Effects of imidacloprid and thiamethoxam at LC_{30} and LC_{50} on the life table of soybean aphid *Aphis glycines* (Hemiptera: Aphididae)

Running title: Effects of imidacloprid and thiamethoxam on *Aphis glycines*

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Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a main pests of soybean that poses a serious threat to its production. Studies were conducted to understand effects of the different concentrations of the insecticides (imidacloprid and thiamethoxam) on the life table of *A. glycines* to provide vital information for its effective management. We found that the mean generation time, adult and total pre-oviposition periods in *A. glycines* specimens exposed to LC_{50} imidacloprid and thiamethoxam were significantly longer than those in the control group. However, when exposed to LC_{30} imidacloprid and thiamethoxam, the adult pre-ovipositional period was significantly shorter than that in the control group. The mean fecundity per female adult, net reproductive rate, intrinsic rate of increase, and finite rate of increase were significantly decreased in individuals exposed to LC_{30} and LC_{50} concentrations of imidacloprid and thiamethoxam, respectively ($P < 0.05$). Both insecticides produce stress effects on *A. glycines,* and specimens treated with LC_{50} concentrations of the two insecticides exhibited a significant decrease in their growth rates than those treated with LC_{30} concentrations. This study provides data that can be used as a reference to predict the effect of imidacloprid and thiamethoxam on the population dynamics in the field, and agricultural producers could attach importance to prevent stimulation the reproduction made by low-lethal concentrations during actually applying pesticides.

Keywords: imidacloprid, thiamethoxam, soybean aphid, life table, reproduction, population

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was first detected in North America in 2000 and had rapidly spread to north-central and northeastern United States and southeastern Canada. Although the damage from *A. glycines* to soybeans is rarely devastating in Asia, it is considered a primary pest in North America (Ragsdale, Voegtlin, & O'neil, 2004; Wang, Kritzman, Hershman, & Ghabrial, 2006; Ragsdale, Landis, Brodeur, Brodeur, & Desneux, 2011; Hopper et al., 2017).

Foliar insecticides have been commonly used to effectively control *A. glycines* (Lee, 2000). By the late 1990s, neonicotinoid insecticides had been introduced worldwide due to their high efficiency, low toxicity, and wide application range (Srigiriraju, Semtner, & Bloomquist, 2010; Basit, Saeed, Saleem, Denholm, & Shah, 2013). The sales of neonicotinoids accounted for more than 25 % of the global insecticide sales in 2014 (Bass, Denholm, Williamson, & Nauen, 2015). Imidacloprid is a first-generation neonicotinoid (Matsuda, Buckingham, Kleier, Rauh, Grauso, & Sattelle, 2001), and thiamethoxam is a second-generation neonicotinoid and has higher activity, better safety, and longer duration of efficacy (Shi, Wang, Liu, Qi, & Yu, 2017).

The insecticidal effect of a particular insecticide is closely related to its type, application method, and application frequency (Xie et al., 2010). The drift and degradation of neonicotinoids themselves in the fields would make the doses of some aphids exposed to lower then those normally recommended for control. The lower doses permit survival of heterozygotes for resistance thereby fixing a resistant gene in a population more rapidly (Nauen & Denholm, 2005). The risk of the rapid development of resistant populations, secondary pest outbreaks, and rapid deterioration of the ecological environment was greatly

increased (Isman, 2006; Khan, Abbas, Shad, & Afzal, 2014; Hanson et al., 2017; Zhao et al., 2018; Somar, Zamani, & Alizadeh, 2019).

A monitoring study performed in 2008 and 2011 reported an increased adaptation of the cotton aphid *Aphis gossypii* Glover to neonicotinoids in the south-central United States (Gore et al., 2013). In addition, cotton aphids from six different locations in Korea also exhibited adaptation to common neonicotinoids (Koo, An, Park, Kim, & Kim, 2014). Imidacloprid was the first commercial neonicotinoid used to control rice planthoppers in the early 1990s; however, after 10 years of its large-scale use in Asia, the sensitivity of rice planthoppers to imidacloprid decreased (Matsumura et al., 2013; Tao et al., 2019). *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) showed a similar response to conventional neonicotinoids (Luo, Jones, Devine, Zhang, Denholm, Denholm, & Gorman, 2010; Nauen et al., 2014).

To solve the recent problem of the rapid increase in pest adaptation to neonicotinoids, it is of great significance to carry out population dynamic analysis and to formulate an accurate application scheme (Feliciangeli & Rabinovich, 1985; Stark & Wennergren, 1995; Gabre, Adham, & Chi, 2005; Bass et al., 2011; Abbas, Shad, & Shah, 2015; Jan, Abbas, Shad, & Saleem, 2015). In this study, based on the life table of *A. glycines*, we evaluated the effects of imidacloprid and thiamethoxam on the reproductive development and population dynamics of *A. glycines* to provide a reference for the accurate use of these insecticides for controlling *A. glycines* populations.

Materials and methods

Laboratory aphid population and chemical agents

The laboratory strain of *A. glycines* used in this study was originally collected from a soybean field in Harbin, Heilongjiang Province, China. This strain had been cultured in the laboratory for several years and never been exposed to any insecticides. Dongnong 52 soybean plants were used to maintain the strain of *A. glycines* at Northeast Agricultural University, China. Soybean plants were grown in pots (15 cm diameter × 17 cm depth), with six plants in each pot kept at 25 ± 1 ℃, a relative humidity of 65–70%, and a photoperiod of 14:10 (L:D) h. The laboratory aphid colony was maintained in the same environmental conditions as the chamber used for plant germination. Twelve pots with soybean plants were placed in a large tray (70 × 60 cm; L × W). Twice a week, one third of the old aphid-infested soybean plants (i.e., the four oldest pots with an aphid infestation) were removed and replaced with new aphid-free ones. Aphids were transferred by placing infested leaves on uninfested plants. This prevented the accumulation of excessive honeydew and sooty mold and ensured the provision of a homogeneous soybean plant for the aphids to feed on (Menger et al., 2020).

Water dispersible granules of insecticides (70% imidacloprid and 50% thiamethoxam) were purchased from North China Pharmaceutical Group Corporation, Hebei, China and Shaanxi Thompson Biotechnology Co., Ltd., Shaanxi, China respectively. Calcium nitrate, potassium nitrate, potassium dihydrogen phosphate, magnesium sulfate, disodium ethylenediaminetetraacetic acid (disodium EDTA), and streptomycin sulfate were all purchased from Shanghai Alighting Biochemical Technology Co., Ltd., Shanghai, China.

Preparation of culture medium

Non-toxic, transparent plastic Petri dishes (6 cm diameter × 1.5 cm height) were used to perform the bioassay on newly hatched nymphs of *A. glycines* and the life table study. The components of the plant nutrient solution concentrate used to prepare the medium were as follows: calcium nitrate (4.1 g), potassium nitrate (2.5 g), potassium dihydrogen phosphate (0.7 g), magnesium sulfate (0.6 g), 1.54% disodium EDTA aqueous solution (5.0 mL), one million units of streptomycin sulfate (0.05 g), and distilled water (5.0 L). The diluent was obtained by mixing the plant nutrient solution concentrate with distilled water at a ratio of 1:3. Agar was prepared by mixing 1% w/w agar powder with diluent and was boiled while constantly mixing. After cooling for approximately 10 minutes, the warm agar was poured into the Petri dishes to a depth of at least 3–4 mm. At least 10 mm distance was allowed between the top of the agar and the rim of the Petri dishes. The metal tube was used to cut leaf discs from clean, untreated leaves. The leaf discs were 2 mm lesser in diameter than the Petri dishes and were attached to the agar medium with the top-side facing down. A metal tube was sharpened and cleaned regularly to ensure the clean cutting of the leaf discs. *A. glycines* on leaf discs fed on the bottom surface. Each Petri dish was then placed upside down to keep *A. glycines* in a natural feeding state. The incision was kept neat to avoid excessive crushing of the tissue at the edge of the leaves when they were cut. This prevented the leaves from rapidly developing mildew.

Dose response bioassay

The dose response bioassays were conducted with newly hatched *A. glycines* nymphs using a leaf dip method recommended by the Insecticide Resistance Action Committee (IRAC;

106 http://www.irac-online.org/resources/methods.asp). Insecticidal stock solutions were prepared in 1%
107 acetone and further diluted to different concentrations using distilled water containing 0.05 % (v/v)
108 Triton X-100 before using in dose response bioassay. According to the preliminary bioassays, seven
109 concentrations of imidacloprid (19.95 mg a.i./L, 13.70 mg a.i./L, 9.10 mg a.i./L, 6.10 mg a.i./L, 3.47 mg
110 a.i./L, 2.35 mg a.i./L, 1.88 mg a.i./L) and thiamethoxam (29.95 mg a.i./L, 24.98 mg a.i./L, 14.97 mg
111 a.i./L, 10.05 mg a.i./L, 4.94 mg a.i./L, 3.64 mg a.i./L, 1.98 mg a.i./L) were prepared respectively. Fresh
112 soybean leaf discs were immersed in solutions of seven concentrations; each leaf disc was immersed in a
113 specific concentration for 10 s, removed from the solution, and placed on paper towels (abaxial surface
114 facing up) to air dry. The control leaf disc was immersed in a solution of distilled water containing 0.05
115 % (v/v) Triton X-100 and 1 % acetone. The air-dried leaf discs were attached to the agar medium with the
116 top-side facing down and newly hatched nymphs were placed on them. Treatment details (insecticide,
117 concentration, and date) were recorded for each Petri dish. A small drop of distilled water was placed on
118 the surface of the agar prior to laying the leaf on the surface to help the leaf stick to the agar surface. Sixty
119 newly hatched nymphs were used for dose response bioassays at each concentration; three replicates
120 were used per concentration and each replicate had 20 newly hatched nymphs. Mortality was determined
121 after 24 h of exposure. The newly hatched nymphs were considered dead if they were found upside down
122 and not moving or if they did not move when prodded with a small paint brush (Cordero, Bloomquist, &
123 Kuhar, 2007). The toxicity of imidacloprid and thiamethoxam to the nymphs were statistically analyzed
124 using SPSS (version 23.0, SPSS Inc., Chicago, IL, USA) and the LC_{50} and LC_{30} values for the newly
125 hatched nymphs were obtained.

Life table study

One hundred and fifty apterous adults were transferred onto fifteen leaf discs using a small paint brush and 10 apterous adults were placed on each leaf disc. Each Petri dish containing a leaf disc was sealed with a close-fitting, ventilated lid. Newly hatched nymphs were selected 24 h later and placed on a leaf disc pre-impregnated with LC_{30} and LC_{50} imidacloprid and thiamethoxam or a leaf disc pre-impregnated with distilled water containing 0.05 % (v/v) Triton X-100 and 1 % acetone. One hundred newly hatched nymphs were exposed to each treatment; each newly hatched nymph was kept in a separate Petri dish, and each Petri dish was treated as one replicate. The growth, survival, mortality, and fecundity of the individuals were observed until all organisms died. After reaching the adult stage, the number of newly hatched nymphs reproduced by each adult every day was recorded and removed after recording.

Life table analysis

The age-stage-specific survival rate (s_{xj}, x = age, j = stage), age-specific survival rate (l_x), age-stage specific fecundity (f_{xj}), and age-specific fecundity (m_x) were calculated as follows (Chi & Liu, 1985; Chi & Getz, 1988; Chi, 1988).

$$s_{xj} = \frac{n_{xj}}{n_{01}}, \tag{1}$$

$$l_x = \sum_{j=1}^{k} s_{xj}, \tag{2}$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}}, \tag{3}$$

where n_{01} stands for the number of newly hatched nymphs and k stands for the number of stages. The net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T) were calculated as follows (Goodman, 1982):

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \,, \tag{4}$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \,, \tag{5}$$

$$\lambda = e^r \,, \tag{6}$$

$$T = \frac{\ln R_0}{r} \,. \tag{7}$$

The life expectancy (e_{xj}), i.e. the time that an individual of age x and stage j is expected to live, was calculated according to Chi & Su (2006) as

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy} \,, \tag{8}$$

where s'_{iy} is the probability that an individual of age x and stage j would survive to age i and stage y. Fisher (1993) defined the reproductive value (v_{xj}) as the contribution of individuals of age x and stage j to the future population. It was calculated as

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} s'_{iy} f_{iy} \,. \tag{9}$$

TWOSEX-MSChart software was used to estimate the mean values and standard errors of the population parameters, mean longevity of first instar to fourth instar nymphs and adults, adult and total pre-ovipositional period, mean fecundity per female adult, and the bootstrap test method was used to compare the differences in all the treatments (B = 100,000; Chi, 2012). All curve graphs were generated using SigmaPlot 12.0.

Results

Dose response bioassay for newly hatched nymphs of *A. glycines*

The LC_{50} concentrations for imidacloprid and thiamethoxam were 4.440 (3.672–5.335) mg a.i./L and 7.049 (5.394–8.998) mg a.i./L respectively, whereas their LC_{30} concentrations were 3.114 (2.425–3.757) mg a.i./L and 4.184 (2.850–5.460) mg a.i./L,respectively (Table 1).

Life history traits

Imidacloprid and thiamethoxam had effects on the development time, longevity, and fecundity of *A. glycines* (Table 2). Compared with that in the control group, exposure to LC_{50} imidacloprid and thiamethoxam resulted in longer first instar development time, adult pre-ovipositional period (APOP), and total pre-ovipositional period (TPOP; $P < 0.05$), but decreased fecundity ($P < 0.05$) and adult longevity ($P < 0.05$).

Compared with that in the control group (1.20 d), the development time of second instar nymphs increased significantly when exposed to LC_{50} imidacloprid (1.43 d, $P < 0.05$), but no significant change was observed in nymphs exposed to LC_{50} thiamethoxam (1.28 d, $P > 0.05$). The development time of the third and fourth instars did not change on exposure to LC_{50} imidacloprid and thiamethoxam ($P > 0.05$).

Exposure to LC_{30} imidacloprid and thiamethoxam had no significant effect on the development time of the first to fourth instars ($P > 0.05$). However, LC_{30} imidacloprid and thiamethoxam decreased the APOP and reduced the fecundity of *A. glycines* compared with that in the control ($P < 0.05$). The longevity of adults exposed to LC_{30} imidacloprid and thiamethoxam was significantly decreased compared with that

177 of the control group (11.02, 8.95, and 9.31 d in control, imidacloprid, thiamethoxam groups,
178 respectively; $P < 0.05$). The TPOP of *A. glycines* exposed to LC_{30} imidacloprid (4.55 d) showed no
179 significant difference ($P > 0.05$) compared to that in the control group (4.66 d), whereas that of *A.*
180 *glycines* exposed to LC_{30} thiamethoxam was significantly decreased (4.29 d; $P < 0.05$).

Life table and fertility parameters

181 The mean fecundity per female adult, R_0, r, and λ decreased significantly in the imidacloprid and
182 thiamethoxam treatment groups compared to the control group ($P < 0.05$, Table 3). The T in LC_{50}
183 imidacloprid (9.50 d) and thiamethoxam (9.16 d) treatment groups was significantly longer compared
184 with that in the control group (8.20 d; $P < 0.05$). In contrast, the T in the LC_{30} thiamethoxam (7.71 d)
185 group was significantly decreased compared with that in the control group ($P < 0.05$), whereas that in the
186 LC_{30} imidacloprid (7.99 d) group showed no significant change ($P > 0.05$, Table 3).

187 Due to the different development rates between individuals, the age-stage specific survival rates
188 curves show obvious overlaps (Fig 1). The relative number of female adults in the LC_{30} imidacloprid and
189 thiamethoxam treatment groups was higher than that in the respective LC_{50} treatment groups.

190 Age-specific survival rate is the probability that a newly hatched nymph will reach an age x, and the
191 curve of the age-specific survival rate is a simplified form of the curve of the age-stage survival rate,
192 disregarding developmental stages. After treatment with imidacloprid and thiamethoxam, the l_x curve
193 decreased significantly (Fig 2).

194 The highest peak of m_x in the control group was higher than that in LC_{30} and LC_{50} treatment groups.
195 The highest peak of m_x in the control group appeared on day 8 (Fig 2), whereas that in the LC_{30}

imidacloprid group appeared on day 7, i.e., a day earlier than that in the control group. The highest peak of m_x in the LC_{30} thiamethoxam group appeared on day 6, two days earlier than that in the control group. The highest peak of m_x in the LC_{50} imidacloprid group appeared on day 10, two days later than that in the control group, while the highest peak of m_x in the LC_{50} thiamethoxam group appeared on day 9; a day later than that in the control group (Fig 2).

The values of age-specific maternity (l_xm_x) were significantly dependent on l_x and m_x, and the maximum l_xm_x values were 8, 8, 9, 7, and 6 d for the control, LC_{50} imidacloprid, LC_{50} thiamethoxam, LC_{30} imidacloprid, and LC_{30} thiamethoxam treatment groups, respectively.

The female reproductive values in the imidacloprid and thiamethoxam treatment groups decreased compared with those in the control group; however, the female reproductive value in the LC_{30} treatment group was higher than that in the LC_{50} group (Fig 3).

The age-stage life expectancy curve (e_{xj}) is shown in Fig 4. In the curve, the highest peak values of the first to fourth instar nymphs and female adults were lower in the treatment groups compared with the control group.

Discussion

The life table parameters used herein reflect the total effect of imidacloprid and thiamethoxam on *A. glycines*. We found that imidacloprid and thiamethoxam at LC_{50} significantly increased the APOP and TPOP and significantly decreased the mean fecundity per female adult compared with that in the control group ($P < 0.05$). In contrast, the APOP in individuals exposed to imidacloprid and thiamethoxam at LC_{30} was shorter than that in the control group ($P < 0.05$, Table 2). In addition, according to the results in

the age-stage two-sex life table, the R_0, λ, and r also decreased significantly ($P < 0.05$, Table 3). Collectively, these results indicate that both imidacloprid and thiamethoxam have inhibitory effects on the reproduction of *A. glycines*.

The l_x curve is a basis for the s_{xj} curve. In this study, we found that the l_x curves of individuals exposed to imidacloprid and thiamethoxam showed a declining trend (Fig 2). During the first stage, *A. glycines* failed to respond effectively when initially exposed to a high dosage of insecticide; consequently, the l_x decreased sharply, and only some surviving individuals entered the second stage. During the second stage, intoxicated aphids refuse to eat or eat in small amounts and spend energy trying to get out of the toxic arena. This was the stage of confrontation between insecticides stress and *A. glycines*. The different effects of imidacloprid and thiamethoxam at LC_{50} and LC_{30} on the life table may be also related to the regulation strategies of the species, such as self-metabolism and detoxification. In the future research, we will be committed to metabolic detoxification, from the physiological indicators and even molecular level to continue understand the deep impact of imidacloprid and thiamethoxam on population dynamics. During the third stage, the survival rate continued to decline, less sharply than in the first stage, but more sharply than in the second stage. During the third stage, the individual longevity may also be one reason for the decrease of the l_x; this is consistent with the decrease in the age-stage life expectancy curve with increasing age (Fig 4).

In the present study, individuals in the LC_{50} thiamethoxam and LC_{50} imidacloprid treatment groups reached their reproductive peaks 1 and 2 days later compared with those in the control group, respectively. In contrast, individuals in the LC_{30} thiamethoxam and LC_{30} imidacloprid treatment groups reached their reproductive peaks 2 and 1 day earlier than the control group, respectively. Different types

and doses of insecticides have different biological and ecological effects on pests. More attention should be paid to the increase of pest reproduction caused by low doses of insecticides (Stark, Tanigoshi, Bounfour, & Antonelli, 1997; James & Price, 2002). For example, low-lethal concentration of spinetoram can decrease the developmental time of *Tetranychus urticae* (Acari: *Tetranychidae*) from egg to adult (Wang, Zhang, Xie, Wu, & Wang, 2016). The rapid increase in the number of *A. glycines* individuals during the productive peak and the fast reproduction of species from generation-to-generation indicate that large outbreaks may occur within a short time. This phenomenon of low-lethal concentration promoting rapid reproduction has brought great pressure on the prevention and control of *A. glycines* in the field.

The actual application doses of neonicotinoids in soybean fields in northeast China was higher than LC_{50}, but the complex environmental conditions in the fields and the drift of neonicotinoids themselves would make the doses of some pests exposed to less than LC_{50}. The risk of low-lethal doses neonicotinoids stimulate the rapid reproduction of field populations would highly possible happen. We will continue to study whether field populations show similar trends after exposure to low-lethal doses neonicotinoids in the future.

Supporting information

S1 Fig. Age-stage specific survival rate (s_{xj}) of *Aphis glycines*. Age-stage specific survival rate (s_{xj}) of

257 *Aphis glycines* exposed to the following treatments: control, imidacloprid LC_{30} treatment group,

258 imidacloprid LC_{50} treatment group, thiamethoxam LC_{30} treatment group, and thiamethoxam LC_{50}

259 treatment group. L1 = s_{xj} of first instar nymphs; L2 = s_{xj} of second instar nymphs; L3 = s_{xj} of third

260 instar nymphs; and L4 = s_{xj} of fourth instar nymphs. (XLS)

261

262 S2 Fig. Age-specific survival rate (l_x), age-specific fecundity of the total population (m_x), and

263 age-specific maternity (l_xm_x) of *Aphis glycines*. Age-specific survival rate (l_x), age-specific fecundity of

264 the total population (m_x), and age-specific maternity (l_xm_x) of *Aphis glycines* exposed to the following

265 treatments: control, imidacloprid LC_{30} treatment group, imidacloprid LC_{50} treatment group,

266 thiamethoxam LC_{30} treatment group, and thiamethoxam LC_{50} treatment group. (XLS)

267

268 S3 Fig. Age-stage specific reproductive values (v_{xj}) of *Aphis glycines*. Age-stage specific reproductive

269 values (v_{xj}) of *Aphis glycines* exposed to the following treatments: control, imidacloprid LC_{30} treatment

270 group, imidacloprid LC_{50} treatment group, thiamethoxam LC_{30} treatment group, and thiamethoxam

271 LC_{50} treatment group. L1 = v_{xj} of first instar nymphs; L2 = v_{xj} of second instar nymphs; L3 = v_{xj} of third

272 instar nymphs; and L4 = v_{xj} of fourth instar nymphs. (XLS)

273

274 S4 Fig. Life expectancy (e_{xj}) of *Aphis glycines*. Life expectancy (e_{xj}) of *Aphis glycines* exposed to the

275 following treatments: control, imidacloprid LC_{30} treatment group, imidacloprid LC_{50} treatment group,

276 thiamethoxam LC_{30} treatment group, and thiamethoxam LC_{50} treatment group. L1 = e_{xj} of first instar

277 nymphs; L2 = e_{xj} of second instar nymphs; L3 = e_{xj} of third instar nymphs; and L4 = e_{xj} of fourth instar

278 nymphs. (XLS)

279

280 S1 Table. Toxic effects of imidacloprid and thiamethoxam on newly hatched nymphs of *Aphis glycines.*

281 SE = Standard error (XLS)

282

283 S2 Table. Mean value (± SE) of life history parameters of *Aphis glycines* exposed to imidacloprid and

284 thiamethoxam. SE = Standard error. Means (±SE) followed by different letters in the same row are

285 significantly different as calculated using the paired bootstrap test at the $P < 0.05$ level. Leaves treated

286 with pure water were used as the control. L1 = mean longevity of first instar nymphs; L2 = mean

287 longevity of second instar nymphs; L3 = mean longevity of third instar nymphs; L4 = mean longevity

288 of fourth instar nymphs; Fecundity = mean fecundity per female adult. (DOC)

289

290 S3 Table. Mean value (± SE) of fertility parameters of *Aphis glycines* exposed to imidacloprid and

291 thiamethoxam. Means (±SE) followed by different letters in the same row are significantly different as

292 calculated using the paired bootstrap test at the $P < 0.05$ level. Leaves treated with pure water were used

293 as the control. (DOC)

294

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299

Author Contributions

All authors conceived research. AZ conducted experiments. AZ, LZ and TL analysed data and conducted statistical analyses. AZ, LH and ZS wrote the manuscript. LH and KZ secured funding. All authors read and approved the manuscript.

References

304 Abbas, N., Shad, S. A., & Shah, R. M. (2015). Resistance status of *Musca domestica* L. populations to
305 neonicotinoids and insect growth regulators in Pakistan poultry facilities. Pak. J. Zool. 47,
306 1663–1671.

307 Basit, M., Saeed, S., Saleem, M. A., Denholm, I., & Shah, M. (2013). Detection of resistance,
308 cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci*
309 (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 106, 1414–1422. https://doi.org/10.1603/ec12414.

310 Bass, C., Puinean, A. M., Andrews, M., Cutler, P., Daniels, M., Elias, J., ... Slater, R. (2011). Mutation
311 of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid
312 insecticides in the aphid *Myzus persicae*. *BMC Neurosci.* 12, 51.
313 https://doi.org/10.1186/1471-2202-12-51.

314 Bass, C., Denholm, I., Williamson, M. S., & Nauen, R. (2015). The global status of insect resistance to
315 neonicotinoid insecticides. Pestic. Biochem. Phys. 121, 78–87.
316 https://doi.org/10.1016/j.pestbp.2015.04.004.

317 Chi, H., & Liu, H. (1985). Two new methods for the study of insect population ecology. B. I. Zool.
318 Acad. 24, 225–240.

319 Chi, H., & Su, H. Y. (2006). Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead)
320 (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with
321 mathematical proof of the relationship between female fecundity and the net reproductive rate.
322 Environ. Entomol. 35, 10–21.

323 Chi, H. (1988). Life-table analysis incorporating both sexes and variable development rate among
324 individuals. Environ. Entomol. 17, 26–34. https://doi.org/10.1093/ee/17.1.26.
325 Chi, H., & Getz, W. M. (1988). Mass rearing and harvesting based on an age-stage, two-sex life table: a
326 potato tuber worm (Lepidoptera: Gelechiidae) case study. Environ. Entomol. 17, 18–25.
327 Chi, H. (2012). TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis.
328 (http://140.120.197.173/Ecology/prod02.htm).
329 Cordero, R. J., Bloomquist, J. R., & Kuhar, T. P. (2007). Susceptibility of two diamondback moth
330 parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus*
331 *sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. Biol.
332 Control. 42, 48–54. https://doi.org/10.1016/j.biocontrol.2007.04.005.
333 Feliciangeli, M. D., & Rabinovich, J. (1985). Vital statistics of triatominae (Hemiptera: reduviidae)
334 under laboratory conditions II. *Triatoma maculata*. *J. Med. Entomol.* 22, 43–48.
335 https://doi.org/10.1093/jmedent/22.1.43.
336 Fisher, R. A. (1993). The genetical theory of natural selection: a complete variorum edition. Oxford
337 University Press, Oxford, United Kingdom.
338 Gabre, R. M., Adham, F. K., & Chi, H. (2005). Life table of *Chrysomya megacephala* (Fabricius)
339 (Diptera: Calliphoridae). Acta Oecol. 27, 179–183. https://doi.org/10.1016/j.actao.2004.12.002.
340 Goodman, D. (1982). Optimal life histories, optimal notation, and the value of reproductive value. *Am.*
341 *Nat.* 119, 803–823.

342 Gore, J., Cook, D., Catchot, A., Leonard, B. R., Stewart, S. D., Lorenz, G., ... Kerns, D. (2013). Cotton
343 aphid (Heteroptera: Aphididae) susceptibility to commercial and experimental insecticides in the
344 southern United States. *J. Econ. Entomol.* 106, 1430–1439. https://doi.org/10.1603/ec13116.
345 Hanson, A. A., Menger-Anderson, J., Silverstein, C., Potter, B. D., MacRae, I. V., Hodgson, E. W., ...
346 Koch, R. L. (2017). Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid
347 insecticides in the upper Midwestern United States. J. Econ. Entomol. 110, 2235–2246.
348 https://doi.org/10.1093/jee/tox235.
349 Hopper, K. R., Lanier, K., Rhoades, J. H., Hoelmer, K. A., Meikle, W. G., Heimpel, G. E., ... Woolley,
350 J. B. (2017). Host specificity of *Aphelinus* species collected from soybean aphid in Asia. *Biol.*
351 *Control.* 115, 55–73. https://doi.org/10.1016/j.biocontrol.2017.09.004.
352 Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an
353 increasingly regulated world. *Annu. Rev. Entomol.* 51, 45–66.
354 https://doi.org/10.1146/annurev.ento.51.110104.151146.
355 James, D. G., & Price, T. S. (2002). Fecundity in two-spotted spider mite (Acari: Tetranychidae) is
356 increased by direct and systemic exposure to imidacloprid. *J. Econ. Entomol.* 95, 729–732.
357 https://doi.org/10.1603/0022-0493-95.4.729.
358 Jan, M. T., Abbas, N., Shad, S. A., & Saleem, M. A. (2015). Resistance to organophosphate, pyrethroid
359 and biorational insecticides in populations of spotted bollworm, *Earias vittella* (Fabricius)
360 (Lepidoptera: noctuidae), in Pakistan. Crop Prot. 78, 247–252.
361 https://doi.org/10.1016/j.cropro.2015.09.020.

362 Khan, H., Abbas, N., Shad, S. A., & Afzal, M. B. S. (2014). Genetics and realized heritability of
363 resistance to imidacloprid in a poultry population of house fly, *Musca domestica* L. (Diptera:
364 Muscidae) from Pakistan. Pestic. Biochem. Phys. 114, 38–43.
365 https://doi.org/10.1016/j.pestbp.2014.07.005.
366 Koo, H. N., An, J. J., Park, S. E., Kim, J. I., & Kim, G. H. (2014). Regional susceptibilities to 12
367 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point
368 mutation associated with imidacloprid resistance. Crop Prot. 55, 91–97.
369 https://doi.org/10.1016/j.cropro.2013.09.010.
370 Lee, C. C. (2000). Sublethal effects of insecticides on longevity, fecundity and behaviour of insect
371 pests: a review. *J. Biosci.* 11, 107–112.
372 Luo, C., Jones, C. M., Devine, G., Zhang, F., Denholm, I., & Gorman, K. (2010). Insecticide resistance
373 in *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) from China. *Crop Prot.* 29, 429–434.
374 https://doi.org/10.1016/j.cropro.2009.10.001.
375 Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., & Sattelle, D. B. (2001).
376 Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends. Pharmacol.
377 Sci. 22, 573–580. https://doi.org/10.1016/s0165-6147(00)01820-4.
378 Matsumura, M., Sanada-Morimura, S., Otuka, A., Ohtsu, R., Sakumoto, S., Takeuchi, H., ... Satoh, M.
379 (2013). Insecticide susceptibilities in populations of two rice planthoppers, *Nilaparvata lugens* and
380 *Sogatella furcifera*, immigrating into Japan in the period 2005-2012. *Pest Manag. Sci.* 70, 615–622.
381 https://doi.org/10.1002/ps.3590.

382 Menger, J., Beauzay, P., Chirumamilla, A., Dierks, C., Gavloski, J., Glogoza, P., ... Koch, R. L. (2020).
383 Implementation of a diagnostic-concentration bioassay for detection of susceptibility to
384 pyrethroids in soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 113, 932–939.
385 https://doi.org/10.1093/jee/toz351.
386 Nauen, R., & Denholm, I. (2005). Resistance of insect pests to neonicotinoid insecticides: current status
387 and future prospects. *Arch. Insect. Biochem.* 58, 200–215. https://doi.org/10.1002/arch.20043.
388 Nauen, R., Jeschke, P., Velten, R., Beck, M. E., Ebbinghaus-Kintscher, U., Thielert, W., ... Raupach,
389 G. (2014). Flupyradifurone: a brief profile of a new butenolide insecticide. *Pest Manag. Sci.* 71,
390 850–862. https://doi.org/10.1002/ps.3932.
391 Ragsdale, D. W., Voegtlin, D. J., & O'neil, R. J. (2004). Soybean aphid biology in North America. Ann.
392 Entomol. Soc. Am. 97, 204–208. https://doi.org/10.1093/aesa/97.2.204.
393 Ragsdale, D. W., Landis, D. A., Brodeur, J., Heimpel, G. E., & Desneux, N. (2011). Ecology and
394 management of the soybean aphid in North America. Annu. Rev. Entomol. 56, 375–399.
395 https://doi.org/10.1146/annurev-ento-120709-144755.
396 Shi, T. F., Wang, Y. F., Liu, F., Qi, L., & Yu, L. S. (2017). Sublethal effects of the neonicotinoid
397 insecticide thiamethoxam on the transcriptome of the honey bees (Hymenoptera: Apidae). *J. Econ.*
398 *Entomol.* 110, 2283–2289. https://doi.org/10.1093/jee/tox262.
399 Somar, R. O., Zamani, A. A., & Alizadeh, M. (2019). Joint action toxicity of imidacloprid and
400 pymetrozine on the melon aphid, *Aphis gossypii*. Crop Prot. 124, 104850.
401 https://doi.org/10.1016/j.cropro.2019.104850.

Srigiriraju, L., Semtner, P. J., & Bloomquist, J. R. (2010). Monitoring for imidacloprid resistance in the tobacco adapted form of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in the eastern United States. Pest. Manag. Sci. 66, 676–685. https://doi.org/10.1002/ps.1929.

Stark, J. D., & Wennergren, U. (1995). Can population effects of pesticides be predicted from demographic toxicological studies? *J. Econ. Entomol.* 88, 1089–1096. https://doi.org/10.1093/jee/88.5.1089.

Stark, J. D., Tanigoshi, L., Bounfour, M., & Antonelli, A. (1997). Reproductive potential: its influence on the susceptibility of a species to pesticides. *Ecotoxicol. Environ. Saf.* 37, 273–279. https://doi.org/10.1006/eesa.1997.1552.

Tao, Y., Phung, D., Dong, F., Xu, J., Liu, X., Wu, X., ... Zheng, Y. (2019). Urinary monitoring of neonicotinoid imidacloprid exposure to pesticide applicators. *Sci. Total Environ.* 669, 721–728. https://doi.org/10.1016/j.scitotenv.2019.03.040.

Wang, L., Zhang, Y., Xie, W., Wu, Q., & Wang, S. (2016). Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Pestic. Biochem. Phys. 132, 102–107. https://doi.org/10.1016/j.pestbp.2016.02.002.

Wang, R. Y., Kritzman, A., Hershman, D. E., & Ghabrial, S. A. (2006). *Aphis glycines* as a vector of persistently and nonpersistently transmitted viruses and potential risks for soybean and other crops. *Plant Dis.* 90, 920–926. https://doi.org/10.1094/pd-90-0920.

Xie, W., Wang, S., Wu, Q., Feng, Y., Pan, H., Jiao, X., ... Zhang, Y. (2010). Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest. Manag. Sci. 67, 87–93. https://doi.org/10.1002/ps.2037.

423 Zhao, Y., Wang, Q., Ding, J., Wang, Y., Zhang, Z., Liu, F., ... Mu, W. (2018). Sublethal effects of
424 chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of
425 *Bradysia odoriphaga* (Diptera: Sciaridae). Pestic. Biochem. Phys. 148, 93–102.
426 https://doi.org/10.1016/j.pestbp.2018.04.003.

Tables

Table 1.

Toxic effects of imidacloprid and thiamethoxam on newly hatched nymphs of *Aphis glycines*

Insecticide	Concentration (mg a.i./L) (95 % CL)$^{-1}$		Slope ± SE	χ^2 (df)
	LC_{50}	LC_{30}		
Imidacloprid	4.440 (3.672–5.335)	3.114 (2.425–3.757)	3.402 ± 0.469	0.464 (5)
Thiamethoxam	7.049 (5.394–8.998)	4.184 (2.850–5.460)	2.314 ± 0.339	0.502 (5)

SE = Standard error

Table 2.

Mean value (± SE) of life history parameters of *Aphis glycines* exposed to imidacloprid and thiamethoxam

Parameters		LC_{30}		LC_{50}	
	Control	Imidacloprid	Thiamethoxam	Imidacloprid	Thiamethoxam
L1 (day)	1.23 ± 0.04c	1.19 ± 0.05c	1.14 ± 0.04c	1.88 ± 0.09a	1.57 ± 0.11b
L2 (day)	1.2 ± 0.04b	1.17 ± 0.05b	1.14 ± 0.04b	1.43 ± 0.08a	1.28 ± 0.07ab
L3 (day)	1.13 ± 0.05a	1.14 ± 0.05a	1.15 ± 0.05a	1.17 ± 0.06a	1.15 ± 0.05a
L4 (day)	1.01 ± 0.01a	1.02 ± 0.02a	1.02 ± 0.02a	1.1 ± 0.05a	1.06 ± 0.04a
Mean longevity of female adult (day)	11.02 ± 0.55a	8.95 ± 0.56b	9.31 ± 0.62b	8.48 ± 0.64b	8.62 ± 0.55b
APOP (day)	0.20 ± 0.04b	0.05 ± 0.03c	0.02 ± 0.02c	1.11 ± 0.15a	1.07 ± 0.15a
TPOP (day)	4.66 ± 0.07c	4.55 ± 0.08c	4.29 ± 0.07d	6.68 ± 0.20a	5.93 ± 0.18b
Fecundity	42.49 ± 1.83a	20.91 ± 1.39b	22.66 ± 1.60b	13.88 ± 1.56c	16.02 ± 1.37c

SE = Standard error. Means (±SE) followed by different letters in the same row are significantly different as calculated using the paired bootstrap test at the $P < 0.05$ level. Leaves treated with pure water were used as the control. L1 = mean longevity of first instar nymphs; L2 = mean longevity of second instar nymphs; L3 = mean longevity of third instar nymphs; L4 = mean longevity of fourth instar nymphs; Fecundity = mean fecundity per female adult.

448 **Table 3.**

449 Mean value (± SE) of fertility parameters of *Aphis glycines* exposed to imidacloprid and thiamethoxam

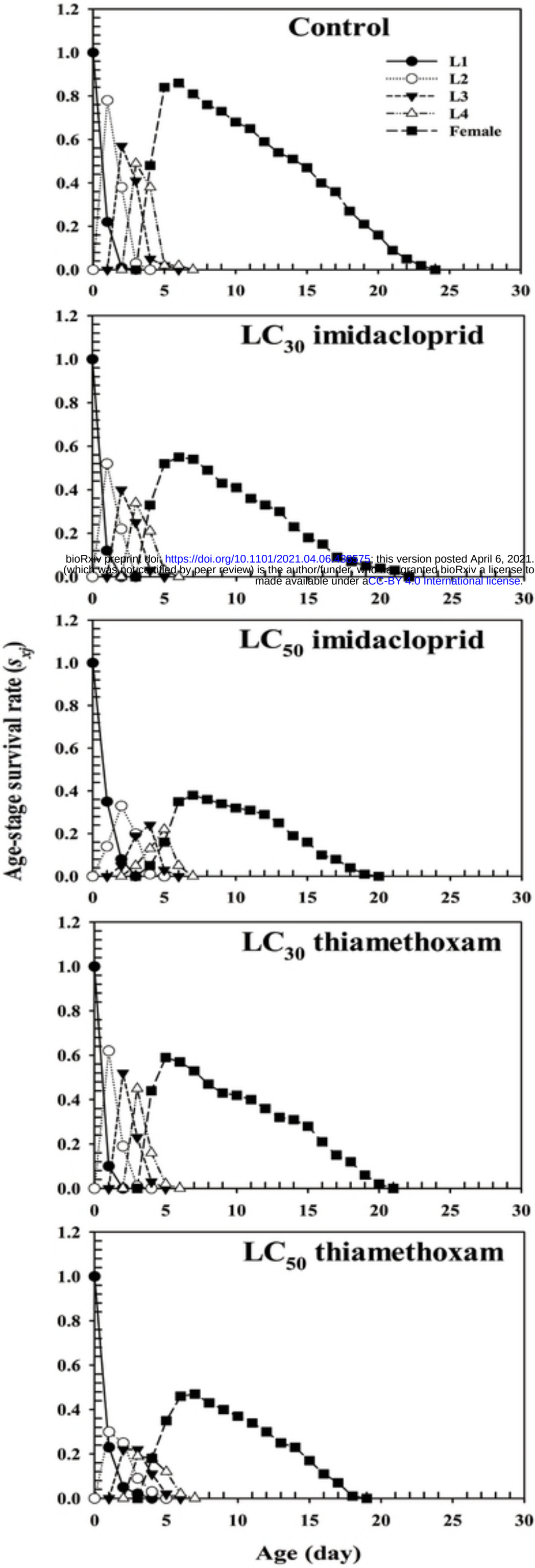
Population parameters	Control	LC_{30}		LC_{50}	
		Imidacloprid	Thiamethoxam	Imidacloprid	Thiamethoxam
Intrinsic rate of increase (r) (d^{-1})	0.439 ± 0.008a	0.310 ± 0.014b	0.341 ± 0.015b	0.180 ± 0.019c	0.223 ± 0.016c
Finite rate of increase (λ) (d^{-1})	1.551 ± 0.012a	1.363 ± 0.019b	1.406 ± 0.021b	1.198 ± 0.023c	1.250 ± 0.020c
Net reproductive rate (R_0)	36.54 ± 2.156a	11.92 ± 1.303b	13.82 ± 1.466b	5.55 ± 0.916c	7.69 ± 1.028c
Mean generation time (T) (d)	8.20 ± 0.076b	7.99 ± 0.107bc	7.71 ± 0.109c	9.50 ± 0.219a	9.16 ± 0.097a

450 Means (±SE) followed by different letters in the same row are significantly different as calculated

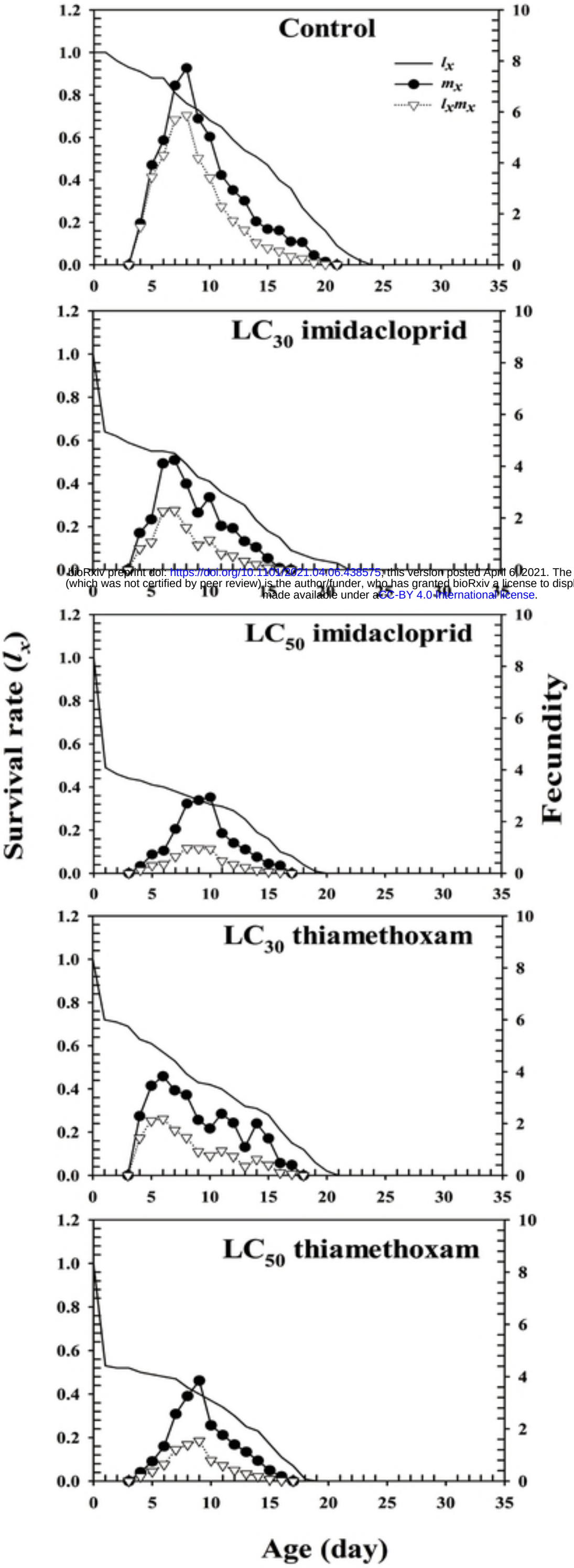
451 using the paired bootstrap test at the $P < 0.05$ level. Leaves treated with pure water were used as the

452 control.

453



Figure



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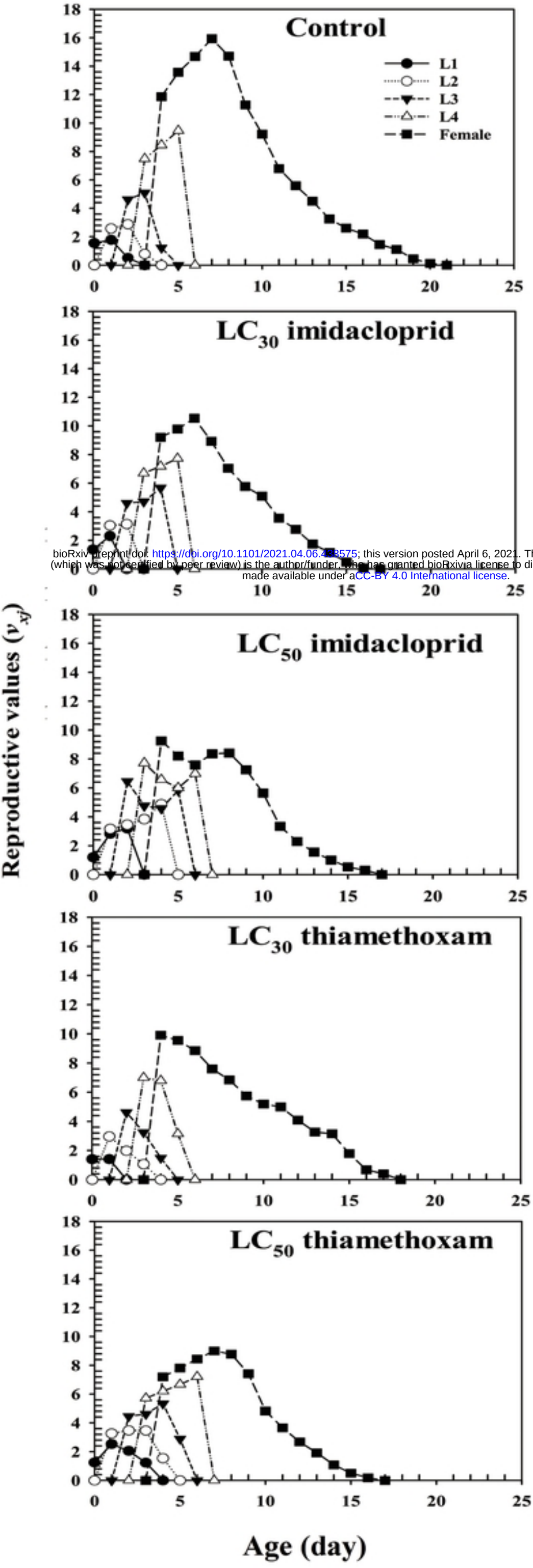

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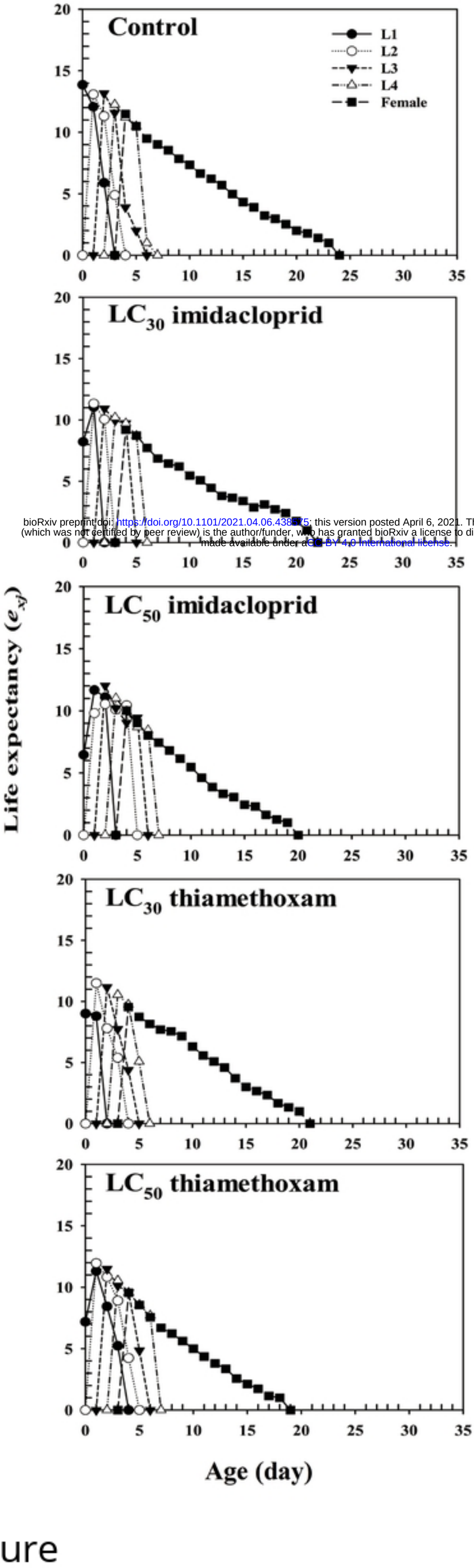

Figure