

1 **Chronic exposure to a neonicotinoid pesticide and a** 2 **synthetic pyrethroid in full-sized honey bee colonies**

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14 **ABSTRACT**

15 In the last decade, the use of neonicotinoid insecticides increased significantly in the
16 agricultural landscape and meanwhile considered a risk to honey bees. Besides the exposure
17 to pesticides, colonies are treated frequently with various acaricides that beekeepers are forced
18 to use against the parasitic mite *Varroa destructor*. Here we have analyzed the impact of a
19 chronic exposure to sublethal concentrations of the common neonicotinoid thiacloprid (T) and
20 the widely used acaricide τ -fluvalinate (synthetic pyrethroid, F) - applied alone or in
21 combination - to honey bee colonies under field conditions. The population dynamics of bees
22 and brood were assessed in all colonies according to the Liebefeld method. Four groups (T, F,
23 F+T, control) with 8-9 colonies each were analyzed in two independent replications, each
24 lasting from spring/summer until spring of the consecutive year. In late autumn, all colonies
25 were treated with oxalic acid against Varroosis. We could not find a negative impact of the
26 chronic neonicotinoid exposure on the population dynamics or overwintering success of the
27 colonies, irrespective of whether applied alone or in combination with τ -fluvalinate. This is in
28 contrast to some results obtained from individually treated bees under laboratory conditions
29 and confirms again an effective buffering capacity of the honey bee colony as a

30 superorganism. Yet, the underlying mechanisms for this social resilience remain to be fully
31 understood.

32

33 **1 INTRODUCTION**

34 Neonicotinoid pesticides are among the most used insecticides during the past decades and are
35 dominating the global market for insecticidal seed dressings (Jeschke et al., 2011; Simon-
36 Delso et al., 2015). However, these neonicotinoids are suspected to be a main driver for the
37 decline of honey bees (Hopwood et al., 2016), wild bees (Potts et al., 2010) and even non-
38 target wildlife in general (Goulson, 2013). Recently, the European Food Safety Authority
39 (EFSA) has updated their risk assessment and now considers the three neonicotinoids
40 imidacloprid, clothianidin and thiametoxam to be “a risk for bees” and suggested suitable
41 amendments to the European Commission (EFSA, 2018). These three nitro-substituted
42 compounds have the highest toxicity to bees among the class of neonicotinoids (Iwasa et al.,
43 2004) and have been already banned for the use in flowering crops by the European Union
44 since the year 2014 (EFSA, 2013).

45 However, other neonicotinoid insecticides with a far lower toxicity to bees - for instance
46 thiacloprid and acetamiprid - are still widely used not only as seed dressings but are even
47 approved as foliar spray in blooming cultures like oilseed rape (Schmuck et al., 2003). This
48 leads to a remarkable high contamination of nectar and pollen and foragers might therefore be
49 continuously exposed to these agents (Genersch et al., 2010; Collison et al., 2016; Rolke et
50 al., 2016; Böhme et al., 2017). There is no doubt about the comparable low acute toxicity of
51 these compounds to bees, however there is a controversial discussion on sublethal and long-
52 term effects. So, it has been shown that thiacloprid can affect the sensitivity of honey bees to
53 the gut parasite *Nosema ceranae* (Vidau et al., 2011; Pettis et al., 2013; Retschnig et al.,
54 2015). More recent publications indicate that sublethal concentrations of thiacloprid alter their
55 social behavior (Forfert and Moritz 2017) and, more importantly, disturb the orientation of
56 foragers (Fischer et al., 2014; Tison et al., 2016, 2017). These studies have been conducted on
57 the level of individual or small groups of bees by performing cage tests or semi-field trials
58 under rather artificial conditions. Therefore, they do not cover important attributes of a social
59 entity, with a more complex perception to its environment. Hence, the transfer of these results
60 to field conditions must be taken with caution. Significantly, the only field study available so
61 far could not confirm negative effects of thiacloprid at the colony level (Siede et al., 2017).

62 Another controversial point is the possible interaction of thiacloprid - considered as “non-
63 toxic for bees” - with active compounds of other chemical classes that are applied by
64 beekeepers to control the parasitic mite *Varroa destructor*, requiring multiple annual
65 treatments (Rosenkranz et al., 2010). In an effective and easy to use application, synthetic
66 pyrethroids were, amongst others, introduced to beekeepers (Watkins, 1997) and are besides
67 the formamidine amitraz the most frequently used acaricides in apiculture (Garrido et al.,
68 2016). The exposure of honey bee colonies to a combination of sublethal doses of such
69 pesticides may increase the susceptibility to pathogens and are suspected to contribute to the
70 worldwide health problems of honey bee colonies (Cornman et al., 2013; Matsumoto, 2013;
71 Wu et al., 2012). To study such possible combination effects we have chronically exposed
72 full-sized colonies to the neonicotinoid thiacloprid and the synthetic pyrethroid τ -fluvalinate
73 (Apistan[®]) in a two-year field study. To our knowledge this is the first study that analyzes the
74 effect of a chronic application of both, a neonicotinoid insecticide and a common acaricide
75 under realistic field conditions at the colony level. An exposure to these two pesticides is very
76 likely under common beekeeping conditions in rural areas. Our crucial endpoints were (i) the
77 overwintering success of treated colonies compared to untreated controls and (ii) the colony
78 population dynamics.

79 **2 MATERIALS & METHODS**

80 **2.1 Experimental colonies**

81 For each treatment group, five experimental colonies were established in early May of the
82 year 2010. The experiment was repeated with three to four new colonies per group in the year
83 2011 (Tab. 1). All colonies were set up at our local apiary at the agricultural experimental
84 station Kleinhohenheim, which is an organic farming facility not using any agro chemicals or
85 common pesticides at all. To standardize our experiment, we used artificial swarms made
86 from stock colonies that were screened for low *Varroa* infestation and lack of virus infections
87 prior to the trials. Freshly reared and mated sister queens of the Hohenheim breeding line
88 were provided to each swarm, respectively. After the colonies successfully showed the first
89 open brood stages, we sprayed all of them with a 3.5 % oxalic acid sugar solution for *Varroa*
90 treatment to have a comparable low mite infestation for all experimental groups at the start of
91 the experiment. We used residue free beeswax foundations to minimize the risk of additional
92 contamination through pesticide residues in the wax (Bogdanov et al., 1998; Wallner, 1999).

93 All colonies were set up on one box of 10 Zander frames, which was extended to two boxes
 94 when necessary during the summer season.

95
 96 **Tab. 1:** List of replications, treatment groups, treatment duration, assessment dates (AD) and no. of colonies (N)
 97 at the time of the assessment.

Year	Treatment	Duration [days]	AD 1)	N	AD 2)	N	AD 3)	N	Winter treatment	N	AD 4)	N
2010-2011	Control	56	23. Jul	5	16. Aug	5	8. Oct	5	30. Nov	4	15. Apr	4
	Thiacloprid			5		5		5		3		3
	Fluvalinate			5		5		5		5		5
	Flu + Thia			5		5		5		4		4
2011-2012	Control	62	21. Apr	3	5. Aug	3	13. Oct	3	29. Dec	3	3. Apr	2
	Thiacloprid			4		4		4		4		4
	Fluvalinate			3		3		3		3		3
	Flu + Thia			3		3		3		3		3

98

99

100 2.2 Thiacloprid application

101 For the application of thiacloprid we used the pure substance (98 % purity, Dr. Ehrenstorfer
 102 GmbH), which was sonicated in pure water for a stock solution. We aimed to use a field-
 103 realistic concentration that was approximately 100-fold lower than the oral LD₅₀ for
 104 thiacloprid (173.2 mg/kg, Würfel, 2008). We therefore diluted thiacloprid in sucrose syrup
 105 (Apiinvert, Südzucker GmbH) in order to receive the respective concentration. The final
 106 solution was quantified by an external lab (Eurofins Dr. Specht Laboratorien GmbH,
 107 Hamburg, Germany) which confirmed a thiacloprid concentration of 1.6 mg/kg (= 1,600 ppb).
 108 This feeding solution was applied to the colonies of the specific treatment groups and control
 109 colonies were fed with untreated sucrose syrup. The duration of the treatment in the year 2010
 110 was 56 days (23rd Jul-17th Sep) and in the year 2011 62 days (21st Apr-22nd Jun) during
 111 summer season. In this time period we fed 1 kg syrup per week with an internal feeding
 112 device, to simulate a chronic exposure. A final amount of 8 kg per colony in 2010 and 9 kg in
 113 2011 was administered in the summer season, respectively. Based on the concentration of
 114 1.6 mg/kg we therefore applied a total amount of 12.8 mg thiacloprid per colony in 8 weeks
 115 (2010) and 14.4 mg thiacloprid per colony in 9 weeks (2011) during the summer season,
 116 respectively. The treatment was resumed when colonies were fed for overwintering at the end
 117 of the season. Every colony was fed with approximately 15 kg of the feeding solution with a
 118 total amount of 24.0 mg thiacloprid in each year for winter feeding. After the treatment period

119 in summer, a pooled sample of food (nectar/honey) from the combs was analyzed for residues
120 at Eurofins Dr. Specht Laboratorien GmbH.

121 **2.3 τ -fluvalinate application**

122 Apistan[®] strips (Vita Europe Ltd, Basingstoke, UK) were used for the τ -fluvalinate treatment.
123 As recommended, one strip per box was applied to the τ -fluvalinate treatment groups during
124 the same time of the thiacloprid application. After the treatment period, a pooled sample of
125 beeswax was analyzed for residues at our own lab in Hohenheim. During overwintering, the
126 strips were again inserted to the colonies to resume a chronic treatment.

127 **2.4 Assessment of population dynamics**

128 The amount of bees and brood cells (open and sealed) were estimated with the Liebefelder
129 Method (Imdorf et al., 1987), which is a feasible tool that provides accurate and reliable
130 results at the colony level (measuring error +/- 10 %). Care was taken that all colonies were
131 evaluated by the same person on all dates to minimize variation. Colony assessments were
132 usually conducted in the morning before bee flight.

133 **2.5 *Varroa* winter treatment**

134 In order to monitor the level of mite infestation in the colonies and to measure the
135 effectiveness of the τ -fluvalinate treatment, we applied 3.5 % oxalic acid sugar solution to the
136 bees in a brood free stage during late autumn or winter time (30th Nov in 2010 and 29th Dec in
137 2011). In both years the temperature was below 3 °C for optimal application to a closely
138 spaced bee cluster. Dead mites were counted approximately one week after the treatment with
139 a sticky board, which was inserted at the same day of treatment, respectively.

140 **2.6 Statistical analysis**

141 The estimated number of bees and brood cells from both years were checked with a Shapiro-
142 Wilk test for normal distribution ($p > 0.05$). Therefore, a one-way ANOVA and a multiple
143 comparison of the means with a post-hoc Bonferroni correction were performed on the four
144 experimental groups, respectively ($\alpha = 0.05$).

145 All tests were performed using WinSTAT (R. Fitch Software, Bad Krozingen).

146

147 **3 RESULTS**

148 **3.1 Overwintering success**

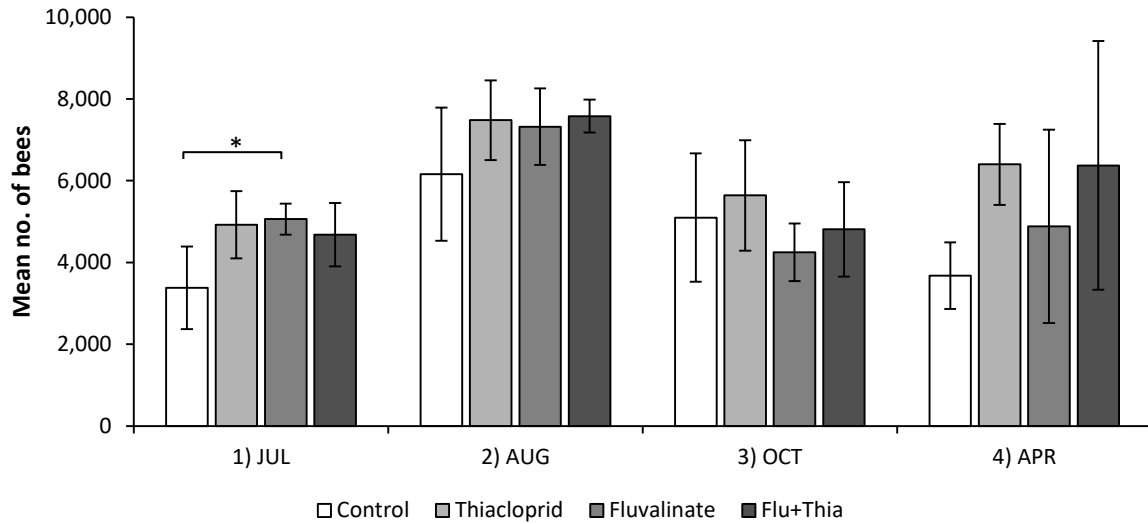
149 In both years, none of the colonies died until the start of wintering in October (Tab. 1). Taken
150 both years together, a total of five of the 33 colonies died over winter. Two of the
151 “Thiacloprid” group (N = 9), one of the “Flu+Thia” group (N = 8), two of the “Control”
152 group (N = 8) and none of the “Fluvalinate” group (N = 8; Tab. 1).

153 **3.2 Population dynamics**

154 **3.2.1 Experiment 1 (2010 - 2011)**

155 The population of bees and brood cells were estimated four times during the whole season
156 (Tab. 1). The results are shown in Fig. 1a for the number of bees and in Fig. 1b for the
157 number of brood cells. We compared the four treatment groups for each date of the estimates
158 and could not see significant differences (ANOVA) for the number of bees in August 2010
159 (“AUG”; $p=0.254$), October 2010 (“OCT”; $p=0.473$) and April 2011 (“APR”; $p=0.388$).
160 Likewise, no significant differences of the amount of brood cells were recorded in October
161 2010 (“OCT”; $p=0.590$) and April 2011 (“APR”; $p=0.128$). However, in July the number of
162 bees of the “Control” were significantly lower compared to “Fluvalinate” ($p=0.029$,
163 ANOVA). The number of brood cells of the “Control” was significantly lower compared to
164 “Thiacloprid” and “Flu+Thia” in July ($p=0.012$, ANOVA) and compared to “Thiacloprid” in
165 August ($p=0.004$, ANOVA).

166



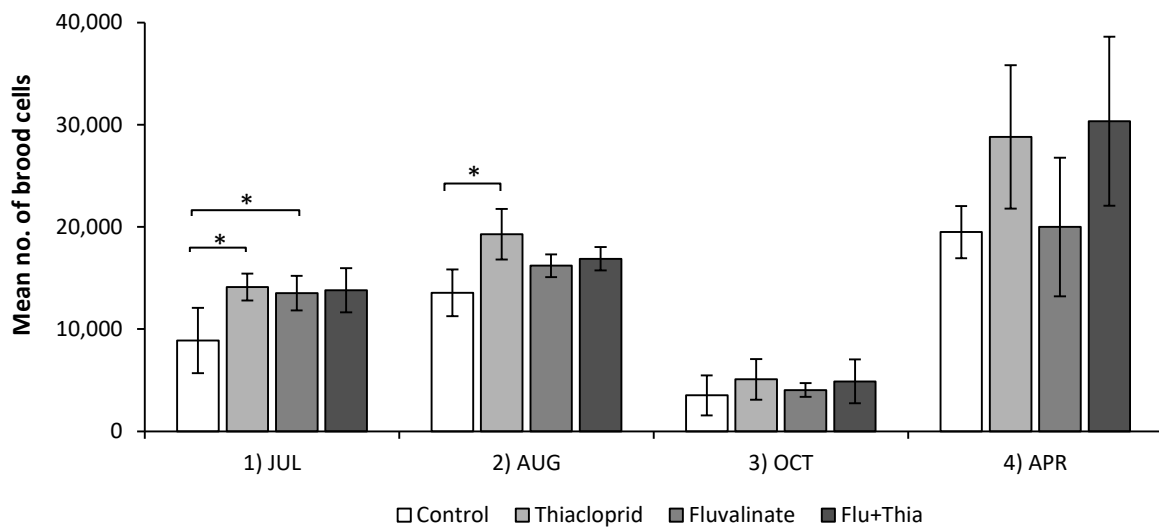
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168 **Fig. 1a:** Number of bees estimated in the colonies in the year 2010-2011 for the four treatment groups at four
169 different assessments. * statistically significantly lower when “Control” compared to “Fluvalinate” ($p < 0.05$,
170 ANOVA).

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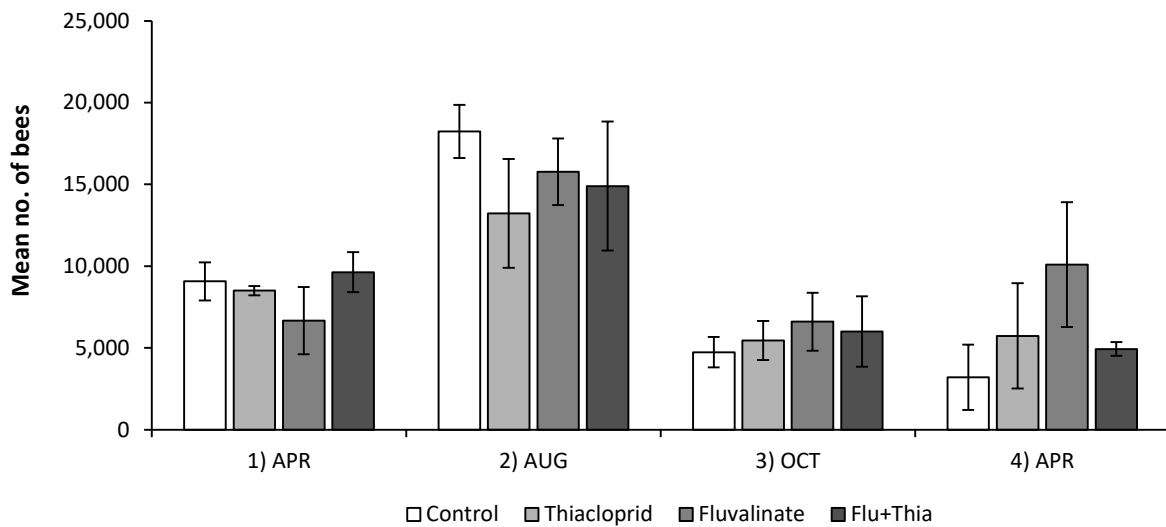
175 **Fig. 1b:** Number of brood cells estimated in the colonies in the year 2010-2011 for the four treatment groups at
176 four different assessments. * statistically significantly lower when “Control” compared to “Thiacloprid” and
177 “Fluvalinate” ($p < 0.05$, ANOVA) in 1), and when “Control” compared to “Thiacloprid” ($p < 0.05$, ANOVA) in 2).

178

179 **3.2.2 Experiment 2 (2011 - 2012)**

180 For the replicate of experiment 1, also four assessments were performed throughout the
181 season. The results are shown in Fig. 2a for bees and in Fig. 2b for brood. We again compared
182 the four groups within each assessment but could not see any significant differences for the
183 number of bees (April 2011 $p=0.174$; August 2011 $p=0.367$; October 2011 $p=0.664$; April
184 2012 $p=0.198$) and no significant differences for the number of brood cells in April 2011
185 ($p=0.071$), October 2011 ($p=0.328$) and April 2012 ($p=0.176$; ANOVA). Solely, in August
186 2011, the number of brood cells in “Thiacloprid” was significantly lower compared to
187 “Control” and “Fluvalinate” ($p=0.017$, ANOVA).

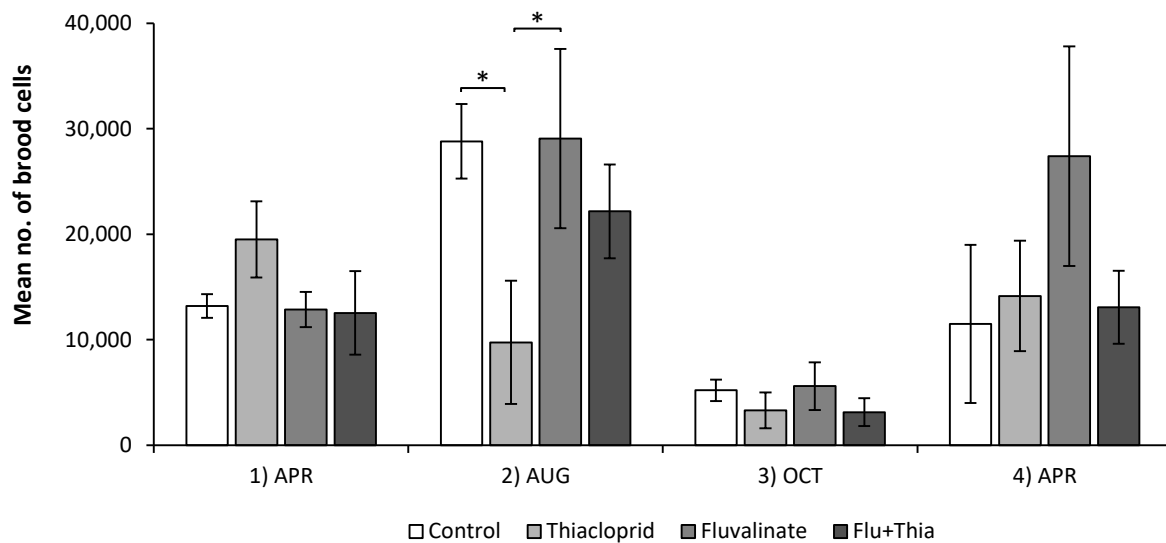
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190 **Fig. 2a:** Number of bees estimated in the colonies in the year 2011-2012 for the four treatment groups at four
191 different assessments. We could not see statistically significant differences within the assessments ($p>0.05$,
192 ANOVA).

193



194

195 **Fig. 2b:** Number of brood cells estimated in the colonies in the year 2011-2012 for the four treatment groups at
196 four different assessments. * statistically significantly lower when “Thiocloprid” compared to “Control” and
197 “Fluvalinate” ($p < 0.05$, ANOVA) in 2).

198

199 3.3 Thiocloprid residues

200 Food from the syrup feeding, which was processed by the bees and stored in honeycombs,
201 was analyzed for thiacloprid residues in both years with QuEChERS method (Limit of
202 Quantification LOQ = 0.01 mg/kg). For the analysis, samples from all colonies and the
203 respective groups per year were pooled. All groups without thiacloprid treatment did not have
204 measurable residues in both years. The pooled samples from the “Thiacloprid” and
205 “Flu+Thia” groups had residues of 0.11 mg/kg and 0.20 mg/kg, respectively, in the year
206 2010-2011 and 0.29 mg/kg and 0.19 mg/kg, respectively, in the year 2011-2012 (Tab. 2).

207

208 3.4 τ -fluvalinate residues

209 Beeswax was analyzed for τ -fluvalinate residues in both years by solid-phase extraction (SPE)
210 and GC-ECD (LOQ = 0.5 mg/kg). For the analysis, samples from all colonies and the
211 respective groups per year were pooled. All groups without τ -fluvalinate treatment did not
212 have measurable residues in both years. Pooled samples from the “Fluvalinate” and
213 “Flu+Thia” groups had residues of > 100 mg/kg and 16.7 mg/kg, respectively, in the year
214 2010-2011 and 14.3 mg/kg and 31.6 mg/kg, respectively, in the year 2011-2012 (Tab. 2).

215

216 **Tab. 2:** Thiacloprid residues in pooled food (syrup) samples, which was processed by the bees and stored in the
 217 honeycombs from all treatment groups in both years (QuEChERS method, LOQ = 0.01 mg/kg). τ -fluvalinate
 218 residues in pooled beeswax samples from all treatment groups in both years (SPE & GC-ECD, LOQ = 0.5
 219 mg/kg).

Year	Treatment	Matrix	Thiacloprid [mg/kg]	Matrix	τ -fluvalinate [mg/kg]
2010- 2011	Control	Food	0	Beeswax	0
	Thiacloprid		0.11		0
	Fluvalinate		0		> 100
	Flu + Thia		0.2		16.7
2011- 2012	Control	Food	0	Beeswax	0
	Thiacloprid		0.29		0
	Fluvalinate		0		14.3
	Flu + Thia		0.19		31.6
Feeding Syrup		Syrup	1.6	-	-

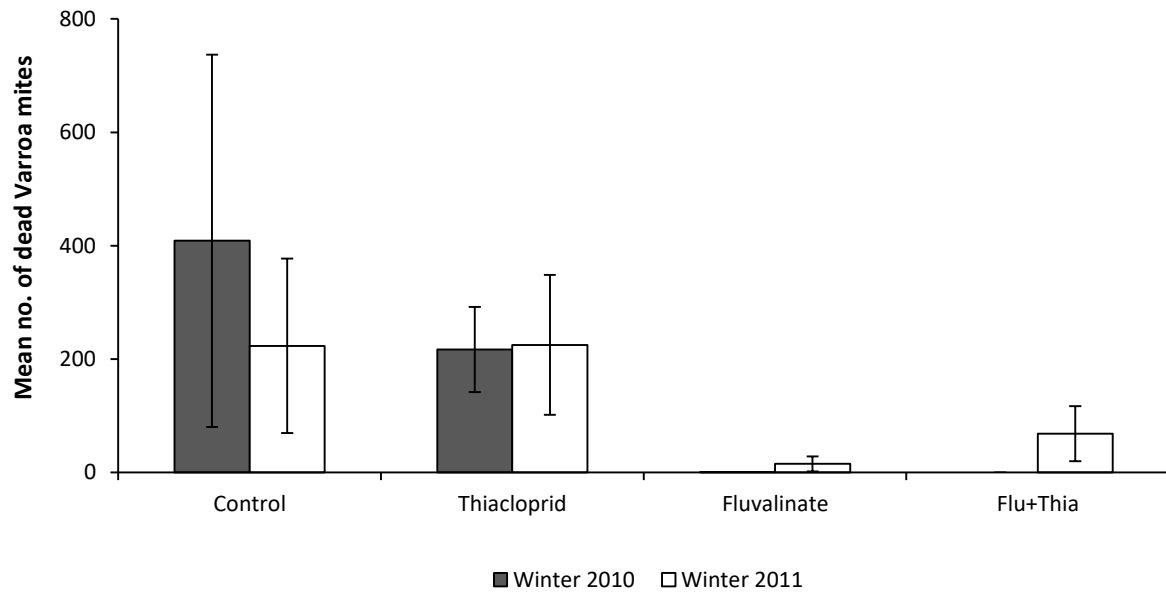
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222 3.5 *Varroa* winter treatment

223 In both years, the winter treatment with oxalic acid killed considerably fewer mites in those
 224 groups that have been continuously treated with the acaricide τ -fluvalinate (Fig. 3). In the
 225 “Control” and “Thiacloprid” groups between 217 to 409 mites were killed through this winter
 226 treatment, on average. In 2010, only one single mite was found in the eight τ -fluvalinate
 227 treated colonies! However, in both τ -fluvalinate treated groups the number of mites killed by
 228 the winter treatment increased in the second year to an average of 15 mites for the
 229 “Fluvalinate” group and 68 mites for the “Flu+Thia” group, respectively.

230



231

232 **Fig. 3:** Graph of the dropped *Varroa* mites approximately one week after oxalic acid treatment during winter
233 time (2010 and 2011). In both years a considerably lower number of dead mites could be detected in the τ -
234 fluvalinate treated vs. the untreated groups.

235

236 4 DISCUSSION

237 We here analyzed the effects of two commonly used pesticides on the population dynamics
238 and the overwintering success of free flying honey bee colonies. The pesticides belong to two
239 different substance classes, one a neonicotinoid insecticide and the other a synthetic
240 pyrethroid widely used as acaricide to combat varroa mites. For both, the insecticide and the
241 acaricide, the applied dosages represent worst case scenarios. Thiacloprid is meanwhile
242 frequently found as residue in pollen and honey, presumably due to the application in
243 flowering oilseed rape and fruit production. Maximum peak concentrations of thiacloprid in
244 bee products such as nectar, honey or pollen range from ~0.05 to 1 mg/kg across the globe
245 (EFSA, 2016; Genersch et al., 2010; Laaniste et al., 2016; Mitchell et al., 2017; Mullin et al.,
246 2012; Pohorecka et al., 2012; Smodis Skerl et al., 2009) but rarely exceed the average level of
247 0.2 mg/kg (reports of the German Bee Monitoring, see Rosenkranz et al., 2016). It should be
248 mentioned that 0.2 mg/kg is also the maximum value for thiacloprid residues accepted for
249 honey in the EU (EFSA, 2016). The continuous long-term feeding of 1.6 mg/kg thiacloprid to
250 our experimental colonies resulted indeed in residue levels of this magnitude ranging from
251 about 0.1 to 0.3 mg/kg in the stored food. It is interesting to note the significant 8-fold-
252 decrease from the concentration in the original feeding syrup to the honey bee processed
253 syrup stored in the honeycombs. This decrease might be due to a dilution effect, as all
254 colonies could forage and had access to various nectar sources. Furthermore, Iwasa et al.
255 (2004) and Brunet et al. (2005) reported that cyano-substituted neonicotinoids such as
256 thiacloprid and acetamiprid appear to be metabolized more quickly by the honey bee
257 compared to nitro-substituted ones (i.e. imidacloprid, clothianidin). The enzyme that
258 metabolizes thiacloprid very efficiently but lacking impact against imidacloprid was recently
259 identified as a single cytochrome P450, CYP9Q3 (Manjon et al., 2018). As we did not analyze
260 metabolites, this could additionally have contributed to decrease the in-hive concentration of
261 the pesticide by bees processing the syrup.

262 For τ -fluvalinate, likewise high maximum residue values are reported. Due to their lipophilic
263 property residues are concentrated and accumulated within the beeswax and can exceed 15
264 mg/kg (Berry et al., 2013) which is in the range of τ -fluvalinate residues in our experimental
265 colonies after long-term treatment with Apistan[®] strips. Bogdanov et al. (1998) confirmed an
266 increase of residues with the duration of the strip exposition with a plateau of about 40 to 60
267 mg/kg after six months whereas other authors found values between 6.6 and 200 mg/kg
268 (Mullin et al., 2010; Adamczyk et al., 2010; Tsigouri et al., 2004).

269 However, even these residue levels of thiacloprid and τ -fluvalinate are considered to have no
270 acute toxicity to bees or brood (Iwasa et al., 2004; Sanchez-Bayo and Goka, 2014). In our
271 worst case approach we examined whether a long-term exposure to field-realistic peak
272 concentrations of the two pesticides - applied alone or in combination - impairs the
273 development of honey bee colonies under field conditions. In two approaches performed in
274 two consecutive years and using an identical experimental setup we could not detect any
275 negative impact of the treatments on the population of bees and brood and on the
276 overwintering of the colonies. Our moderate overwintering losses of about 15 % (20 % in the
277 first and 8 % in the second winter) are within the range of common winter losses in free flying
278 colonies in Germany and United States (Genersch et al., 2010; Lee et al., 2015) and affected
279 all except the “Fluvalinate” group. Probably, the higher mite load in the untreated groups has
280 contributed to these slightly higher overwintering losses. The mite infestation was quantified
281 in late autumn/winter by an oxalic acid treatment which is known to be highly effective
282 against *Varroa* mites, given that bees are in their winter cluster without brood (Rademacher
283 and Harz, 2006). With the treatment we could also verify that the colonies treated with τ -
284 fluvalinate were sufficiently exposed to this compound during the season, resulting in lower
285 dead mite drops compared to the two groups not treated with τ -fluvalinate. Remarkably, in the
286 winter treatment of the second season our colonies already showed signs of an established τ -
287 fluvalinate resistance in the *Varroa* mite population at our apiary. Such resistance was often
288 reported in the past all over the world (Lodesani et al., 1995; Elzen et al., 1999; Gracia-
289 Salinas et al., 2006; Alissandrakis et al., 2017).

290 In both years the population of bees and brood was evaluated eight times in a total of 8 - 9
291 colonies per treatment group. Only in very few cases significant group differences were
292 recorded. In the first year (2010/2011), the control colonies were slightly weaker at the start of
293 the experiment in spring/summer but revealed no differences any more in the autumn and
294 after-winter evaluations. Although all experimental colonies were established from artificial
295 swarms of approximately the same weight it is not unusual that there are small differences in
296 the first weeks of development in newly established honey bee colonies (Imdorf et al., 2008).
297 In the second year (2011/2012) the “Thiacloprid” group had a significant lower number of
298 brood cells in August, however without differences in the two consecutive assessments and
299 without significant effects on the adult bee population. More importantly, there were no group
300 differences at all in the assessments before and after overwintering, indicating no effects of
301 the pesticide treatment on this crucial colony performance. In a previous study performed in
302 observation hives we could already confirm that behavioral traits like flight activity,

303 antennation, grooming and trophallaxis are not affected by the chronic exposure to high
304 concentrations (1 mg/kg) of thiacloprid (Retschnig et al., 2015). The authors therefore
305 assumed a rather weak impact of the pesticide treatment.

306 Our results are also in agreement with a three-year study of Siede et al. (2017) who
307 chronically applied two different thiacloprid concentrations (0.2 mg/kg and 2 mg/kg) and
308 could also not confirm any negative impairment on colony health and winter survival.
309 Interestingly, they also found a significant lower amount of brood cells in colonies fed with
310 the high thiacloprid concentration but equally to our results no effect on the colony strength or
311 overwintering was noticed. In contrast to other neonicotinoids (Blacquiere et al., 2012) there
312 has been no prove of acute toxicity of thiacloprid to brood; however, according to our results
313 and those of Siede at al. (2017) this aspect should be considered in future approaches. Berry et
314 al., (2013) could also show for τ -fluvalinate, that exposure to high concentrations in beeswax
315 did not have measurable effects on the amount of brood, amount of honey, foraging rate, time
316 required for marked bees released to return to their hive, percentage of released bees that
317 return to the hive, and colony *Nosema* spore loads. In addition, we here could prove for the
318 first time that a combination of this acaricide with the neonicotinoid insecticide did not have
319 measurable synergistic effects at the colony level.

320 However, our study is in contrast to many laboratory and semi-field studies providing
321 evidence for negative effects of thiacloprid such as elevated mortality under stress (Doublet et
322 al., 2015) or in combination with pathogens (Vidau et al., 2011), impaired navigation (Fischer
323 et al., 2014), reduced immunocompetence (Brandt et al., 2016), disrupted learning and
324 memory functions (Tison et al., 2017) as well as affected social behavior (Forfert and Moritz
325 2017; Tison et al., 2016). In most of these studies individual bees were exposed to different
326 concentrations of thiacloprid over a certain time period and subsequently challenged to
327 various physiological tests. The findings were then extrapolated to the colony level without
328 confirmation under field conditions. For example, Tison et al. (2016) found foraging behavior
329 and social communication impaired when applying a concentration of 4.5 mg/kg thiacloprid
330 over one week in a free flying feeder experiment. This exposure corresponds to a 23-fold
331 higher concentration than the maximum value for thiacloprid residues accepted for honey in
332 the EU (0.2 mg/kg; EFSA, 2016). It seems unlikely that honey bees are chronically exposed
333 to such high concentrations under realistic field conditions. Additionally, it makes a
334 difference whether pesticides are applied to individual bees under artificial conditions or to
335 bees within a free flying colony. Obviously, the damage threshold of the honey bee colony as

336 a huge social entity is different from the threshold calculated from the effects on individual
337 bees. This “buffering effect” of the colony has frequently been discussed, however without a
338 final explanation of the underlying mechanisms (Straub et al., 2015; Sponsler and Johnson,
339 2017). Recently, Odemer et al. (2018) could demonstrate that even the highly bee toxic
340 neonicotinoid clothianidin is significantly less toxic when applied to bees that are kept within
341 the social environment of a colony.

342 Our results might contribute to the current discussion about the ban of neonicotinoids in
343 agricultural practice which recently led to an assessment of the EFSA considering three
344 neonicotinoids (clothianidin, thiametoxam and imidacloprid) a “risk to bees” (EFSA, 2018). It
345 is an important issue for the agricultural production and for environmental protection, whether
346 neonicotinoids with substantially lower bee toxicity should also be banned. Our results
347 indicate that at least for honey bees the risk is low. It is likely that wild bees or other
348 pollinating insects are more susceptible to thiacloprid as it has been shown already for bumble
349 bees (Ellis et al., 2017), however more field data on the population level of wild pollinators
350 are necessary for a reliable risk assessment of thiacloprid.

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356 **Disclosure statement**

357 No potential conflict of interest was reported by the authors.

358

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