Research Article

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Sub-lethal effects of four neonicotinoid seed treatments on the demography and feeding behaviour of the wheat aphid *Sitobion avenae*

Jin Miao,[a†] Zhen-Bao Du,[a,b†] Yu-Qing Wu,[a*] Zhong-Jun Gong,[a] Yue-Li Jiang,[a] Yun Duan,[a] Tong Li[a] and Chao-Liang Lei[b]

Abstract

BACKGROUND: Neonicotinoids are widely used as seed treatments in wheat fields against the grain aphid (*Sitobion avenae* F.) in China. Due to the degradation of neonicotinoids in wheat plants, wheat aphids are more likely to be exposed to low concentrations of neonicotinoids over long periods. It is therefore expected that neonicotinoids, aside from acute (lethal) effects, may also cause a range of sub-lethal effects on this pest.

RESULTS: The growth and fertility of *S. avenae* feeding on wheat plants treated with a sub-lethal concentration (LC_{10}) of imidacloprid, dinotefuran, thiacloprid and thiamethoxam were not greatly affected. However, the population growth parameters of *S. avenae* were significantly reduced at median lethal concentration (LC_{50}). Electronic penetration graph recordings showed a higher percentage of no probing phase and shorter phloem sap ingestion phase on the wheat plants treated with LC_{10} and LC_{50} concentrations.

CONCLUSION: The results indicate that even low concentrations of neonicotinoid treatments on wheat seeds have long-term, adverse effects on wheat aphid. As such, neonicotinoid seed treatments have far greater effects on wheat aphids than estimated by acute toxicity tests. These results benefit our understanding on the subtle effects of the four tested neonicotinoids when applied as seed treatments.

Keywords: sub-lethal effect; neonicotinoid insecticide; *Sitobion avenae*; demographic growth; feeding behaviour

1 INTRODUCTION

Neonicotinoids are the most important class of chemical insecticides introduced to the global market since the synthetic pyrethroids. They are among the most effective insecticides for control of sucking insect pests such as aphids, whiteflies, planthoppers, thrips, some micro-Lepidoptera, and a number of coleopteran pests.[1] This insecticide class displays characteristics including efficiency, safety, broad insecticidal spectrum activity, long duration and no cross-resistance to conventional insecticides such as pyrethroids, organophosphates and carbamates. In addition, neonicotinoid insecticides possess a high degree of versatility not observed in other chemical classes. Most neonicotinoids can be used as foliar sprays and introduced via soil application. More recently, those strategies have been surpassed by the seed-coating technique, which is effective against a broad range of soil-inhabitating, root-, stem- and leaf-feeding pests from different orders, including Coleoptera, Lepidoptera, Diptera, Homoptera, Hemiptera and Hymenoptera.[1] Upon application on the seed surface, the active compound is translocated and distributed throughout the whole plant, conferring a substantial and long-lasting control of insects and protecting young plants from sucking leafhoppers and aphids,[2] which are potential vectors for plant viruses.[3]

Neonicotinoids are widely used for seed treatment in cotton (*Gossypium* spp.), sugar beet (*Beta vulgaris* L.), oilseed rape (*Brassica napus* L.), corn (*Zea mays* L.), and other cereals and crops.[4] The reduced load of insecticide per field unit, allowed by confining it to the seed, confers major advantages in environmental terms compared with former products requiring whole-soil or thorough applications. Recently, imidacloprid has been a major neonicotinoid insecticide for seed treatment controlling the grain aphid (*Sitobion avenae* F.), an important pest insect of wheat, *Triticum aestivum* (L.), in China.[5] With the degradation of neonicotinoids in wheat plants, wheat aphids are likely to be exposed to sub-lethal concentration of neonicotinoids over relatively long periods. It is therefore expected that neonicotinoids,

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[*] *Correspondence to: Yu-Qing Wu, 1Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou 450002, P.R. China, E-mail: yuqingwu36@hotmail.com*

[†] *Jin Miao and Zhen-Bao Du contributed equally to this work.*

[a] *Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou 450002, P.R. China*

[b] *College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, P.R. China*

aside from a direct (lethal) effect on the grain aphid, may also cause a range of sub-lethal effects on this pest.

Exposure to sub-lethal concentration/dose of pesticides may result in both behavioural and physiological effects on individuals that survive these treatments, as well as on their offspring.[6,7] Clark and Haynes[8] reported that sub-lethal concentration/dose of cypermethrin affected chemical communication, courtship and oviposition of the cabbage looper, *Trichoplusiani* (Hübner). Life-table parameters (e.g. the intrinsic rate of increase, r_m, and finite capacity for increase, λ) are useful for obtaining a complete measure of sub-lethal effects at the population level.[9] For example, Lashkari *et al.*[10] reported that a sub-lethal concentration/dose of imidacloprid and pymetrozine caused a considerable reduction in the intrinsic rate of increase of cabbage aphid, *Brevicoryne brassicae* L.

No method has been more useful in revealing detailed information about the stylet penetration behaviour of Homopteran insects than the electrical penetration graph (EPG) technique. This method was first developed for aphids by McLean and Kinsey[11,12] using an AC circuit and later modified by Tjallingii[13] using a DC circuit. Employing these EPG systems, detailed information on feeding behaviour has been obtained for over 50 Homopteran species, mostly from the sub-order Sternorrhynchi.[14] EPG has been used in studies on host plant resistance to sap-feeding insects,[15,16] on evaluation of genetically modified plants,[17] on the mode of action of insecticides[18,19] and on the identification of stylet activities that are critical for virus transmission by aphids.[20,21]

In this article, we assessed the concentration mortality response of four neonicotinoid (imidacloprid, thiamethoxam, thiacloprid and dinotefuran) seed treatments to the grain aphid, aiming to establish sub-lethal and median concentrations (LC_{10} and LC_{50}). Our study focuses on the effects of sub-lethal and median concentrations of these seed treatment pesticides on demographic growth and feeding behaviour of the grain aphid. Consequently, this study provides some available information for neonicotinoids seed treatments for controlling wheat aphids in China.

2 MATERIALS AND METHODS

2.1 Insects

Wheat aphids were obtained from the Institute of Plant Protection (Chinese Academy of Agricultural Science, Beijing). All aphid clones were maintained on winter wheat (*Triticum aestivum* L., Zhengmai 9023) in an environmental chamber at 22 ± 1 °C, 70 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

2.2 Insecticide applications and concentration mortality response

Trials started in spring 2011. The neonicotinoid insecticides used in the study were imidacloprid (Gaucho70 WS; Bayer CropScience, Monheim, Germany); thiamethoxam (Cruiser 70WS; Syngenta International AG, Basel, Switzerland); thiacloprid (Calypso 70 WS; Bayer CropScience) and dinotefuran (20% SG, Shenzhen Sun Rising Industry, Shenzhen, China). These insecticides were tested because they are all registered for use in wheat fields in China. Preliminary experiments were carried out to determine the range of concentrations to be further tested. This was done by spraying wheat seeds with decreasing concentrations of four neonicotinoids according to the recommended field application rate (on wheat) until mortality rates lower than 100% were observed.[22] To determine the dose-mortality regression lines, wheat seeds were treated with five concentrations of each neonicotinoid, i.e. 62.5, 125, 250, 500 and 1000 mg AI kg^{-1} seed, and with uncoated seeds as controls. All wheat seeds were grown in plastics cups (width 5 cm, height 10 cm) with a normal soil mixture, in an environmental chamber at 23 ± 1 °C, 70 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Seven-day-old wheat plants were used for testing the concentration mortality response of four neonicotinoid seed treatments to the grain aphid. Wheat plants of each treatment and control were infested with 50 adult apterous aphids and kept at 22 ± 1 °C, 70 ± 5% RH, and with a photoperiod of 16:8 (L:D) h for 24 h. After this period, mortality was assessed, i.e. aphids not moving when touched with a fine brush were considered dead. Each treatment was replicated five times.

2.3 Demographic effects

Using an identical experimental protocol to that described above, we exposed wheat aphids to sub-lethal concentrations of four neonicotinoids. To ensure low mortality, concentrations were chosen at the LC_{10} and LC_{50} values as obtained previously in the previous section. Initially, 10 adult aphids were placed onto wheat plants for each treatment. When >30 offspring were produced, all adult aphids were removed and 30 first-instar nymphs were kept to initiate a cohort. Each treatment was replicated five times. Thereafter, the development and reproduction of aphids on each treatment and control were checked and recorded daily. During the reproductive period of the resulting adults, the newborn nymphs were counted and then removed daily. The procedure was continued until all the adult aphids died. All experiments were conducted at 22 ± 1 °C, 70 ± 5% RH, and at a photoperiod of 16:8 (L:D) h. The life-table parameters, which include the net reproductive rate (R_0), average generation lifespan (T), intrinsic rate of natural increase (r_m), finite rate of increase (λ), and population doubling time (DT) were calculated as follows for each treatment:[9]

$$R_0 = \sum l_x m_x \tag{1}$$

$$T = \frac{\sum x l_x m_x}{\sum l_x m_x} \tag{2}$$

$$r_m = \frac{\ln R_0}{T} \tag{3}$$

$$\lambda = e^{r_m} \tag{4}$$

$$\mathrm{DT} = \frac{\ln 2}{r_m} \tag{5}$$

where the age-specific survival rate (l_x) is the proportion of individuals in the initial cohort alive at age x time (days), and the age-specific fecundity (m_x) is the mean number of female progeny produced per female alive at the age interval, x days.

2.4 Sub-lethal effects on feeding behaviour

This experiment was conducted to assess potential changes, wheat seed treated with LC_{10} and LC_{50} of neonicotinoids, on the feeding behaviour of *S. avenae*. The probing behaviour of adult apterous aphids was recorded by using a DC EPG amplifier (type

Table 1. Toxicity of the four neonicotinoid seed treatments (imidacloprid, thiamethoxam, thiacloprid and dinotefuran) on *Sitobion avenae*

Neonicotinoid	LD-P line	Correlation coefficient	LD_{50} (mg kg^{-1})	95% CL
Imidacloprid	$Y = 4.8 + 0.66x$	0.996	188	94–282
Thiamethoxam	$Y = 5.5 + 0.54x$	0.976	135	67.5–203
Thiacloprid	$Y = 5.2 + 0.69x$	0.986	48	24.2–72.6
Dinotefuran	$Y = 4.9 + 0.7x$	0.977	13	6.25–19.4

Table 2. The mean rates (± SE) of stable population parameters of *Sitobion avenae* exposed to LC_{10} and LC_{50} of the four neonicotinoid seed treatments (imidacloprid, thiamethoxam, thiacloprid and dinotefuran)

Concentration	Treatment	R_0	T	r_m	λ	DT
0	Control	5.04 ± 1.15^a	11.71 ± 0.51^a	0.132 ± 0.024^a	1.14 ± 0.02^a	5.40 ± 0.78^a
LC_{10}	Imidacloprid	3.33 ± 0.32^a	13.11 ± 0.44^a	0.091 ± 0.008^a	1.10 ± 0.014^a	7.83 ± 1.07^a
	Thiamethoxam	4.88 ± 1.15^a	13.83 ± 0.52^a	0.114 ± 0.013^a	1.12 ± 0.02^a	6.77 ± 1.17^a
	Thiacloprid	3.90 ± 0.38^a	11.51 ± 0.56^a	0.122 ± 0.009^a	1.12 ± 0.014^a	6.11 ± 0.06^a
	Dinotefuran	4.31 ± 1.74^a	12.38 ± 1.52^a	0.109 ± 0.034^a	1.09 ± 0.03^a	6.87 ± 2.57^a
LC_{50}	Imidacloprid	0.91 ± 0.46^b	8.13 ± 4.06^a	0.021 ± 0.015^b	0.68 ± 0.34^{ab}	42.46 ± 13.36^b
	Thiamethoxam	1.33 ± 0.70^b	9.62 ± 4.87^a	0.028 ± 0.016^b	0.69 ± 0.35^{ab}	22.83 ± 4.91^b
	Thiacloprid	0.78 ± 0.39^b	6.88 ± 3.44^{ab}	0.013 ± 0.005^b	0.67 ± 0.34^{ab}	69.88 ± 14.44^b
	Dinotefuran	0.37 ± 0.20^b	2.97 ± 1.33^b	0.003 ± 0.001^b	0.31 ± 0.12^b	203.11 ± 23.84^b

Means followed by different letters in columns indicate significant differences compare to the control ($P < 0.05$).
R_0, net reproductive rate; T, average generation lifespan; r_m, rate of natural increase; λ, finite rate of increase; DT, doubling time.

Giga-8). A gold wire 2–3 cm long and 10 μm in diameter was glued to the thorax of an aphid using a water-based silver conductive paint (Dr W.F. Tjallingii, Wageningen, The Netherlands). Wheat seed were treated with LC_{10} and LC_{50} of four neonicotinoids (see the Results section) using the method described in the section 'Insecticide applications and concentration mortality response'. Seven-day-old wheat plants were used for testing feeding behaviour of the grain aphid. Experiments were carried out in a Faraday cage at 25 ± 1 °C. The probing behaviour was recorded after the aphid had been starved for 2 h, and the aphid was connected to the amplifier before being placed on a leaf. The EPG signal of each aphid was monitored continuously for 12 h, with each aphid being recorded on a single wheat plant. For each treatment, at least 15 adult aphids were recorded successfully. The electrical signals were scored based on the following categories as described previously by Tjallingii: np, nonprobing; C, reflecting an extracellular stylet pathway; G, reflecting ingestion in xylem; E1, reflecting salivary secretion into the sieve element; and E2, reflecting phloem sap ingestion and concurrent salivation.[13]

2.5 Statistical analysis

We estimated the concentration–mortality response and median lethal concentration (LC_{50}) for apterous adult aphids using probit analysis (POLO-PC).[23] The one-way ANOVA test was used to testing the effects of the sub-lethal concentration (LC_{10}) and the median lethal concentration (LC_{50}) of four neonicotinoid seed treatments on the life-table parameters (see the section 'Demographic effects') and EPG parameters (see the Results section) of the grain aphid , and means were separated using Tukey's honestly significant difference (HSD) test ($P < 0.05$). All data in this paper are expressed as mean ± standard error (SE). The SPSS 10.0 (SPSS, Chicago, IL, USA) software was used for all statistical analyses.

3 RESULTS

3.1 Concentration mortality response

The mortality of adult apterous aphids increased with increasing doses of each neonicotinoid. The median lethal concentration (LC_{50}) of imidacloprid, thiamethoxam, thiacloprid and dinotefuran seed treatments were determined to be 188, 135, 48 and 13 mg AI kg^{-1} seed, respectively (Table 1). The acute toxicity of the neonicotinoids tested was as follows: dinotefuran > thiacloprid > thiamethoxam > imidacloprid.

3.2 Demographic effects

Life-table parameters of aphids were not greatly affected by LC_{10} concentration of all neonicotinoids examined compared to the control (Table 2).

The net reproductive rates (R_0) of LC_{50} concentration treatments were significantly lower than those of controls ($F_{4,24} = 10.386$, $P = 0.0005$), yet there was not a significant difference among the four neonicotinoids ($F_{3,19} = 0.686$, $P = 0.6434$). For the mean generation time (T) of LC_{50} concentration treatments, only dinotefuran was significantly lower compared to the control and other treatments ($F_{4,24} = 9.751$, $P = 0.0007$). The intrinsic rate of increase (r_m) of aphids was significantly affected at the LC_{50} concentration for all neonicotinoids examined compared to the control ($F_{4,24} = 8.672$, $P = 0.0015$). The lowest value of r_m measured was 0.003 ± 0.001 in the dinotefuran treatment. The finite rate of increase (λ) was not significantly affected by LC_{50} concentration of imidacloprid, thiamethoxam and thiacloprid compared to the control ($F_{3,19} = 2.693$, $P = 0.0929$). On the other hand, λ values of dinotefuran treatments were significantly lower than those of the control and other treatments ($F_{4,24} = 6.751$, $P = 0.0124$). The doubling time (DT) for each treatment at the LC_{50} concentration was also significantly longer than that of the control ($F_{4,24} = 10.138$, $P = 0.0006$). The highest values of DT were 203.11 ± 23.84 days in the dinotefuran treatment.

Table 3. Electrical penetration graph (EPG) parameters of *Sitobion avenae* stylet penetration activities on wheat plants treated with LC_{10} and LC_{50} of the four neonicotinoid seed treatments (imidacloprid, thiamethoxam, thiacloprid and dinotefuran)

Concentration	Treatment	C	E1	E2	F	G	np
0	Control	184.11 ± 14.19^{a}	50.11 ± 6.64^{a}	363.33 ± 28.01^{a}	22.33 ± 7.55^{a}	23.56 ± 11.91^{a}	71.78 ± 17.90^{a}
LC_{10}	Imidacloprid	227.56 ± 26.58^{a}	25.11 ± 6.06^{b}	202.64 ± 28.38^{b}	32.11 ± 14.08^{a}	1.22 ± 1.22^{b}	214.78 ± 29.26^{b}
	Thiamethoxam	226.67 ± 24.62^{a}	29.56 ± 6.24^{b}	171 ± 29.66^{b}	26.44 ± 19.09^{a}	4.22 ± 2.88^{b}	142.11 ± 13.87^{b}
	Thiacloprid	220.22 ± 29.02^{a}	32.08 ± 6.08^{b}	202.78 ± 28.38^{b}	19.47 ± 6.15^{a}	8.89 ± 5.47^{ab}	112.72 ± 17.22^{b}
	Dinotefuran	221.22 ± 29.02^{a}	25.44 ± 6.83^{b}	236.44 ± 36.37^{b}	18.56 ± 5.50^{a}	0^{b}	148.89 ± 30.72^{b}
LC_{50}	Imidacloprid	338.56 ± 29.02^{ab}	16.67 ± 5.04^{b}	89.33 ± 17.49^{b}	19.29 ± 5.53^{a}	5 ± 5.00^{b}	378.33 ± 22.00^{b}
	Thiamethoxam	330.11 ± 20.22^{ab}	24.11 ± 8.73^{b}	91.56 ± 16.90^{b}	19.33 ± 5.27^{a}	11.89 ± 1.29^{b}	382.89 ± 35.32^{b}
	Thiacloprid	310.44 ± 25.12^{ab}	11.3 ± 2.44^{b}	101.5 ± 19.40^{b}	31.67 ± 6.49^{a}	0^{b}	265.67 ± 27.21^{b}
	Dinotefuran	282.11 ± 22.46^{a}	18.67 ± 1.64^{b}	133.56 ± 27.75^{b}	21.12 ± 8.17^{a}	3.89 ± 19.92^{b}	296.67 ± 34.87^{b}

The data in the table represents the mean ± SE of 15 aphids continuously recorded for 12 h.
Different superscript letters in columns indicate significant differences compared to the control ($P < 0.05$).
The EPG parameters are: C, reflecting an extracellular stylet pathway; E1, reflecting salivary secretion into the sieve element; E2, reflecting phloem sap ingestion and concurrent salivation; F, reflecting mechanical probing difficulties; G, reflecting ingestion in xylem; np, nonprobing.

3.3 Sub-lethal effects on feeding behaviour

Sub-lethal effects of the four neonicotinoids on EPG parameters are shown in Table 3. Percentages of the accumulated duration of the E1 and E2 waveform (which reflect the proportion of time spent by aphids salivating or ingesting into/from phloem) were significantly lower on LC_{10} and LC_{50} concentration treatments than on control. The C waveform times, which reflect the amount of time spent probe penetrating, were not significantly affected by LC_{10} and LC_{50} concentration for all neonicotinoids examined compared to the control. The durations of the no-probing periods (the np waveform value) for the LC_{10} and LC_{50} concentration treatments were significantly longer than those of the control. Moreover, the aphids on LC_{10} and LC_{50} concentration treatments reduced the time of ingestion inxylem (G waveform).

4 DISCUSSION

The aim of chemical control within an Integrated Pest Management (IPM) context is to keep pest numbers below an economic threshold. In practice, however, in order to achieve the desired control effect, and a higher economic return, people often blindly increase the quantity and frequency of insecticide usage. These practices can directly lead to an accelerated rate of resistance development, environmental pollution and actual harm to the health of people.[24–28] Our research shows that in addition to an acute toxicity to *S. avenae*, seed treatment with neonicotinoids also caused a range of sub-lethal effects on life-table parameters and feeding behaviour.

Daniels *et al.*[29] reported that *Rhopalosiphum padi* (L.) born on wheat treated with thiamethoxam reached reproductive maturity more slowly and produced significantly fewer nymphs than aphids born on wheat treated with distilled water. Similarly, reductions in fitness, including increased development times and decreased fecundity, have also been observed in *Aphis gossypii* (Glover) and *Brevicoryne brassicae* (L.) feeding on plants treated with a sub-lethal dose of imidacloprid.[10,30] In our study, the growth and fertility of *S. avenae* feeding on wheat plants treated with sub-lethal concentrations (LC_{10}) of imidacloprid, dinotefuran, thiacloprid and thiamethoxam was not greatly affected. However, the longevity, fertility and population increase of *S. avenae* was reduced significantly at the median lethal concentration (LC_{50}) of the four neonicotinoids, particularly dinotefuran.

The effects on fecundity after exposure to sub-lethal pesticide concentration may be ascribed to physiological and behavioural effects.[6] Research on the behavioural effect of neonicotinoids is important in preventing an increase in the levels of insecticide resistance and avoiding off-target effects on non-target organisms. Examples of such sub-lethal effects on behaviour have been quantified in some species, e.g. foraging of honey bees and bumblebee,[31,32] chemical communication of the cabbage looper, the pink bollworm moth and *Trichogramma brassicae* wasps[8,33,34] courtship of the cabbagelooper[35,36] and oviposition of the mirid bug, *Trichogramma brassicae* wasps and *Encarsia formosa* wasps.[7,34,37] Disrupted feeding behaviour in response to sub-lethal concentration/dose of neonicotinoids have been observed in *Myzusper sicae* (Sulzer),[38] *Myzus nicotianae* (Blackman)[39] and *Bemisia tabaci* (Gennadius)[40] in response to imidacloprid. Our study concurs with these findings in indicating that the feeding behaviour of *S. avenae* is greatly affected by sub-lethal concentrations of the four insecticide seeding treatments. EPG recording showed a higher percentage of no probing phase and a shorter phloem sap ingestion phase on the wheat plants treated with sub-lethal concentration of four neonicotinoids, suggesting that the aphids spend less time feeding from phloem. In addition, xylem feeding times of *S. avenae* reared on wheat treated with sub-lethal concentration were significantly reduced compared to aphids feeding on controls. Similar results have been reported in *R. padi*.[29] We hypothesise that the reduction in phloem and xylem feeding may result in severe detrimental effects on aphid performance, specifically growth and fecundity.

5 CONCLUSIONS

Our work shows that even low concentration of dressing seeds with imidacloprid, dinotefuran, thiacloprid and thiamethoxam have long-term, adverse effects on wheat aphid feeding, survival, fecundity and population increase. However, to fully assess sub-lethal effects on wheat aphids under field conditions, additional research is needed. This will require relating sub-lethal effects of neonicotinoids on wheat aphids with the degradation dynamics of those pesticides in wheat plants. Meanwhile, a long-term study should evaluate the potential sub-lethal effects on natural enemies of wheat aphids, which could help in form integrated pest management programmes with emphasis on biological control for this pest.[6] This study helps assess the value of dressing seeds with neonicotinoids as a control tool for wheat aphids, and it provides the basis for a more rational use of those pesticides in China.

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