Multigenerational and transgenerational side-effects of an insecticide on eggs of *Folsomia candida* (Collembola)

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ABSTRACT

Environmental toxicants such as insecticides are able to provoke epigenetic alterations which can be inherited to future generations. The aim of the current study was to assess whether the insecticide Trebon 10F (containing the active ingredient etofenprox) causes multigenerational and/or transgenerational effects on the egg traits of the collembolan *Folsomia candida*. The parent generation was kept in soil treated with three concentrations of the insecticide. The hatched offspring from each treatment were divided into two groups and were treated in the same manner as their parents (multigenerational effect), or remained untreated (transgenerational effect). The parents reacted with smaller eggs to the insecticide in a concentration dependent manner. Both multigenerational and transgenerational effects were detected in the offspring generations. While neither the number of eggs nor the ratios of the egg diameters (shortest/longest diameter) changed, the egg size increased as the Trebon 10F concentration increased. This is an indication that parents living under insecticide stress transmit this information to their offspring, who react with higher quality egg production. Such alterations in egg traits may have important consequences on the *F. candida* population dynamic.

INTRODUCTION

Transgenerational effect

The transgenerational effects of environmental factors have become of interest to ecologists in the past decade (Whitham *et al.* 2006, Bossdorf *et al.* 2008, Ledón-Rettig *et al.* 2013). These effects may result in epigenetic processes which lead to the non-genetic alteration of phenotype (Youngson and Whitelaw 2008). The term epigenetics is still ill-defined and has several meanings (Bird 2007). Currently, Deans and Maggert (2015) recommend a definition for epigenetics as follows: "*the study of phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression that are not dependent on changes to DNA sequence*." Evidence indicates that in addition to classic genetic mechanisms, epigenetic transgenerational inheritance can play an important role in the population dynamics (Uller 2008), plant-herbivore interactions (TerHorst and Lau 2012), biodiversity changes (Bonduriansky *et al.* 2012), as well as the effects of chemical contamination on populations (Bickham *et al.* 2000). However, transgenerational epigenetic effects are essentially disregarded in ecotoxicology.

Several environmental factors were identified as triggers of transgenerational epigenetic effects. In the case of *Drosophila melanogaster*, the exposure of the parent population to temperature changes was shown to generate effects influencing territorial success in male flies and fitness of the offspring (Zamudio *et al.* 1995; Gilchrist and Huey 2001). Food availability and nutritional status induced epigenetic alterations in the development and phenotype of the honey bee (*Apis mellifera*) (Kucharski *et al.* 2008) and European earwig (*Forficula auricularia*) (Raveh *et al.* 2016). Moreover, weight and immune reactivity of the filial generation of the moth *Plodia interpunctella* was influenced by the food quality of the parents (Triggs and Knell 2012). The fact that predator induced transgenerational effects can influence predator-prey systems is a focal point of population ecology (Agrawal *et al.* 1999).

The epigenetic and transgenerational effects of toxicants were summarised in the reviews by Vandegehuchte and Janssen (2011, 2014) and Head *et al.* (2012). In the case of insects, cadmium exposure in the diet of the blow fly (*Protophormia terraenovae*) upregulated their immune system. This effect was observable in the uncontaminated subsequent generation as well (Pölkki *et al.* 2012). Besides temperature and electric field, contact of the parents with lithium ions also provoked short antennae in the subsequent five generations of the moth *Ephestia kuehniella* (Pavelka and Koudelová 2001). Offspring of the beetle *Leptinotarsa decemlineata* exhibit lower adult body mass, and higher lipid content and resting metabolic rate if their parents were exposed to low concentrations of the insecticide deltamethrin (Piiroinen *et al.* 2014). Application of the insecticides Engeo Pleno® (lambda-cyhalothrin and thiamethoxam) and Tracer® (spinosad) on the parent population resulted in alterations to the emergence and the sex ratio of the F1 generation at the egg stage in the parasitoid wasp *Trichogramma galloi* (Costa *et al.* 2014). Guo *et al.* (2013) found that the life span of the diamondback moth *Plutella xylostella* F1 generation was extended remarkably when compared to that of the chlorantraniliprole-exposed parents. Bingsohna *et al.* (2016) found the red flour beetle (*Tribolium castaneum*) as suitable species for early warning subject of transgenerational epigenetic risk of pharmaceuticals.

Transgenerational problems have rarely been discussed in a soil zoological context. Hafer *et al.* (2011) demonstrated that the food availability of the mother and grandmother generation influenced the time to maturity and number of eggs in their first clutches in *F. candida*. A similar effect for food was detected by Triggs and Knell (2012). The transgenerational effects of four insect growth regulators were tested in standard reproduction tests with *F. candida* by Campiche *et al.* (2006). It was found that methoprene and teflubenzuron had an impact on the number of hatched juveniles for two generations, although only the parent generation was in contact with the toxicants. No such effect was detected in the case of fenoxycarb and precocene II.

Multigenerational effect

The continuous exposure of populations to toxic substances is a common event in ecosystems under anthropogenic pressure. This chronic exposure to toxic chemicals can have many multigenerational effects (parent and filial generations were exposed as well), and has the potential for altering the structure and/or function of the ecosystem (Marvin-DiPasquale *et al.* 2000). This is a focal point because in addition to scientists, the general public is also interested in the long-term effects of the pollutants.

Unlike air and water, the movement and distribution of toxicants in the soil is restricted. Moreover, the dispersion abilities of soil animals are also limited. This is why soil animals may be affected by multigenerational exposure more seriously than above-ground species. In light of this fact, some studies were performed in order to observe the multigenerational effects of toxicants on soil animals. Besides earthworms (Hertel-Aas *et al.* 2007, Andre *et al.* 2009), nematodes (Contreras *et al.* 2013, Schultz *et al.* 2016), ground beetles (Mozdzer *et al.* 2003), and collembolans were also used (Bakonyi *et al.* 2011). Van Gestel (2012) and Filser *et al.* (2013) pointed out the importance of the multigenerational studies with collembolans because of the deficiency of data in this field.

A growing body of data indicates that endocrine disruptors may cause adverse effects to an organism or population. Campiche *et al.* (2007) found that exposure of the *F. candida* parent generation to the pesticides methoprene and teflubenzuron decreased the number of juveniles in the F_2 generation, even though the F_1 generation was not in contact with these chemicals. In another study, ten generations of *F. candida* were exposed continuously to phenanthrene, a polycyclic aromatic hydrocarbon in a laboratory study (Paumen *et al.* 2008). Drastic changes were observed after the fourth generation, which showed the importance of multigenerational experiments.

Aims and hypothesis of the study

Our aims were to reveal whether the insecticide Trebon 10F causes (i) multigenerational and/or (ii) transgenerational effects on egg traits in the laboratory populations of *F. candida*, and (iii) is this effect influenced by the concentration of the insecticide and (iv) which egg traits is most affected?

We hypothesised that the insecticide Trebon 10F has multigenerational as well as transgenerational effects on the egg traits of *F. candida*, but the manifestation of the effects will be different, while in the multigeneration line the effect will accumulate, in contrast of the transgenerational line, where the effect will fade.

MATERIALS AND METHODS

Model animal

Folsomia candida Willem 1902 (Collembola, Isotomidae) used in this study was obtained from the stock population reared in the laboratory of the Szent István University, Department of Zoology and Animal Ecology for the past 20 years. Collembolans were kept in Petri dishes with a diameter of 9 cm based on the method of Goto (1960), so the Petri dish was poured with plaster of Paris mixed with activated charcoal (10:1 volume ratio). The animals were kept at a temperature of 20 ± 0.2°C, with ~100% humidity and in total darkness. Plastic boxes and Petri dishes were watered if needed in order to maintain the humidity at a constant level. The collembolans were fed with dry baker's yeast once per week ad libitum in all treatments. During this operation they were aerated. All phases of the experiment were performed under the mentioned environmental conditions. Collembolans were transported with the aid of a small aspirator.

Basic settings

The experiment was performed in OECD standard soil (OECD 2009). The composition of the soil was 74% sand, 20% kaolin clay, 5% sphagnum peat, and 1% calcium carbonate, at pH 7.29. Trebon 10F is a new insecticide, which has low toxicity to mammals and a short pre-harvest interval. The active ingredient is etofenprox (2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether), a synthetic pyrethroid insecticide. It is recommended against pests affecting vegetables, cereals, fruits, and flowers. The half-life of Trebon 10F in aerobic soils is 7–21 days (Mitsui Chemicals, Inc.). This is why the duration of the test was set to 20 days. In this study, the pesticide Trebon 10F (Mitsui Chemicals, Inc.) was mixed with OECD soil at three ratios, (i) field concentration (F), (ii) tenfold diluted (0.1F), and (iii) tenfold concentrated (10F) of the average recommended field concentration in orchards. The recommended field concentration of this insecticide in orchards is 0.883 ml Trebon 10F l^{-1} water. Control treatments (C) with tap water were also used. Altogether four treatments were set up.

Parent generation

Synchronized, 10–12 days old individuals (n = 90 per treatment) were placed in plastic boxes (5 cm high, 5.3 cm wide, and 6.6 cm long). The boxes were filled with 24.5 g of dry soil and mixed with 5.5 ml of pesticide solution or tap water in order to achieve 60% water holding capacity and the planned pesticide concentrations. Altogether, four boxes were used, three for the treatments (0.1F, F, and 10F) plus one for the control (C). After 20 days all of the 90 individuals were carefully separated from the soil, and 30 animals were chosen randomly from each treatment. These 30 collembolans were placed individually in Petri dishes (9 cm in diameter) prepared based on Goto (1960). This transport induced egg laying in most cases. The boxes were opened for aeration, feeding (ad libitum), and cleaning the mould if needed once each week. So, each animal was kept alone during the measurements and was assigned an identification number.

On the ninth day after transporting, when the eggs were 7–9 days old, the clutches were spread carefully with a wet brush and a digital photo was taken of each (Olympus C7070 Wide Zoom camera with Olympus C5060

ADL optic). The eggs were numbered on the photo. Thereafter, ten eggs were chosen randomly from each clutch. The shortest and longest diameters of the eggs relative at a 90° angle to each other were measured with the aid of the ImageJ software (Schneider *et al.* 2012).

Offspring generation

The following procedure was applied in the case of all four treatments. The hatched offspring of the individually kept parents in all 30 Petri dishes were separated into two equal groups. The *first* group was handled the same way as the parents. This group was added to freshly prepared soil with Trebon 10F identically as was done in the case of the parents. Sibling individuals were kept in the same box. Each box was marked with the appropriate parent's identification number (from 1–30 per treatment). Using this method the identification of a given offspring's parent was unambiguous. This treatment was regarded as the multigenerational effect of the insecticide (group M). Collembolans were left for 20 days in the boxes. Thereafter 30 individuals were selected randomly from each of the 30 boxes and placed individually in a Petri dish (three treatments plus control × 30 = 120 Petri dishes). The 7–9 days old eggs were photographed on the ninth day after translocation as described above. The *second group* of collembolans was exposed to no further insecticide treatments. This treatment was regarded as the transgenerational effect (group T; only the parental effects manifest). Collembolans were left for 20 days in the Petri dishes without repeated insecticide treatment. Thereafter, thirty offspring were placed individually in new Petri dishes, so altogether 120 dishes were handled for the T treatment as well. The 7–9 days old eggs were photographed on the ninth day after translocation as described above.

The following abbreviations were applied for the treatments. If the offspring were treated in the same manner as their parents MC = control on soil, M0.1F = treated with a tenfold diluted insecticide, MF = treated with a field concentration of insecticide, and M10F = treated with a tenfold concentrated insecticide. If the offspring were non-treated TC = control in a Petri dish, T0.1F = parents treated with a tenfold diluted insecticide, TF = parents treated with a field concentration of insecticide, T10F = parents treated with a tenfold concentrated insecticide. We handled 120 Petri dishes in the parent generation and 240 Petri dishes in the offspring generation. The main steps of the experimental procedure are summarized in Fig. 1.

The volume of each egg was calculated according to the prolate spheroid's volume formula ($V = 4/3\ \pi \times a \times b^2$, where "*a*" is the longer and "*b*" is the shorter diameter, (Satterly 1960). This measure may be related to offspring fitness (Awmack and Leather 2002) and is called egg size, hereafter. The ratio of the egg diameters is a measure of viability in a sense that a lower ratio value equates with higher viability (Martin *et al.* 2009).

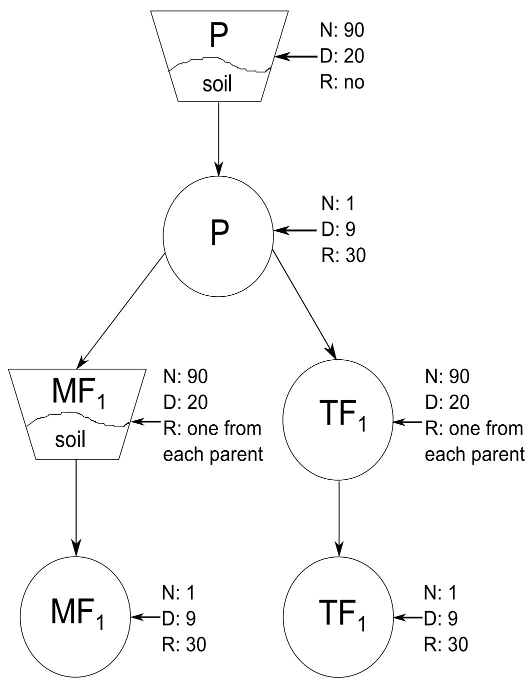

Fig. 1. Schematic diagram of the experimental set-up. Collembolans were handled like this in all treatments and the control. Trapeze: plastic box filled with soil and mixed with pesticide solution or tap water. The pesticide was applied on the first experimental day in the soil treatments. Circle: Petri dish with plaster of Paris mixed with activated charcoal. N: number of individuals in one box or Petri dish. D: how long (days) the animals were kept in the box or Petri dish. R: number of replicates. P: parent generation. F_1: offspring generation. M: multigenerational treatment. T: transgenerational treatment.

Statistics

All of the statistical analyses were made with the R Statistical program 3.1.1 (R Core Team 2012). The effect of the insecticide concentration on the number of eggs was analysed with the Wilcoxon-Mann-Whitney rank correlation test (ANOVA or linear model was not applicable because of the instability of the residual variance).

A mixed effect model was applied for all further analyses. In order to gain normality of the data, the concentration values were log transformed ($\log_{10}$) and the cube root of the egg size was calculated. After the transformations, all of the data sets of the experiment met the requirements of normality according to the diagnostic plots (Residual variances, QQ plot, Cook distance plot). The measurements on the eggs from the same clutch were not independent, which is why the model corrects the calculation accordingly (nlme package from R, (Pinheiro *et al.* 2013)). The identification number of the individuals was the random effect, which showed that the eggs had originated from the same dish.

The pesticide Trebon 10F concentration and number of eggs effect on the ratio of the egg diameters (shortest/longest diameter) and egg size were analysed in the mixed effect model. The group T and group M data were analysed separately. The effect of the number of eggs on the ratio of the egg diameters was not significant in either treatment; therefore it was left out from the analysis and is not discussed later. The difference from the control group was also tested with the mixed effect model.

To test the accuracy of the measurements the intraclass correlation coefficient (ICC) (irr package of R (Gamer *et al.* 2012)) was calculated. This statistical method can be used as a measure of the repeatability of a quantitative measurement (Lessells and Boag 1987). If the one sided 95% confidence interval (CI) value is under 0.4 the repeatability or quality is unacceptable, if it is over 0.75 it is excellent. Egg diameter measurements were performed as described above, then after half an hour the procedure was repeated in order to gain basic data for the interclass correlation calculation (ICC). All of the measured eggs were handled in such a way. In this study, in the case of the parent (CI = 0.683, F = 11.4) as well as the offspring generation (CI = 0.69, F = 0.923), the CI values were acceptable.

RESULTS

Parents

The total number of eggs in the parent generation was not affected by the insecticide concentration ($P = 0.971$). The egg size was affected by the concentration ($P < 0.001$) as well as by the total number of eggs ($P = 0.005$). The data clearly show that as the Trebon 10F concentration increases, the egg size decreases (Fig. 2). Similarly, with the increase of the total number of eggs the egg size decreases. When compared to the control group, both the field concentration and the tenfold concentrated insecticide significantly decreased egg size (Table 1). The ratio of the egg diameters was significantly affected by the concentration as well. As the Trebon 10F concentration increased, the ratio de-

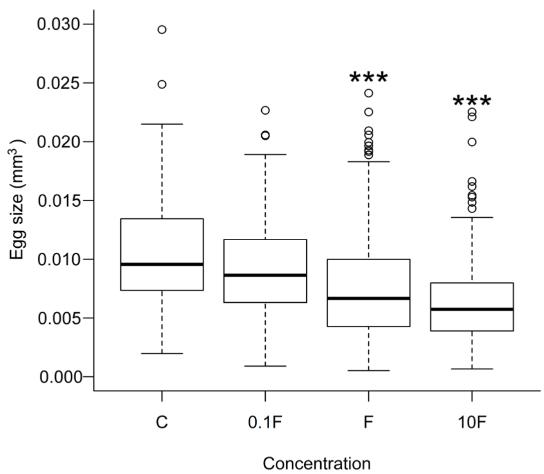

Fig. 2. Effects of the insecticide concentration on *Folsomia candida* egg size in the parent generation. The band inside the box is the median. The bottom and the top of the box are the first and third quartile. The ends of the whiskers are the minimum and maximum excluding outliers. Open circle: outlier (more than 3/2 times of the upper or lower quartile). C = control. 0.1F = tenfold diluted insecticide. F = field concentration of insecticide. 10F = tenfold concentrated insecticide. Asterisks indicate the significant difference at $P < 0.001$ from the control.

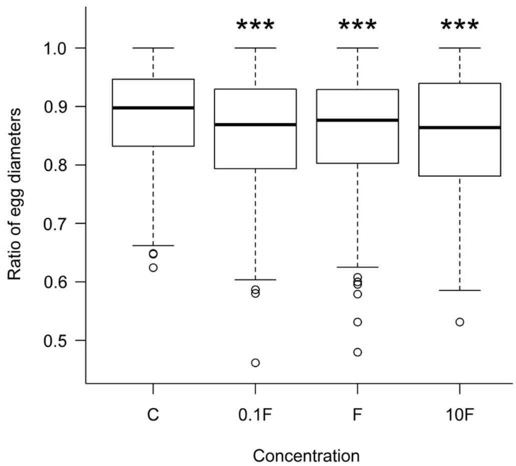

Fig. 3. Effects of the insecticide concentration on the ratio of the *Folsomia candida* egg diameters in the parent generation. C = control. 0.1F = tenfold diluted insecticide. F = field concentration of insecticide. 10F = tenfold concentrated insecticide. Asterisks indicate the significant difference at $P < 0.001$ from the control. For more explanations see Fig. 2.

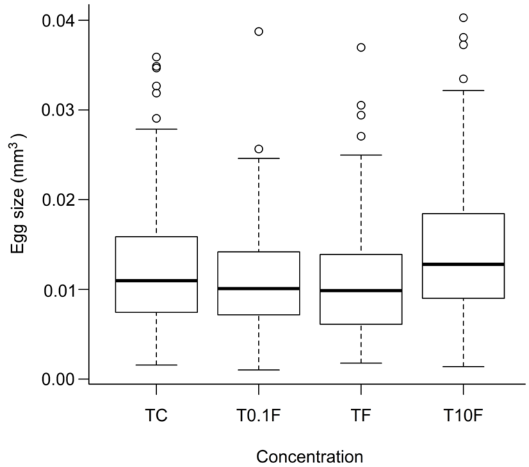

Fig. 4. Effects of the insecticide concentration on *Folsomia candida* egg size in group T (see Fig. 1). TC = control in Petri dish. T0.1F = parents treated with tenfold diluted insecticide. TF = parents treated with the field concentration of insecticide. T10F = parents treated with tenfold concentrated insecticide. Asterisks indicate the significant difference at $P < 0.001$ from the control. For more explanations see Fig. 2.

creased ($P = 0.037$; Fig. 3). All three concentrations applied in the study decreased the ratio of the egg diameters, inclusive of the tenfold diluted insecticide solution (Table 1).

Offspring

The insecticide concentration did not have a significant effect on the total number of eggs in group T ($P = 0.220$) nor in group M ($P = 0.963$). However, the egg size was affected by the concentration in both the T and M offspring groups. As the concentration of Trebon 10F increased the egg size also increased in both cases. The egg size was affected by the total number of eggs in both the T and M offspring groups. As the total number of eggs increased, the egg size decreased in both cases.

In group T egg size was significantly affected by concentration ($P = 0.003$) (Fig. 4) and by the total number of eggs ($P = 0.002$). Concentration had a positive (Fig. 4) while the total number of eggs a negative effect. No significant differences were found in the egg size between the treatments and the control (Table 1).

Similar to group T, in group M both the concentration effect on the egg size ($P < 0.001$) (Fig. 5), and the effect of the total number of eggs ($P = 0.014$) were significant. The concentration had a positive effect (Fig 5.), while the total number of eggs had a negative effect. Significant differences were found in the average egg size between the ten-

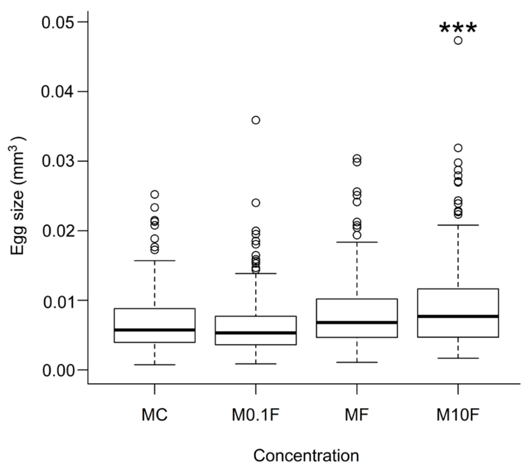

Fig. 5. Effects of the insecticide concentration on *Folsomia candida* egg size in group M (see Fig. 1). MC = control on soil. M0.1F = treated with tenfold diluted insecticide. MF = treated with the field concentration of insecticide. M10F = treated with tenfold concentrated insecticide. Asterisks indicate the significant difference at $P < 0.001$ from the control. For more explanations see Fig. 2.

Table 1. The effect of the Trebon 10F concentrations on *Folsomia candida* egg size and on the ratios of the egg diameters of *Folsomia candida*. Treatments are compared with the control in the linear mixed effect model. Significant effects are marked in bold. 0.1F = tenfold diluted insecticide. F = field concentration of insecticide. 10F = tenfold concentrated insecticide. Offspring's parents treated with T0.1F = tenfold diluted insecticide. TF = field concentration of insecticide. T10F = tenfold concentrated insecticide. Offspring treated too with M0.1F = tenfold diluted insecticide. MF = field concentration of insecticide. M10F = tenfold concentrated insecticide.

The effect:	Treatment	df	t-value	*P*-value
on egg size	0.1F	81	-1.144	0.256
	F	81	-3.900	**<0.001**
	10F	81	-5.344	**<0.001**
	T0.1F	88	-1.209	0.230
	TF	88	-1.640	0.105
	T10F	88	1.708	0.091
	M0.1F	95	-0.966	0.337
	MF	95	1.680	0.096
	M10F	95	3.424	**0.001**
on the rate of egg diameter	0.1F	81	-3.238	**0.002**
	F	81	-3.475	**0.001**
	10F	81	-3.370	**0.001**
	T0.1F	88	-0.591	0.556
	TF	88	1.562	0.122
	T10F	88	-0.102	0.919
	M0.1F	95	-1.389	0.168
	MF	95	-1.103	0.273
	M10F	95	-2.010	**0.047**

fold concentrated insecticide treatment and the control group (Table 1).

The concentration did not have an effect on the ratios of the egg diameters in group T ($P = 0.872$), nor in group M ($P = 0.12$). A similar figure was found when the treatments were compared to the control group. The only exception was the tenfold concentrated treatment. In this case, the ratio of the egg diameters was marginally significantly higher than that of the control group.

DISCUSSION

An inverse relationship between the total number of eggs and egg size in insect species is a common phenomenon as a result of the trade-off between production and reproduction (Mousseau and Fox 1998). However, a dose-response relationship was not found between Trebon 10F and the number of *F. candida* eggs, neither in the parent nor in the offspring populations. In the case of *F. candida* controversial data exists when various pesticides were tested. A concentration-dependent decrease in the total number of eggs was found by Crommentuijn *et al.* (1997) when the insecticide chlorpyrifos and the fungicide triphenyltin hydroxide were tested on *F. candida*. On the contrary, Widarto *et al.* (2007) observed that nonylphenol (an adjuvant of pesticides) enhanced egg production significantly. According to Cutler (2013) the correlation between egg production and concentration in the mentioned study followed the hormetic dose-response model. Enhanced egg production was observed at concentrations of 4, 5.5, and 7 mg kg^{-1} of carbaryl when compared to the control (Cardoso *et al.* 2014). Our results were not in line with these observations, because the total number of eggs was not influenced by the insecticide concentration in the parent nor in the offspring populations. The most probable reason for this is that (a) the tested concentrations were out of the effective range (namely that the concentrations applied were lower than the lowest effective concentration, or LOEC of Trebon 10F for the number of *F. candida* eggs), or (b) a different pesticide was used in our experiments, which has a dissimilar mode of action. Etofenprox, for example, blocks the ion gates of the sodium channel of nerve cells during re-polarization (Soderlund *et al.* 2002). Unlike etofenprox, both of the insecticides chlorpyrifos (Crommentuijn *et al.* 1997) and carbaryl (Cardoso *et al.* 2014) are inhibitors of cholinesterase enzymes (Karanth and Pope 2000, Metcalf *et al.* 1966).

Modification of the egg size due to Trebon 10F application showed different results than were seen for egg production. In the case of the parent generation, the insecticide decreased the egg size in a concentration-dependent manner. This is in agreement with earlier findings which predict that low reproductive investment is presumed if the environmental conditions are poor (Fox *et al.* 1997, Fox and Czesak 2000). According to this expectation, *F. candida* would produce smaller eggs if the population density were high and food was in shortage (Tully and Ferrière 2008). The insecticide Trebon 10F caused a similar effect in the parent generation in this study. Moreover, the negative effect of the number of eggs on egg size as demonstrated by Tully and Ferrière (2008) was found in our study as well. The negative influence of Trebon 10F on embryonic development was shown in this study by the decrease in the ratios of the egg diameters. This indicates that the eggs were less spherical as the insecticide concentration increased in the parent generation. Hafer and Pike (2010) demonstrated that the egg shape of *F. candida* is related to the viability, as spherical eggs were less viable when compared to discoid (spheroid) eggs (Hafer and Pike 2010). All of these results show that due to Trebon 10F application less parental investment was allocated in the subsequent generation. This finding can be a sign of the energy trade-off between the costs of defence mechanisms for the parent individuals against the Trebon 10F application *versus* investment into offspring. However, this hypothesis should be tested further.

Completely different results for the effects of the insecticide on the egg size and shape were found for both the T and M offspring generations. Neither the number of eggs nor the ratio of the egg diameters changed, but the egg size increased as the Trebon 10F concentration increased. This is a clear sign that the insecticide exposure disturbed the epigenetic status of the parent generation in a concentration-dependent manner. Results such as these have rarely been found in ecotoxicology (Vandegehuchte and Janssen 2011, Head *et al.* 2012). In addition, egg size positively correlated with the viability of the *F. candida* offspring (Tully and Ferrière 2008). Similar results were found in the case of several herbivore insect species (Awmack and Leather 2002). The results of our study suggest that parents living under insecticide stress transmit this information to their offspring, who react with higher quality egg production. Epigenetic variation induced by pesticides can be manifested (e.g. in histone modification and DNA methylation). Nevertheless, the true mechanisms of the information transmission in the case of *F. candida* due to Trebon 10F application are still to be revealed.

Similar to our results, Campiche *et al.* (2007) found a sublethal multigenerational effect with the endocrine disruptor fenoxycarb to *F. candida* if both parents and the first offspring generation were exposed to the toxicant. Such multigenerational effects of pesticides, industrial chemicals, and pharmaceuticals is a common phenomenon, as it was demonstrated by Martin *et al.* (2009) on the basis of 329 multigeneration studies. The effect is dependent on the mode of action of the xenobioticum (Walker 2014).

Trebon 10F is a pyrethroid-type insecticide (FAO 2006). The active ingredient is etofenprox, a pyrethroid type insecticide which disturbs the sodium channels of insect neurons (EFSA 2008). The exact mode of action of etofenprox on collembolans is still unexplained. However, the egg size data generated in this study demonstrates that the effects of the insecticide are visible in the egg size and during embryonic development as the egg shape alteration indicated.

If only the parent generation of collembolan were exposed to the insecticide (group T) the outcome was very similar, as in the multigenerational treatment. This is straightforward evidence that in this study the transgenerational effect of the insecticide Trebon 10F exists. Transgenerational effects of insecticides were previously proven for wasp (*Trichogramma galloi*) (Costa *et al.* 2014), and for freshwater gastropod (*Physa pomilia*) (Kimberly and Salice 2014). Hafer *et al.* (2011) showed transgenerational effects for food availability, and Campiche *et al*. (2007) showed transgenerational effects for the insect growth regulators methoprene and teflubenzuron for *F. candida*. Contrary to the previously mentioned pesticide, etofenprox, the active ingredient in Trebon 10F has a high octanol/water partition coefficient (log K_{ow} of 6.9), which suggests the possibility of bioaccumulation in fat (e.g. *F. candida* egg cytoplasm and developing embryo) (Hopkin 1997, Walker *et al*. 2006). If this is the case, the significant effect of Trebon 10F on egg size can be explained. However, the mechanisms of etofenprox action during collembolan embryonic development are not yet identified.

CONCLUSION

The data presented in this study show that Trebon 10F has multigenerational and transgenerational effects on the egg traits of *F. candida*, and it seems that the size of the egg is the trait which is primarily affected. Moreover, these effects are dose-dependent. We presented different arguments in the discussion section that alterations in egg traits may have important consequences on the *F. candida* population dynamic. Similarly, it has been proven previously by Benton *et al*. (2005) that differences in egg sizes lead to differences in growth rate in soil mite *Sancassania berlesei* populations.

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