Research Article

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Neonicotinoids and bumblebees (*Bombus terrestris*): effects on nectar consumption in individual workers

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Abstract:

BACKGROUND: The objective of this study was to quantify whether the presence of three different neonicotinoid insecticides (imidacloprid, thiamethoxam or clothianidin) in sucrose solution results in antifeedant effects in individual worker bumblebees (*Bombus terrestris*), and, if so, whether this effect is reversible if bees are subsequently offered untreated feed.

RESULTS: Bees exposed to imidacloprid displayed a significant dose-dependent reduction in consumption at 10 and 100 μg L^{-1}, which was reversed when untreated feed was offered. No consistent avoidance/antifeedant response to nectar substitute with thiamethoxam was detected at the more field-realistic dose rates of 1 and 10 μg L^{-1}, and exposure to the very high 100 μg L^{-1} dose rate was followed by 100% mortality of experimental insects. No reduction in food intake was recorded at 1 μg clothianidin L^{-1}, reduced consumption was noted at 10 μg clothianidin L^{-1} and 100% mortality occurred when bees were exposed to rates of 100 μg clothianidin L^{-1}.

CONCLUSION: This study provides evidence of a direct antifeedant effect of imidacloprid and clothianidin in individual bumblebees but highlights that this may be a compound-specific effect.

Keywords: bumblebees; neonicotinoids; consumption; antifeedant; avoidance

1 INTRODUCTION

In recent years there has been an unprecedented increase in the number of studies of the effects of neonicotionid insecticides on bees when applied as seed treatments.[1] These have yielded reports of a range of biological responses that might result in adverse effects at both the individual and colony levels, such as reduced queen production in bumblebees[2] and impaired foraging activity[3] and nest building.[4,5] However, accurate interpretation of the impact of these responses on colonies nesting near to commercial crops is hampered by the high exposure rates used in many laboratory and semi-field experiments, uncertainty regarding the significance at the colony level of the sublethal endpoints used in some studies to assess pesticide effects and the high incidence of studies of *Apis* spp., together with the corresponding significant lack of information on other pollinator species.[6] In addition, much of the published research has been conducted using imidacloprid, but usage of imidacloprid as a seed treatment on oilseed rape has decreased rapidly in the United Kingdom.[7] Data from both honeybees[8] and bumblebees[9–12] suggest that the effects elicited following exposure to imidacloprid may not reflect those of thiamethoxam and clothianidin, making extrapolation of potential effects to other members of the neonicotinoid group unreliable without further targeted research.

A potential exposure route following seed treatment results from systemic translocation of active substances to the pollen and nectar collected by foraging pollinators, and it is known that both the quality and quantity of such food sources can directly influence the development of bumblebee colonies.[13–15] However, whether the presence of pesticides in nectar and pollen influences the rate of collection and consumption of food, as has been shown to occur in some biological control agents,[16] is uncertain and requires further investigation.

Published laboratory studies have reported collection rather than consumption of treated feed by *Bombus* workers from colonies or microcolonies that only have access to pesticide-treated nectar or pollen. The objective of this study was to quantify whether the presence of three different neonicotinoid insecticides (imidacloprid, thiamethoxam or clothianidin) in sucrose solution used as a nectar substitute results in modified consumption rates, and, if so, whether this effect is reversible if

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bees are subsequently offered untreated feed. Such responses might be generated by mechanisms such as avoidance (a primary response based on smell or taste) or antifeedant (a secondary response following ingestion) effects in individual worker bumblebees (*Bombus terrestris*).

2 MATERIALS AND METHODS

Bumblebees (*B. terrestris audax*) were obtained from Koppert, the Netherlands. All bumblebees were anaesthetised for a maximum of 60 s under nitrogen prior to handling and maintained at 21 °C with a 8:16 h light:dark cycle. Sucrose solution ('nectar substitute'; 30% w/v to mimic oilseed rape nectar) was prepared in deionised water. Imidacloprid, thiamethoxam and clothianidin were obtained as Pestanal analytical standards (>99% purity; Sigma Aldrich, UK). Stock solutions of the pesticides were prepared in acetone (Analar; Sigma Aldrich, UK) which was then diluted in 30% w/v sucrose solution to provide solutions of 0 (control), 1, 10 and 100 µg neonicotinoid L^{-1}. The two lower (field-realistic) concentrations (1 and 10 µg neonicotinoid L^{-1}) were selected because they span the range reported in nectar following use of the insecticides as seed treatments in the field,[17–19] and the highest level represented a suprafield concentration identified by previous studies as likely to produce effects.[4]

2.1 Feeding rates following pesticide exposure to thiamethoxam and clothiandin

Individual bees were housed in cages similar to those used by Elston *et al.*,[12] consisting of a 500 mL plastic container containing a preweighed 1.5 mL Eppendorf tube with a feeding hole drilled at one end and filled with thiamethoxam- or clothiandin-treated sucrose solution. The feeding tube was replaced daily and was weighed on removal to determine the mass of sucrose solution consumed during each 24 h period. Controls utilised the same method, but bees were offered untreated sucrose solution. Owing to the naturally wide variations in the size of bumblebee workers,[20] all bees were weighed at the end of the exposure phase and the sucrose intake converted to g sucrose g^{-1} bee. The study was conducted for four consecutive days, and there were 20 replicates per dose.

2.2 Feeding rates during and following exposure to imidacloprid

To assess the effects of imidacloprid-treated feed on consumption of sucrose solution both during and post-exposure, the exposure study was run twice. The first study was conducted for four consecutive days with imidacloprid-treated sucrose using the technique described above. The second study was then run as previously but for 3 days of exposure to imidacloprid-treated sucrose followed by a 2 day recovery period during which the bees were offered only untreated sucrose. The mass of sucrose solution consumed during each 24 h period was recorded and converted to g sucrose g^{-1} bee.

2.3 Data analysis

Data were subjected to repeated-measures ANOVA using MINITAB, with time (day) as the repeated measure and dose as the treatment factor. Where significant effects of dose were detected, the total consumption over the treatment period was compared by Student's *t*-test.

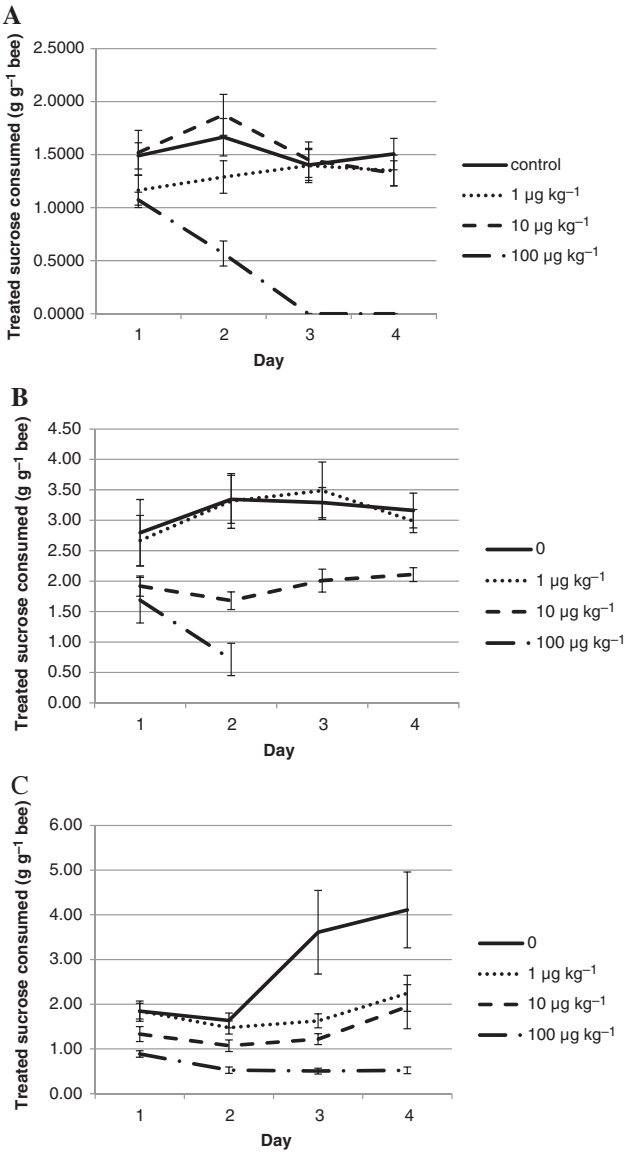

Figure 1. Mean consumption per day of sucrose treated with 0, 1, 10 and 100 µg L^{-1} of neonicotinoid insecticide on days 1 to 4 by individually housed bumblebees ($n = 20$; bars = ± SE). A: thiamethoxam; B: clothiandin; C: imidacloprid.

3 RESULTS

3.1 Feeding rates following pesticide exposure

Following the 4 day exposure to the 0 (control), 1 and 10 µg thiamethoxam L^{-1} treatments, 10% mortality was recorded in each treatment. As exposure to 100 µg thiamethoxam L^{-1} resulted in 55% mortality by day 2 and 100% mortality by day 3, these data were excluded from the statistical analysis. There was no significant effect of day ($F = 2.59$, df = 3, $P = 0.058$) or dose ($F = 1.14$, df = 2, $P = 0.327$) on consumption in the 0, 1 and 10 µg thiamethoxam L^{-1} treatments (Fig. 1A) over the 4 day experimental period. There was also no significant effect of thiamethoxam on consumption on day 1 (one-way ANOVA: $F = 1.94$, df = 3, $P = 0.131$). These data suggest that no avoidance/antifeedant effects of thiamethoxam-treated feed by *B terrestris* were detected.

Mortality was less than 10% following exposure to the 0 (control), 1 and 10 µg clothiandin L^{-1} treatments. However, as 80% mortality was recorded by day 2 and 100% mortality by day 3 in the 100 µg

clothiandin L^{-1} treatment, these data were excluded from the statistical analysis. There was no significant effect of day ($F = 1.32$, df =3, $P = 0.273$), but there was a significant effect of dose (0, 1 or 10 μg L^{-1}) on consumption ($F = 11.16$, df = 2, $P < 0.001$) (Fig. 1B). When compared with the control, the total intake of the 1 μg clothiandin L^{-1} was not significantly different ($P = 0.97$), but the total intake of the 10 μg clothiandin L^{-1} treated sucrose over the 4 days was significantly lower ($P < 0.001$).

Following 4 day exposure to imidacloprid, 5% mortality was recorded in the 1 and 10 μg imidacloprid L^{-1} treatments, and 25% of the bees died following exposure to 100 μg imidacloprid L^{-1}, and therefore the latter data were included in the analysis. There was a 15% mortality in controls after the 4 day exposure period. There were significant effects of both day ($F = 6.02$, df = 3, $P = 0.003$) and dose ($F = 18.32$, df = 3, $P < 0.001$) on consumption of imidacloprid-treated sucrose, with a significant interaction between day and dose ($F = 2.63$, df = 9, $P = 0.021$) (Fig. 1C). Overall intake over the 4 day exposure period was significantly lower in the 10 and 100 μg imidacloprid L^{-1} treatments than in controls ($P = 0.002$ and $P < 0.001$ respectively) but not in the 1 μg imidacloprid L^{-1} treatment ($P = 0.057$).

3.2 Feeding rates on untreated sucrose

Provision of untreated sucrose for 2 days after a 3 day exposure to imidacloprid resulted in a recovery in sucrose consumption rates by the bees (Fig. 2). Following 3 day exposure to imidacloprid, 5% mortality was recorded in the 1 μg imidacloprid L^{-1} treatments, and 15% of the bees died following exposure to 10 and 100 μg imidacloprid L^{-1}. There was a 15% mortality in controls by the end of the 3 day exposure period. There was no additional mortality in any of the treatments or controls during the following 2 days when untreated sucrose was provided. During the treatment period (days 1 to 3) there was no significant effect of day on consumption ($F = 1.64$, df = 2, $P = 0.198$), but there was a significant effect of dose ($F = 13.78$, df = 3, $P < 0.001$). During the subsequent 2 days when they were fed untreated sucrose there were significant effects of both day ($F = 10.24$, df = 1, $P = 0.002$) and the dose administered during the earlier treatment period ($F = 7.27$, df = 3, $P < 0.001$) on consumption.

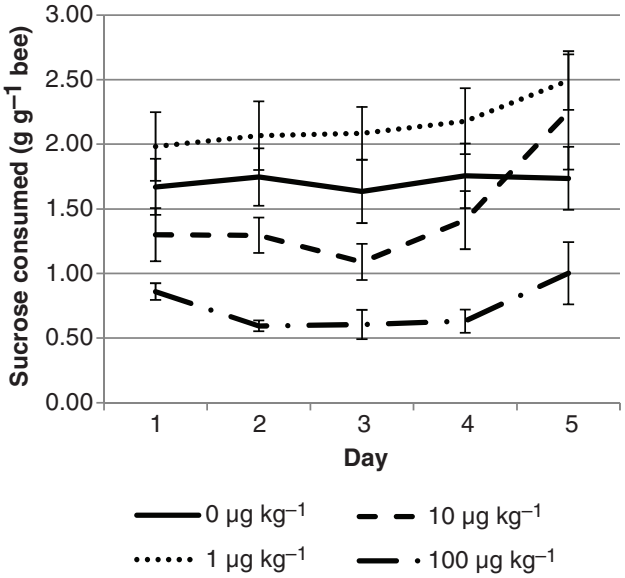

Figure 2. Mean consumption per day of sucrose treated with 0, 1, 10 and 100 μg L^{-1} of imidacloprid on days 1 to 3 and untreated sucrose on days 4 and 5 by individually housed bumblebees ($n = 20$; bars = ± SE).

4 DISCUSSION AND CONCLUSIONS

The use of realistic dose/exposure rates of pesticides is important if the results of laboratory experiments are to be relevant predictors of effects under field conditions.[1] The field-realistic exposure rates used in this study were established using a similar approach to previous work[12] and were based on residue data held by European Member States and collated by EFSA.[17–19] The highest dose rates utilised (100 μg L^{-1}) were included to take account of high levels identified by previous studies as likely to produce effects.[1,6] Diverse forage sources utilised by *B. terrestris* coupled with discontinuous distribution of treated plants in the agri-environment offer the opportunity to feed on untreated plants, further reducing exposure, and it has been postulated that antifeedant or avoidance behaviour in other bee species following contact with pesticide-contaminated plant material may enhance this effect.[21,22] However, further studies were required to establish whether neonicotinoids stimulate such responses under laboratory or field conditions.

Imidacloprid has well-established antifeedant properties in other invertebrate species, including *Gammarus* pulex,[23] *Chironomus*,[24] mayflies and oligochaetes,[25] *Myzus persicae* (peach potato aphid),[26] *Bemisia tabaci* (tobacco whitefly),[27] Coleoptera [*Anoplophora glabripennis* and *Plectrodera scalator* (cottonwood borer)][28] and Coccinellids.[29] Reversibility of these antifeedant effects within 24 h has been reported in Coccinellids[29] and aphids[26] when individuals are given access to untreated feed. If similar antifeedant responses are induced in bees by neonicotinoid insecticides present in the nectar at field-realistic levels, then they may explain, in part, why semi-field and field studies have resulted in variable colony level responses.

In this study, no consistent avoidance/antifeedant response to nectar substitute with thiamethoxam was detected at the more field-realistic dose rates of 1 and 10 μg L^{-1}, and all bees exposed to the very high 100 μg L^{-1} dose rates consumed sufficient to cause mortality. There are variable reports in the literature regarding responses of queenless microcolonies to thiamethoxam. When microcolonies were provided with thiamethoxam-treated sucrose and pollen for 28 days, intake was significantly reduced at 10 μg thiamethoxam L^{-1} but not at 1 μg thiamethoxam L^{-1}.[12] In a second study, when only thiamethoxam-treated sucrose was provided at a dose rate of up to 15.7 μg kg^{-1} (approximately 20 μg L^{-1}), consumption was not affected during a 14 day exposure period.[11] Interpretation of microcolony intake data is complicated by the potential for storage of collected sucrose in cells within the nest. Accurate assessment of true consumption by workers is difficult unless stored nectar is quantified during or at the end of the study and used with measurements of nectar collected to estimate intake by individual or small groups of bees. The design of the present work eliminated the potential complication of nectar storage by using individually housed workers, which do not construct storage cells. Reports of laboratory studies with microcolonies or colonies should include assessments of both the collection of the treated sucrose and that remaining within the colony at the end of the study to allow assessment of true levels of consumption.

Contrasting results were obtained when bumblebees were exposed to clothianidin- or imidacloprid-treated sucrose solution. Limited evidence was recorded that exposure to clothianidin may induce reduced nectar consumption. Whereas at dose rates of

1 μg L^{-1} no reduction in food intake was recorded when compared with untreated controls, and 100% mortality occurred when bees were exposed to rates of 100 μg L^{-1}, reduced consumption was noted when bees were exposed to the likely environmental maximum of 10 μg L^{-1} clothianidin in sucrose solution. Bees exposed to imidacloprid displayed a significant dose-dependent reduction in consumption rate.

Consistent results have been reported from queenless microcolonies, with dose-dependent reductions (in response to dose rates of up to 10 μg imidacloprid L^{-1}) of 10–30% in sugar solution consumption.[4] In the present study the rate of consumption of sugar solutions in the controls varied between experiments investigating the effects of different active ingredients. However, this variation is consistent with the range of consumption rates reported for *Apis* bees under similar laboratory conditions in previous studies[9,30] and day-to-day consumption rates reported in bumblebees.[9] Data generated by the present study also appear to show that the effect of imidacloprid is reversible when bumblebees that have fed on treated sucrose solution encounter untreated feed (although delays in this response resulted in the total intake over the 2 day post-exposure period remaining significantly below the intake by controls), reflecting the reversibility of effects in brood production reported in microcolonies[10] and the clearance rate of ingested imidacloprid in bumblebees.[9] The absence of significant mortality and increased intake of the follow-on untreated sucrose in the present work suggested that the treatment was a reversible antifeedant response (a generalised reduction in intake following ingestion of the treatment), which limited the intake at the field maximum concentration.

This study provides evidence of a direct antifeedant effect of imidacloprid in individual bumblebees, but highlights that this may be a compound-specific effect that is not reflected in thiamethoxam at the dose rates tested. However, further experimentation at a wider range of dose rates near to or just above the 10 μg L^{-1} level investigated here may be required to confirm whether a similar response exists for clothiandin and thiamethoxam. Further, experiments in which bees are offered a choice between sugar solutions dosed with pesticides and those without pesticides are also required to determine whether switching to uncontaminated nectar sources occurs instead of, or in addition to, feeding depression.

Existence of an avoidance/antifeedant response to a neonicotinoid insecticide such as imidacloprid is an important consideration in habitats offering a wide range of forage. It is known that bumblebees do not show the same level of flower constancy as some other species,[31] and in agricultural environments this can result in pollen and nectar being taken from a wide range of plants rather than restricting foraging to a nearby mass-flowering crop. Thus, utilisation of florally rich off-crop habitats that have been developed to provide alternative forage for bumblebees may be enhanced by such responses, contributing to the further development of sustainable farming methods.[32] Although the existence of antifeedant responses may result in reduced pollination services, a wide range of wild invertebrate species are known to contribute to pollination of most crops,[33] mediating the impact of potential reduction in bumblebee activity.

Existence of avoidance/antifeedant behaviour may also contribute to the understanding of the results of a recent field study in which *B. terrestris audax* colonies were placed at the edge of a field of oilseed rape grown from imidacloprid-treated seed.[34] In that study the colonies were significantly smaller than those exposed to oilseed rape grown from thiamethoxam-treated oilseed rape, and assessments of flying activity at the colony entrance and palynology suggested foraging activity was lower. However, further work is required to confirm whether individual bees show antifeedant responses under field conditions.

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