sac production in *O. apicatus* spiders (Dinter et al. 1998).

We also found that carapace length in female spiders from the integrated field was smaller compared to specimens from the conventional and organic fields and even smaller than carapace length in male spiders from the same field. However, no significant differences of body and abdomen length or carapace and abdomen width were observed between female specimens from the integrated and organic fields. It seems that onetime treatment with Trebon® on the integrated field at the beginning of April 2011 did not affect female morphology traits in the way that multiple applications of various pyrethroid insecticides on the conventional field over a longer period of time did. Pyrethroids are known to have the ability to induce hormesis in predators and pests (Forbes 2000, Zanuncio et al. 2013), so based on the obtained result we might only hypothesise that their combined use over a longer time span can produce this effect in females of *O. apicatus*. It should be noted that prey is more active when insecticides are sprayed (Fernandes et al. 2016). As a result, more prey is available for generalist predators such as spiders and this could cause a higher growth rate.

Our findings are in contrast with the results presented in papers by Wisniewska & Prokopy (1997), Deng et al. (2006), Tahir et al. (2010), Peng et al. (2010) and Mukhtar et al. (2013), who have concluded that higher levels of application of various pesticides (pyrethroids, organophosphates, endosulfan, etc.) have a negative impact on spider growth (carapace width) and lead to smaller body size. Nevertheless, evidence of hormesis in spiders is still very rare and any possible hypothesis in that regard should be further tested. Male individuals, more agile and always on the move in search for females (Marc et al. 1999), did not seem to be affected by this phenomenon.

The conducted geometric-morphometric analyses also pointed out clear differences of carapace shape between female individuals from the conventional and integrated OSR fields, on the one hand, and ones from the organic field, on the other. Those findings reflect an association between carapace shape and agrochemical treatment among females, while it seems that male specimens are not so affected. The difference of carapace shape among females of *O. apicatus* might arise from a higher number of moultings (and consequently a higher rate of growth) on the conventional and integrated fields because rigid structures in spiders like the carapace can only grow in that way (Foelix 2011). This result is in line with the findings in this study obtained via traditional morphometric analysis.

Overall, it can be concluded that agrochemical treatment with different types of pyrethroid insecticides over a longer period of time on the investigated conventional OSR field led to statistically significant morphological differences of morphological traits (body length, carapace and abdomen length, carapace and abdomen width and carapace shape) in *O. apicatus* females compared to females from the integrated and organic fields. Morphological measurements proved that female specimens from the conventional field were larger in all aspects, which can be attributed to the higher growth rate confirmed also by the regression analysis. Male individuals were not affected by agrochemical treatments, which can be attributed to their mobility. Thus, our results indicate that pesticide application is a multifaceted phenomenon which does not necessarily have a negative effect on the agrobiont spider fauna. This complex and important biological issue needs to be further investigated.

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