Combined thermal and insecticidal stresses on the generalist predator *Macrolophus pygmaeus*

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Abstract

Ecotoxicological risk assessments of pesticides on non-target arthropods are often carried out under constant and optimal temperature regimes. However, living organisms rarely experience these conditions in real field situations. , Understanding the impact of pesticides on non-target beneficial arthropods under temperature stresses is especially important in terms of global warming. We assessed the lethal and sublethal effects of four modern insecticides (chlorantraniliprole, cyantraniliprole, spinetoram, spinosad), on the generalist predator *Macrolophus pygmaeus* (Hemiptera: Miridae) under a range of temperatures (from 10 to 40°C) frequently experienced in a real field scenario. A reduction coefficient (Ex) was calculated by summarizing the mortality and predator reproductive capacity and, the chemicals were classified according to the International Organization for Biological Control (IOBC) toxicity classes. The insecticides showed a marked synergistic effect with temperature, as the predator mortality and reproductive outputs were significantly correlated with increasing temperatures. Spinosyns interacted significantly with temperature causing the highest mortality and lowest fertility rates. Anthranilic diamides showed a safer ecotoxicological profile compared to spinosyns, with cyantraniliprole being more harmful than chlorantraniliprole. These results suggest that temperature should be taken into account in pesticide ecotoxicology studies within the framework of integrated pest management and the recent climate changes.

Keywords: Biopesticide; Chlorantraniliprole; Climate change; Cyantraniliprole; Spinosyn; *Tuta absoluta.*

1. Introduction

Pesticides are used for arthropod pest control globally and often play a key role in many integrated pest management (IPM) strategies (Guedes et al. 2016). However, they have a negative impact on non-target beneficial arthropods, which play a vital role in the ecosystem (Desneux et al., 2007; Kim et al., 2017; Newman 2014; Biondi et al. 2015). Comprehensive studies on pesticide side effects are therefore required to limit the adverse effects on non-target fauna.

Regulations for placing plant protection products on the market are guided by documents issued by non-governmental institutions. In the EU, the ecotoxicological risk assessment (ERA) of pesticides for non-target arthropods was developed in the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002), which, in the case of non-bee arthropods, follows the recommendations of the SETAC/ESCORT (Barret et al. 1994, see also EFSA 2010). Such risk assessments include laboratory tests in which the non-target organism is constantly exposed to toxicants under optimal environmental factors (Newman 2014). However, optimal conditions are rarely experienced by the organism in the field where variable conditions often coexist (Holmstrup et al., 2010; Gergs et al., 2013). Stressors affecting non-target organisms include physical, chemical, biological, and nutritional factors. Assessing the risk of a single stressor may therefore underestimate the real hazard that the non-target organism is likely to be exposed to (Gergs et al., 2013).

Environmental change tends to be the main physical stressors s, with temperature being the most prominent, and which also modulates other stress factors (Schiedek et al., 2007; Fischer et al., 2013). The main challenge in pesticides ERA is thus to include combined stressors in the evaluation schemes (Holmstrup et al., 2010). Despite the growing interest in the risk assessment of pesticide mixtures (Thompson 1996; Panizzi et al. 2017), there has been little focus on the combined effects of thermal stress and chemicals on non-target

arthropods. However, the inclusion of temperature stress in pesticide ERA is of great interest given that the temperature is predicted to increase by 1.8–4.0 °C under a range of greenhouse gas emission scenarios (Giorgi 2006; Lavalle et al., 2009; Noyes et al. 2009; Aregbesola et al. 2019).

Interactions between temperature and chemicals have mainly been studied on water organisms and have revealed significant interactions between heat and chemical stress. However few studies have characterized such interactions in terms of them being synergistic, antagonistic or additive (Holmstrup et al., 2010). Insect susceptibility to pesticides at different temperatures has been principally investigated on target pests (Ladas, 1972; Bednarska and Laskowski, 2009; Laskowski et al., 2010; Yu et al., 2012; Saeed et al., 2018). On the other hand, few studies have examined the combined stress on beneficial arthropods of toxicants and temperatures (Ladas, 1972; Bednarska and Laskowski, 2009; Bednarska et al., 2009; Medrzycki et al., 2010; Abbes et al., 2015). Understanding the interaction between chemicals and heating temperatures on non-target predators may help in using these species within an IPM framework and the global warming scenario.

The present study assesses whether the combination of four chemical insecticides, routinely used for pest control, with seven increasing levels of temperature impacts on the biological life traits of the generalist insect predator *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae). We carried out a series of laboratory bioassays exposing adults of the predator to dry pesticide residues on tomato leaves. The survival and reproduction of the exposed insects were used to estimate reduction coefficients and toxicity categories specific for each insecticide and temperature combination.

2. Material and methods

2.1. Biological material

73 The tomato plants (*cv* Shiren) used in the experimental trials were 40 cm high, 40-d old,
74 obtained from seeds in 2L pots without any pesticide application. Tomato plants were grown
75 in greenhouse conditions (25° ± 5 °C, 60 ± 10% R. H.) in the facilities of the Department of
76 Agriculture, Food and Environment of the University of Catania (Italy).
77 A colony of *M. pygmaeus* was established by individuals provided by Koppert
78 Biological Systems (The Netherlands) in commercial bottles containing 500 adults dispersed
79 in inert material (MIRICAL®). Insect rearing was conducted using tomato plants and *ad*
80 *libitum* frozen eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera:
81 Pyralidae) (marketed as Entofood®, Koppert Biological Systems), kept under the following
82 laboratory conditions 25°C, 60% R.H., 14:10 L:D of photoperiod. The 24-48 day old *M.*
83 *pygmaeus* adults used in the experiments were obtained by isolating coetaneous nymphs in
84 separate rearing cages.
85 **2.2. Insecticides**
86 Two anthranilic diamides (i.e., chlorantraniliprole and cyantraniliprole) and two spynosins
87 (i.e., spinetoram and spinosad) were included in this study because they are commonly used
88 to control economically important phytophagous insects (e.g., moths, thrips and whiteflies) in
89 IPM programmes (especially for tomato crop protection), wherein the predator *M. pygmaeus*
90 often provide biological control sevices (Calvo et al., 2009; Campos et al., 2017; Biondi et al.
91 2018).
92 These insecticides were also chosen because in field practice for *M. pygmaeus* and/or
93 other non-target insect natural enemies (e.g., generalist mirid predators, parasitoid wasps) are
94 highly likely to be exposed to a combination of them (Table 1). Commercial insecticides used
95 in the experiments were applied at their highest field recommended dosage for tomato
96 greenhouse crops at a volume rate of 1000 L Ha^{-1} (Biondi et al., 2012b). Tomato plants were
97 sprayed using a 2L hand sprayer (Matabi®, Antzuola, Guipuzcoa, Spain) and then dried for

two hours in laboratory conditions before insect exposure. Table 1 details the insecticides tested.

2.3. Lethal effect: acute toxicity on adults

To assess the simultaneous impact of insecticides and temperature on the survivorship of the predator, *M. pygmaeus* adults were exposed to dry insecticide residues on plants under seven temperature regimes. Trials were conducted in climatic cabinets maintaining seven different constant temperatures: an extreme low and an extreme high temperature (10 and 40°C), a low and a high temperature (15 and 35°C), two suboptimal temperatures (20 and 30°C) and an optimal temperature (25°C) for zoophytophagous mirids (Martínez-Garcia et al., 2017; Ziaei Madbouni et al., 2017a). Standard 60% R.H. and 14:10 L:D photoperiod conditions were kept in all climatic cabinets.

The upper plant part (15 cm high) from sprayed tomato plants was cut and fixed with odorless modeling clay (Jovi®, Barcelona, Spain) into the two-cup bioassay arena (Biondi et al., 2012b). A total of 0.05g of *E. kuehniella* sterilized eggs were sprinkled onto tomato leaves as factitious prey for predators (Biondi et al. 2016), and distilled water was provided *ad libitum* in an Eppendorf tube filled with moistened cotton. Five coetaneous couples (i.e., five females and five males) of *M. pygmaeus* were then introduced into the described arena and kept in the climatic cabinet under the fixed temperature regime for three days. The numbers of dead females and dead males in each cup were recorded and removed daily for three days. Predators were considered dead when they did not move after being touched with a fine paint brush. For each pesticide-temperature combination, an untreated control was included with demineralized water-sprayed plants. The experiment was replicated between 10 and 15 times for each pesticide-temperature combination and the control.

2.4. Sublethal effect: impact on reproduction

The sublethal effects were assessed of the combined exposure to insecticides at different temperatures on the fertility of the surviving females from the previous bioassay. Adults that survived three days in the acute toxicity trial were removed to stop egg laying. Female mirids are known to lay eggs inside plant tissues especially in tender stems, thus making it difficult to detect eggs laid inside tissue . Thus, to assess the combined impact of temperature and insecticides on the reproductive fitness of females (i.e., total produced progeny), treated tomato leaves from the acute toxicity trial bearing *M. pygmaeus* eggs at different temperatures, were kept in their corresponding bioassay arenas and incubated in a climatic chamber at 25°C and 60 ± 10 % R.H, 14:10 L:D until egg hatching. After an incubation period of 10-12 days, newly emerged nymphs were counted daily and removed from the bioassay arena to prevent any cannibalism. The experiment ended when no further nymphs had emerged for four consecutive days.

2.5. Data analysis

Raw datasets were first tested for normality and homogeneity of variance using the Kolmogorov–Smirnov D test and Cochran's test, respectively, and log-transformed when needed. A General Linear Model (GLM) full factorial univariate analysis was performed to test the effects of the insecticide, temperature and their interactions on the percentages of dead predators and on the number of nymphs produced during the exposure period by *M. pygmaeus* females. One-way ANOVA followed by Tukey's honest significant difference (HSD) post-hoc test for multiple comparisons inside the different temperature sub-datasets were also carried out.

Predator mortality percentages were corrected for the control mortality (Abbott, 1925) and then analyzed using linear regression, with the temperature as the explanatory variable. The quality of fitting was evaluated by the coefficient of determination (R^2), and the

146 regression was assumed to be significant when the slope was significantly different from 0,
147 with $P<0.001$.

148 For each pesticide and temperature tested, a reduction coefficient *Ex* was estimated
149 including in the formula, the female corrected mortality (*Emx*) (i.e., corrected for control
150 mortality) and the reduction percentages of female offspring (*Efx*), i.e., difference between the
151 number of eggs laid by females exposed to insecticide residues and the number of eggs laid
152 by untreated females (see Biondi et al., 2012b for the formulae).

153

154 **3. Results**

155 **3.1. Acute toxicity**

156 The mortality of the predators during the three days of exposure varied significantly among
157 treatments (*insecticide*: $F_{4,284}=60.501$; $P<0.001$) and among the tested temperatures
158 (*temperature*: $F_{6,282}= 122.050$; $P<0.001$). There was also a significant interaction between the
159 factors, insecticide and temperature ($F_{24,264}= 3.836$; $P< 0.001$), with the temperature affecting
160 the insecticide toxicity to *M. pygmaeus* in different ways (Figure 1). Data recorded at 40 °C
161 were not considered for analysis in the reproduction experiment because the control also
162 showed a high mortality rate (～ 80%).

163 Corrected mortality correlated linearly with the increasing temperature, while
164 according to the regression analyses, the corrected mortality regressed significantly with the
165 temperatures (*insecticide:* $R^2= 0.350$; $y = 1.989x -4.613$, $t= -0.980$, $P<0.001$). Based on the
166 regression coefficients, a higher increase in mortality was observed with a temperature
167 increase for the spynosin insecticides (i.e., spinosad and spinetoram) compared to diamide
168 insecticides (i.e., chlorantraniliprole and cyantraniliprole) (Figure 2 and Table 2).

169 **3.2. Impact on reproduction**

170 The number of nymphs produced during the 3-day exposure varied significantly among the
171 chemicals (*insecticide*: $F_{4,163}$= 6.79; P< 0.001) and among the temperatures (*temperature*:
172 $F_{6,161}$= 42.53; P< 0.001). However, no significant interaction between insecticide and
173 temperature was found ($F_{24,144}$= 1.88; P =0.297). The highest number of nymphs produced
174 was recorded for the control at 25°C (39.4 ± 8.4). However, for the same thermal regime, this
175 value decreased after exposure to spinosad (24.78 ± 5.99), spinetoram (25.44 ± 5.58) and
176 chlorantraniliprole (28.10 ± 8.96) (Figure 3). Interestingly, a 5°C negative shift in temperature
177 significantly reduced the fertility of *M. pygmaeus* also for the untreated control (20°C: 8.2 ±2;
178 30°C: 6.8± 2.7).
179 The effect of the lowest (i.e., 10°C) and the highest (i.e., 40°C) temperature regime
180 was not considered because no eggs were laid at 10°C, while a high mortality of *M. pygmaeus*
181 females was recorded for the chemicals and the untreated control under the highest thermal
182 input.
183 **3.3. Reduction coefficient (*Ex*)**
184 According to the regression analyses, the reduction coefficients regressed significantly with
185 the temperature (R^2= 0.355; y = 0.162x –16.442, t= 11.458, P<0.001) (Table 3). The
186 reduction coefficient induced by temperature changes resulted in different IOBC class shifts
187 (Table 4). As a consequence, insecticides ranked as harmless or slightly harmful (class 1)
188 under low temperatures (10-15 °C) were thus classified as moderately harmful and harmful
189 (class 2 and 3, respectively) for temperatures above 20°C. Interestingly, spinosyn insecticides
190 (i.e., spinosad and spinetoram) showed the highest reduction coefficients in all the
191 temperature regimes (Table 4).
192
193 **4. Discussion**

Generalist mirid predators breed naturally in economically important horticultural crops and are largely used for biological control applications worldwide, despite their controversial zoophytophagous behavior (Alomar et al., 2006; Zappalà et al., 2013; Biondi et al., 2016; Mansour et al., 2018; Sanchez et al. 2018; Ingegno et al., 2019; Siscaro et al. 2019). In the West Palaearctic area, *M. pygmaeus* successfully controls several key pests, including whiteflies and the invasive South American tomato pinworm (Calvo et al., 2009; Campos et al., 2017; Ziaei Madbouni et al., 2017a; Biondi et al., 2018; Han et al., 2018; Cherif et al., 2019). Several studies have assessed the non-target risks of pesticides on *M. pygmaeus* and similar mirid predators (Arnó and Gabarra 2011; Zappalà et al., 2012; Martinou et al., 2014; Martinou and Stavrinides 2015; Ziaei Madbouni et al., 2017b; Papanikolaou et al. 2018; Passos et al., 2018; Soares et al., 2019a; Soares et al., 2019b); however, none of them have investigated the combined effect of chemicals and increasing temperatures.

The impact of anthropogenic-induced climate change on terrestrial organisms has led to increasing concerns regarding the challenge it may pose for toxicological studies on agricultural systems (Lavalle et al., 2009; IPCC, 2013). In the present study, we investigated the combined stress effects of temperatures and insecticides on *M. pygmaeus*, a generalist mirid predator widely used in tomato production agroecosystems. Taking into account the effect of temperature regimes (i.e., the control results), an increase in the predator mortality was observed. Our results showed that exposure to the highest temperature (40°C) that is likely to be experienced by mirid predators in tomato cropping systems in the Southern Mediterranean (Siscaro et al. 2019), resulted in high mortality rates. By contrast, the highest fertility occurred at intermediate temperatures, as 25°C is the optimum temperature for egg laying. However, the fertility of predators suffered sharp decreases within a five-degree range shift from the optimum.

Insects are ectotherm organisms and their physiology, behavior and fitness are influenced by the environmental temperature (Hamby et al., 2016; Chen et al., 2019; Sánchez-Ramos et al., 2019). As a consequence, the biological impact of rising temperatures strictly depends on the insect's physiological sensitivity to temperature fluctuations (Neven, 2000; Colinet and Hance, 2009; Colinet et al., 2015; Sulmon et al., 2015). For example, vital motor patterns become deficient in meeting the increased oxygen demand in social insects facing high temperatures (Robertson, 2004; Armstrong and Robertson, 2006; Verberk et al., 2013). Changes in nervous function and metabolic pathways can affect the endocrine system (Verberk et al., 2013; King and MacRae, 2015), inducing alterations in insect development and reproduction (Neven, 2000). Thermal performance curves in insects are typically nonlinear and asymmetric depending on whether the temperature varies above or below the optimal range (Ruel and Ayeres, 1999; Angilletta, 2009; Collinet et al., 2015). Changes in reproductive outputs after stressful temperatures in *M. pygmaeus* might be due to impaired oocyte development (Gruntenko et al., 2003), decreased mating success (Collinet and Hance, 2009), impaired sperm production, and/or decreased sperm viability (Rinehart et al., 2000). Moreover, in various insect species, many kinds of heat shock proteins may be upregulated due to heat or cold stresses (Shen et al., 2014), and thermoregulation is often achieved with negative physiological consequences at the reproductive level (Silbermann and Tatar, 2000; Hercus et al., 2003).

The use of pesticide ERA on non-target arthropods has long relied on endpoints directly related to key demographic processes, such as survival, growth and reproduction under standardized environmental conditions (Jam and Saber, 2018; Tanning et al., 2019; Varikou et al., 2019). However, little is known about the combined effects of environmental stressors (e.g., temperature) and chemical toxicants in the context of global climate change (Fisher et al., 2013). Pesticide-temperature interactions are very complex because temperature

243 can affect the active substances (Ishaaya, 1993; Glunt et al., 2013; Khan and Akram, 2014),
244 the metabolism of the species and/or both (Perdikis and Lykouressis, 2000; Vivan et al., 2003;
245 Sanchez et al., 2009; Pazyuk et al., 2014). Yu et al. (2012) found that triazophos-treated males
246 of *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) under high temperatures through mating
247 may enhance the fecundity of treated females more than untreated males. Saeed et al. (2018)
248 discovered pyrethroid and spynosin-based insecticides to be more effective in controlling
249 *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) under low temperatures than
250 organophosphates and neonicotinoids, indicating that the latter are not suitable for protecting
251 crops under cold conditions.

252 In our experiments, exposure to insecticides at increasing temperatures may have had
253 a synergistic effect on predator mortality because of the combined action of the two stressors.
254 The cumulative effect of pesticides and temperatures was significant above 20°C and
255 increased for spinosyn-based formulations in comparison to anthranilic diamides. In addition,
256 the exposure to insecticides significantly reduced the progeny at increasing temperature
257 regimes. Our results for the two spinosyns confirmed their acute toxicity and sublethal effects
258 for insect species belonging to different taxa. Although many toxicological studies have
259 assessed the risk of spinosad on natural enemies, showing various negative physiological and
260 behavioral effects (Biondi et al., 2012a; Varikou et al., 2019), only a few have focused on
261 spinetoram assessed its lethal and sublethal effects on beneficial arthropods.

262 Abbes et al. (2015) found a high mortality caused by spinetoram in the generalist
263 parasitoid, *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), while the survivorship of
264 two other parasitoid species, *Ganaspidium nigrimanus* (Kieffer) (Hymenoptera: Figitidae) and
265 *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), decreased significantly
266 after exposure to spinetoram (Hernández et al., 2011). Together these results suggest that the

newer spinosyn, similarly to spinosad, does not have a positive toxicological for natural insect enemies.

In line with previous studies, the two diamides showed a safer ecotoxicological profile than the spinosyns because their acute toxicity was lower across the tested temperature range and, chlorantraniliprole was less toxic than cyantraniliprole. Chlorantraniliprole has been reported to have no or negligible adverse effects on the predatory bugs, *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Biondi et al., 2012b), *M. pygmaeus* (Martinou et al., 2014) and on *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) (Zhang et al., 2015). Similarly, no negative impact of the cyantraniliprole insecticide has been detected for several natural insect enemies, such as *B. nigricans* (Abbes et al., 2015), *O. insidiosus*, *O. pumilio* (Funderburk et al., 2013), *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) (Liu et al., 2012), also including three predatory mites (Reis et al., 2014). For these reasons and in agreement with our findings, we suggest that diamide insecticides should be included in IPM programs for tomato crops.

In addition to the application rate and the mode of action of the chemicals (Desneux et al., 2007; Guedes et al., 2016; Tanining et al., 2019), insect physiological stresses triggered by pesticides can be influenced by environmental factors, such as temperature, resulting in multiple stressor effects (Noyes et al., 2009; Schiedek et al., 2007; Fisher et al., 2013). In our study, the lack of a combined effect of the insecticides and temperature at the lowest thermal regime (10°C) followed by the synergetic action under higher temperatures, in particular for spinosyns, suggest a positive effect of the temperature on the insecticide's bioactivity (Harwood et al., 2009; Khan and Akram, 2014; Whiten and Peterson, 2015; Michalko and Košulič, 2016; Maliszewska and Tęgowska, 2017). Although heat can alter pesticide properties and increase the constitutive toxicity, the lethal effect caused by spinosad has been

reported to be greater at lower temperatures than higher temperatures (Khan and Akram, 2014).

Conversely, the increased toxicity recorded for *M. pygmaeus* at the higher temperatures tested could be a consequence of a temperature-dependent increased activity of the predator (Ziaei Madbouni et al., 2017a; Siscaro et al. 2019) on contaminated leaves, which could have increased the chemical uptake through a wider plant surface covered by the insect (Gul et al. 2019). Similarly, if the increased temperature determines a higher leaf intake, we could also hypothesize that the toxicity to target pests could change accordingly in the absence of pest-resistance phenomena. Given that these aspects could have different practical consequences, further studies are needed on the behavior of pests and their associated trophic networks under multiple stressors in order to establish appropriate IPM strategies.

5. Conclusions

Our results indicate that future ecotoxicological studies should also consider natural stressors, and their effects on the demographic traits of pest and beneficial insects as well as their interactions. In addition, our review of the literature highlights the importance of integrating the impact of abiotic factors, demographic traits, long-term effects on beneficial insects and the possibilities of post-treatment recovery in insecticide risk assessment protocols.

In conclusion, the decision to use the four tested insecticides should be carefully considered on a case-by-case basis in relation to the environmental temperatures. Further studies are warranted on the non-target toxicity of these modern insecticides to the beneficial insects of horticultural crops in order to better understand their potential impact on both the pests and their natural enemies in a multiple stressor scenario.

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626 **Table 1.** Insecticides tested for their side effects on *Macrolopus pygmeus* under different
627 temperature regimes.
628 [1] The maximum label rate for greenhouse tomato crop protection was applied
629 [2] Insecticide mode of action classification according the Insecticide Resistance Action
630 Committee (IRAC), www.irac-online.org.
631

Active ingredient (insecticide class)	% active ingredient	Trade name	FApplication rate[1]	Crops	Target pests	Mode of action (IRAC group[2])	No. of maximum application per year
Chlorantraniliprole (Anthranilic diamide)	35	Rynaxypyr Altacor® Du Pont de Nemours	(120 g ha^{-1}	Tomato, sweet pepper, eggplant, cucurbits, Brassica leafy vegetables, leafy vegetables, French beans, carrot, radish, fresh herbs	Coleoptera, Diptera, Lepidoptera	IRyanodine receptor modulator (Group 28)	2
Cyantraniliprole (Anthranilic diamide)	10.2	Cyazypyr Exirel® Du Pont de Nemours	(1500 mL ha^{-1}	Brassica crops, bulb vegetables, cucurbits, fruiting vegetables, leafy vegetables, bushberries, citrus fruit, pome fruit, stone fruit, tree nuts	Lepidoptera, Coleoptera, leafminers, fruit flies, aphids, psyllids, thrips, whiteflies	Ryanodine receptor modulator (Group 28)	2
Spinetoram (Spinosyn)	12	Radiant® SC Dow Agrosciences	(300 mL ha^{-1}	Asparagus, Brassica crops, bulb vegetables, cereal grains, corn, cotton, curcubits, fruiting vegetables, herbs, leafy vegetables, legume vegetables, peanut, pineapple, root and tuber vegetables, strawberry, soybean, spices	Moths, thrips, leafminers	DNicotinic acetylcholine receptor channel agonist (Group 5)	1
Spinosad (Spinosyn)	44.2	Laser® Dow Agrosciences	(250 mL ha^{-1}	Tomato, eggplant, sweet pepper, strawberry, potato, fennel, legumes, garlic, onion, leek, stone fruits, cucurbit crops, artichoke, leafy vegetables, caper, pome fruits, stone fruits, grape, small fruits, tree nuts, ornamental plants, grass	Moths, thrips, leafminers, fruit flies	Nicotinic acetylcholine receptor channel agonist (Group 5)	3

632 **Table 2.** Regression parameters for corrected mortality with the increasing temperature
633 estimated for *Macrolophus pygmaeus* exposed separately to four insecticide residues for three
634 days.
635

Insecticide	Model	Parameters		*t*	*P*	R^2
		a	*b*			
All insecticides		1.989	-4.613	-0.980	<0.001	0.350
Chlorantraniliprole		1.602	-11.239	4.998	<0.001	0.297
Cyantraniliprole	$y=ax+b$	1.532	-1.125	5.008	<0.001	0.298
Spinetoram		2.152	3.216	6.115	<0.001	0.394
Spinosad		2.594	-6.718	9.892	<0.001	0.629

636

637 **Table 3**. Regression parameters for reduction coefficients (*Ex*) in number of nymphs with the
638 increasing temperature estimated for *Macrolophus pygmaeus* exposed separately to four
639 insecticide residues for three days.
640

Insecticide	Model	Parameters		*t*	*P*	R^2
		a	*b*			
All insecticides		0.162	-16.442	11.458	<0.001	0.355
Chlorantraniliprole		0.137	18.745	4.648	<0.001	0.268
Cyantraniliprole	$y=ax+b$	0.149	18.070	4.416	<0.001	0.248
Spinetoram		0.184	14.439	6.675	<0.001	0.430
Spinosad		0.216	11.410	9.135	<0.001	0.598

641

642 **Table 4.** Reduction coefficient *Ex* (mean±SE) (Biondi et al. 2012a) and IOBC toxicity classes (Sterk et al. 1999) estimated for *Macrolophus*
643 *pygmaeus* exposed to insecticide residues for three days.
644

Insecticide	*10 °C*		*15 °C*		*20 °C*		*25 °C*		*30 °C*		*35 °C*		*40 °C*	
	Ex	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**	***Ex***	**IOBC toxicity class**
Chlorantraniliprole	6.60 ± 4.11	1	8.96 ± 3.88	1	75.11 ± 11.76	2	39.68 ± 12.59	2	65.30 ± 11.10	2	36.30 ± 9.81	2	75.20 ± 13.31	2
Cyantraniliprole	4.88 ± 2.35	1	19.95 ± 5.91	1	76.40± 9.69	2	45.12 ± 9.20	2	52.10 ± 9.76	2	53.40 ± 8.16	2	63.26 ± 15.60	2
Spinetoram	14.13 ± 4.42	1	23.49 ± 7.81	1	80.26 ± 5.90	3	40.82 ± 8.96	2	85.94 ± 2.87	3	64.02 ± 13.06	2	86.34 ± 8.95	3
Spinosad	17.45 ± 7.03	1	20.73± 4.46	1	83.63 ± 6.96	3	46.69 ± 9.26	2	90.45 ± 2.91	3	90.77 ± 4.05	3	93.75 ± 4.98	3

645

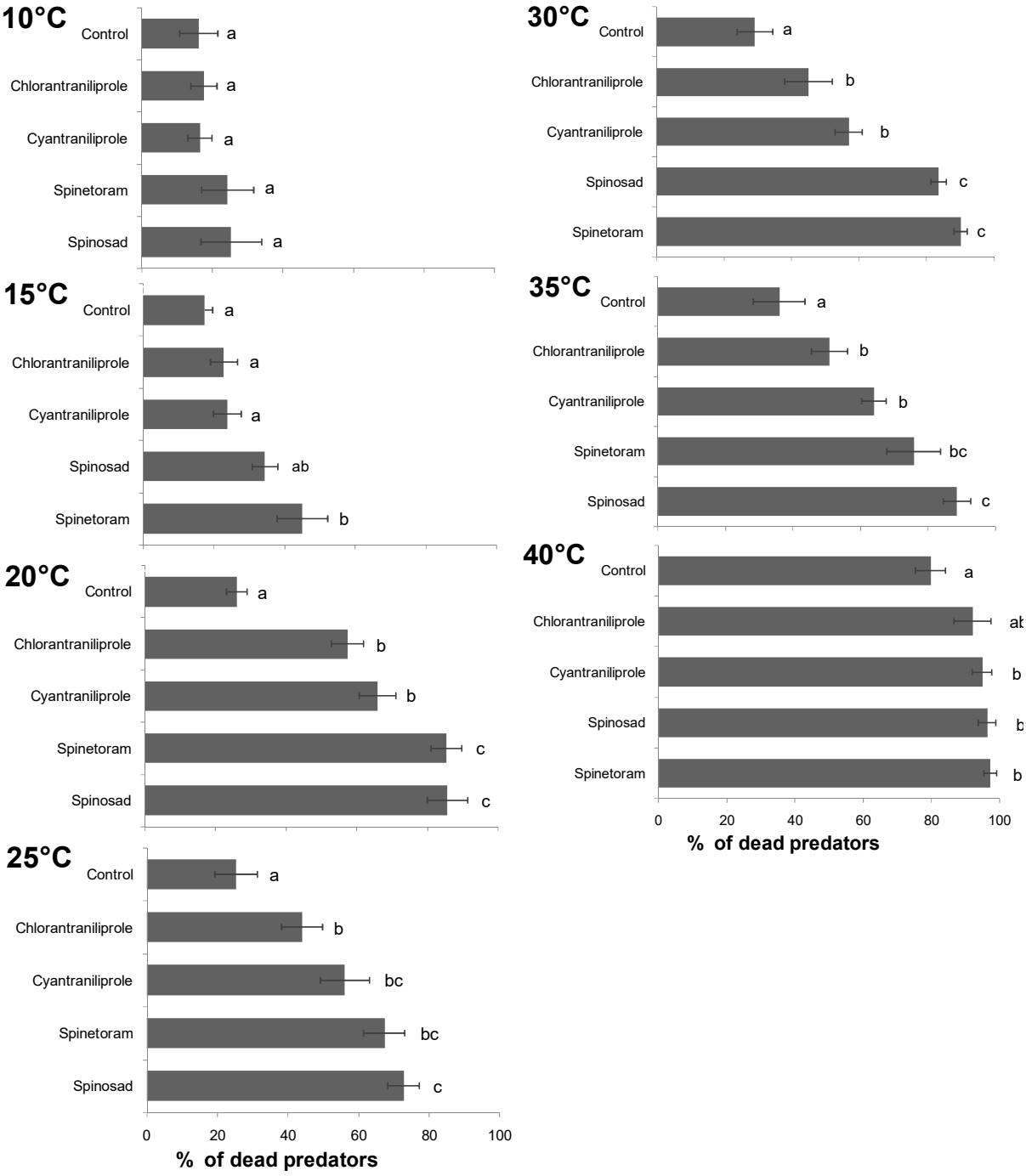
646 **Figure captions**

647 **Figure 1**. Mean (±SE) of mortality percentages of *Macrolophus pygmaeus* after three days of
648 exposure to insecticide residues on tomato sprouts under seven different temperature regimes.
649 For each sub-figure, bars followed by the same letter are not significantly different ($P \leq 0.05$;
650 ANOVA with Tukey HSD post hoc test for multiple comparisons).

651 **Figure 2.** Correlation curves of the corrected mortality with the increasing temperatures
652 estimated for *Macrolophus pygmaeus* exposed separately to four insecticide residues for three
653 days.

654 **Figure 3.** Total mean number (±SE) of nymphs produced of *Macrolophus pygmaeus* after three
655 days of exposure to insecticide residues on tomato sprouts. For each sub-figure, bars followed
656 by the same letter are not significantly different ($P < 0.05$; ANOVA with Tukey HSD post hoc
657 test for multiple comparisons).

658 **Figure 1**



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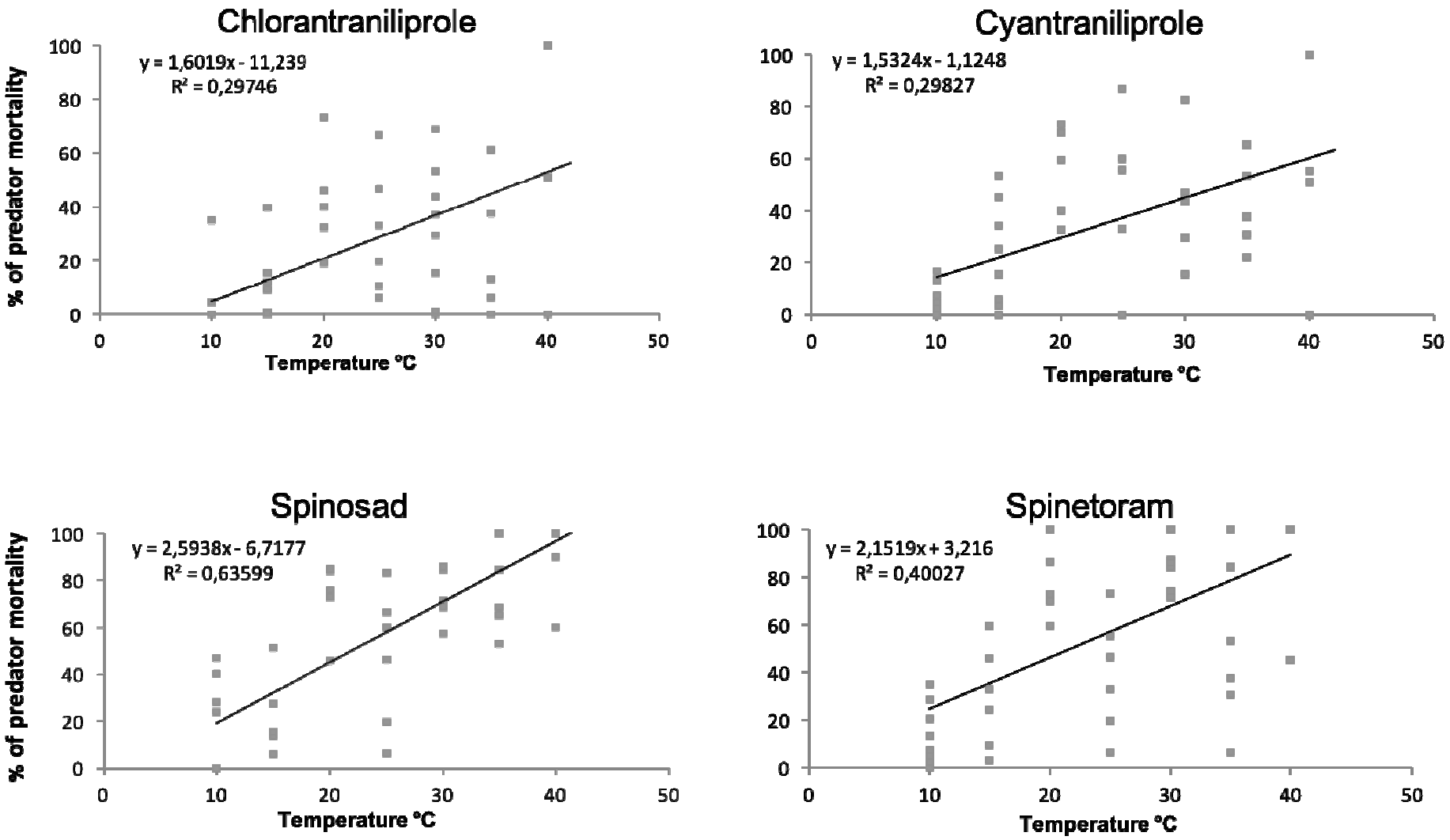
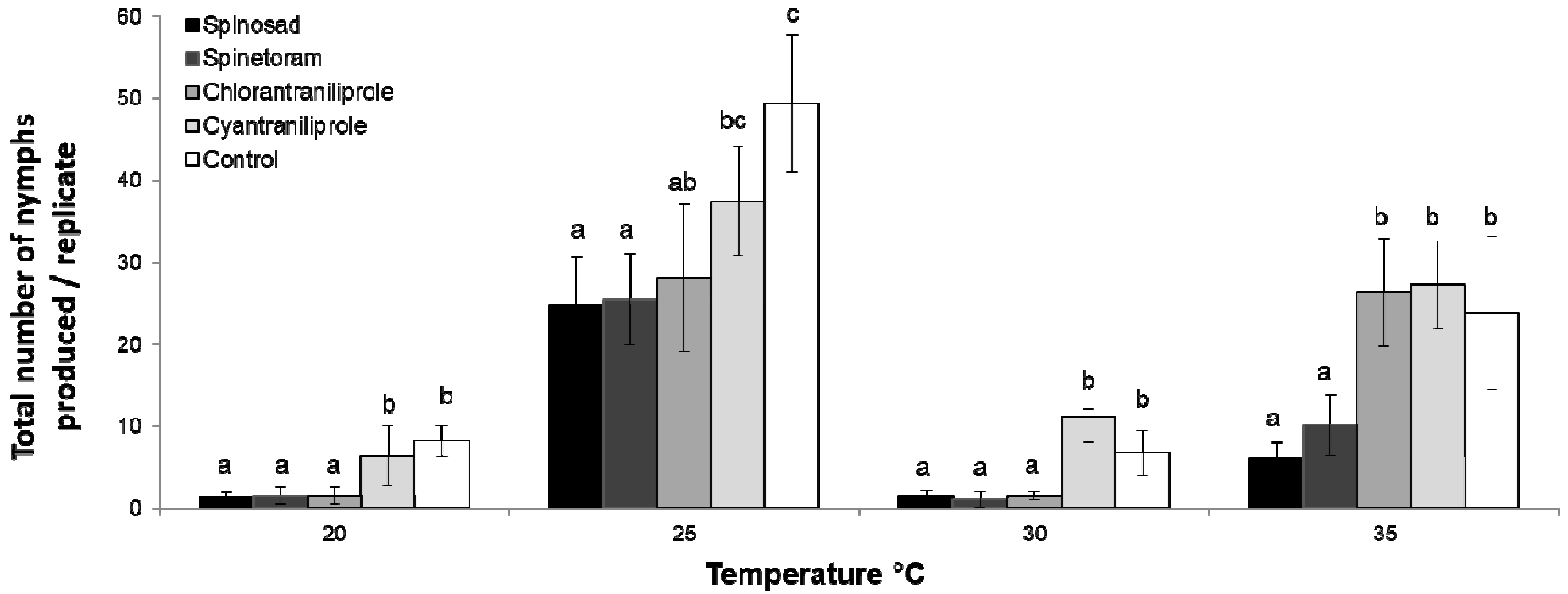
660

661 **Figure 2**



662

663 **Figure 3**



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