**Effects of exposure to imidacloprid direct and poisoned cotton aphids *Aphis gossypii* on ladybird *Hippodamia variegata* feeding behavior**

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Imidacloprid is a pesticide used to control aphid infestations of cotton plants. However, poisoned aphids also serve as food for the ladybird natural predator *Hippodamia variegata*. We investigated whether imidacloprid-treated eggs, pupae, and adults of *H. variegata* and poisoned aphids altered ladybird predatory behavior. Laboratory bioassay results demonstrated that 0.72 g/L imidacloprid was lethal to ladybirds. Imidacloprid significantly reduced the hatching and emergence rates of *H. variegata*, and these effects were time and dose dependent. Predation was most adversely affected when the ladybirds directly consumed poisoned aphids and less so when directly exposed to the insecticide at sublethal concentrations. Imidacloprid use in cotton fields should be restricted to the initial stages of aphid infestation to avoid the period when adult ladybirds are present.

**Keywords:** imidacloprid, *Hippodamia variegata*, toxicity, cotton aphid, *Aphis gossypii*.

**Introduction**

The cotton aphid *Aphis gossypii* is a Hemiptera that is a significant global crop pest. The aphid injures plants by sucking the sap and can transmit plant viruses or other pathogens by this behavior.1–3 An outbreak can rapidly damage plants, since the aphid has a short reproductive cycle and a large reproductive capacity under the appropriate environmental conditions. The control of *A. gossypii* has depended heavily on chemical insecticides. However, the extensive and frequent use of pesticides has resulted in high resistance, and *A. gossypii* infestations have become unmanageable.4,5 Neonicotinoids, which account for 27% of the global pesticide market and are registered in more than 120 countries,6 are generally applied as a foliar spray to control a range of sucking insects, including *A. gossypii* and the whitefly.5,7,8 Imidacloprid was introduced in the early 1990s and showed high insecticidal activity and a unique mechanism of action. However, extensive application of these insecticides over the past decades has led to the development of pesticide resistance in these cotton aphids.9 The increased insecticide applications in attempts to control these resistant strains pose substantial environmental risks, including toxicity to nontarget organisms such as natural predators.10–12

Predatory natural enemies play important roles in integrated pest management (IPM). These IPM systems are designed to keep aphid populations below economic damage thresholds.13,14 There are many examples of the use of natural enemies to control insect pests.15–18 The ladybird *Hippodamia* (Adonia) *variegata* (Goeze) is a coleopteran that is a dominant natural enemy insect of cotton pests in China’s cotton-growing region. It is a strong predator, but the extensive and frequent use of pesticides has adversely affected the development and reproductive behavior of this and other natural predators.19,20 Research on the risk of pesticides such as imidacloprid to natural insect populations has been conducted in several countries. For example, the fecundity of the Tasmanian lacewing (*Micromus tasmaniae*) and the transverse ladybird (*Coccinella transversalis*) was reduced by pirimicarb and imidacloprid treatments. In addition, pyridoxine was lethal to 97.6% of these predators before they matured.21 The insecticides imidacloprid, abamectin, and alpha-cypermethrin at sublethal doses (LC20) had an inhibitory effect on the parental and F1 generations and inhibited the predation of *H. variegata*.22 On the other hand, imidacloprid has been judged generally less toxic to ladybirds than other insecticides, and this may provide an opportunity to achieve balance between aphids and natural enemies.23 Few studies have focused on whether the consumption of contaminated prey affects ladybird predation behavior.24,25 Therefore, our objective was to clarify whether imidacloprid affects the eggs, pupae, and adults of *H. variegata* and whether *H.
variegata is affected after consuming imidacloprid-contaminated prey, including live, freshly killed, and mummy aphids. We also assessed whether different toxicity (stomach toxicity and contact toxicity) of imidacloprid affected the predation of H. variegata.

Materials and Methods
1. Insects and formulation of imidacloprid
Eggs, pupae, and adults of H. variegata were collected from cotton fields near Changji in Xinjiang, China, that had not been treated with any agricultural insecticide because the area was a new field previously not used for farming. Aphis gossypii adults were collected from an experimental cotton field at the campus of Xinjiang Agricultural University, and pesticide use is not allowed near the campus. Bayer Crop Science (China) supplied the technical insecticides used for bioassays. The commercial insecticide formulation used for all of these experiments was imidacloprid as water-dispersible granules at 200 g a.i./kg (Bayer Crop Science, Thayer, India) except for the data in Fig. 3, where the neat formulation was used. Distilled water was used as a control. All tests were conducted in a climate-controlled room at 25±2°C with relative humidity of 60±15% and a 14-hr L:10-hr D photoperiod.

2. Effects of imidacloprid on ladybirds
Imidacloprid was used at 0.06, 0.12, 0.24, 0.48, and 0.72 g/L as an active ingredient, and distilled water alone was used as a blank control treatment. Imidacloprid was applied to H. variegata adults by pesticide exposure method and to eggs and pupae by the dipping method. Each test consisted of adults, eggs, and pupae in individual petri dishes (4.5 cm diameter × 0.7 cm height) with 10 replicates per group. Pre-hatch eggs (black and gray) and pre-emergence pupae (yellow) were used for testing. Survival and mortality were recorded at 24, 48, and 72 hr after treatment and observed via light microscopy. Mortality in adults was judged to be when the legs did not move and the color of pupae and eggs turned black. All experiments were carried out in triplicate.

3. Aphid predation assay of H. variegata
The predation rate tests for H. variegata were conducted in petri dishes (70 mm diameter) using test aphids treated as follows: (A) Insecticide-free aphids (distilled water treatment), (B) live aphids treated with imidacloprid, (C) fresh dead aphids treated with imidacloprid (immobile legs, body color unchanged), and (D) mummy aphids treated with imidacloprid (no leg movement, body dry and black). Experimental cotton aphids were exposed to imidacloprid (0.09 g/L) via the dipping method (see above). One adult ladybird was used per 50 aphids in each dish, and the number of aphids consumed was checked at 8, 16, 24, 32, and 40 hr with 10 replicates per group.

Aphids were treated with imidacloprid (0.09 g/L), and feeding times were evaluated using one H. variegata adult in a petri dish with 100 fresh dead aphids killed with imidacloprid. Aphid consumption was recorded at 3, 6, 12, 24, and 48 hr using 10 replicates per group.

4. Effect of imidacloprid on the predation of H. variegata adults
The effect of imidacloprid on the predation of H. variegata adults was tested using two methods: contact toxicity refers to exposure to pesticides through the body wall and stomach poisoning by ladybirds feeding on poisoned aphids. The assays were carried out in petri dishes (15 cm) with an aphid density of 200 heads and 1 adult H. variegata per dish. Distilled water alone was used as a control. The experiment was divided into two groups. In the first group, H. variegata adults were pretreated with different concentrations (0.06, 0.24, and 0.72 g/L) of imidacloprid using contact toxicity. After 24 hr, the live H. variegata adults were selected to determine their predation on aphids that had not been treated with imidacloprid. In the second group, the prey was pretreated with different concentrations (0.06, 0.24, and 0.72 g/L) of imidacloprid using stomach toxicity. After 24 hr, the live aphids were picked for predation tests using H. variegata adults that had not been treated with imidacloprid. The number of aphids consumed was checked at 48 hr, with 10 replicates per group. Treated under the same conditions, and changed aphids in new petri dishes every day.

5. Statistical analysis
The data were compiled and tabulated for statistical analysis. The Windows-based SPSS Statistics v22 program was used for analysis of variance (ANOVA) to determine the statistical significance of treatment. Corrected mortality was carried out using Abbott’s formula.

Results
1. Effects on adults
We examined the time and dose dependence (0.06, 0.12, 0.24, 0.48, and 0.72 g/L) of imidacloprid on our test insects in a laboratory study. We found that imidacloprid at 24 and 48 hr at the lowest concentration 0.06 g/L was not lethal to adults, but 3.3% mortality was observed up to 72 hr. Treatment concentrations >0.12 g/L resulted in mortality of 56–86% across the dose range by 72 hr, and the mortality significantly increased with the increase in insecticide dose (Table 1).

Table 1. Toxicity of imidacloprid to H. variegata adults

| Treatment | Dose (g/L) | N  | Corrected mortality (Mean) |
|-----------|------------|----|----------------------------|
|           | 24 hr      | 48 hr | 72 hr                     |
| Water     | 0.06       | 0.00 | 0.00 | 0.00 | 3.33 |
|           | 0.12       | 13.33 | 26.67 | 46.67 |
| Imidacloprid | 0.24       | 20.00 | 40.00 | 56.67 |
|           | 0.48       | 26.66 | 50.00 | 76.67 |
|           | 0.72       | 55.17 | 72.41 | 86.21 |

*p<0.05; N, number of insects tested.
2. Effects on eggs
The effects of imidacloprid on eggs were assessed by measuring the egg hatching rate. The hatching rate was 100% at 0.06 g/L imidacloprid and decreased to 88% at 0.12 g/L and 51% at 0.72 g/L. The highest concentration of imidacloprid (0.72 g/L) significantly reduced the hatching rate when compared with the control ($p<0.01$), but the inhibition rate did not reach 50% (Table 2).

3. Effects on pupae
The effects on pupae began to be apparent at 0.24 g/L imidacloprid, and the emergence rates and numbers were significantly reduced as the concentration was increased. We found significant differences between the treatment groups (0.72 g/L) and the control ($p<0.01$). However, 0.06 and 0.12 g/L imidacloprid had little effect on eclosion, and the emergence number was the same for both groups, with no significant differences between the treatment groups and the control ($p>0.05$) (Table 3).

4. Effects of treated aphids on ladybird feeding
Our preliminary experiments indicated that ladybird adults would feed on poisoned aphids. We therefore examined this behavior with aphids killed by imidacloprid (0.09 g/L). Adult ladybirds did not feed on mummy aphids, but the initial predation rate of adults for live aphids treated with imidacloprid was 4% and after 40 hr was 21.8% (60% less). We found significant differences between the treatment groups and the control after 32 hr ($p<0.05$) (Fig. 1). These results demonstrated that feeding on imidacloprid-treated aphids decreased the predation of ladybirds.

5. Effects of aphid death time on ladybird adult feeding
The above studies indicated that ladybird predation was significantly reduced after feeding on imidacloprid-treated aphids. We found a feeding rate in our controls that ranged from 12.8 to 25.2% and showed an overall upward trend, as expected. The feeding rate of the treatment groups ranged from 5.9 to 0% over time, and this difference was highly significant ($p<0.01$). Therefore, altering pesticide treatment times also altered ladybird feeding rates (Fig. 2).

6. Effects of imidacloprid on ladybird adult predation
The above tests demonstrated that the ladybird predation rate is negatively affected by imidacloprid exposure. Thereafter, whether exposure of the ladybirds to imidacloprid directly or by feeding on poisoned aphids was the cause of decreased aphid feeding were tested. After exposure of the ladybirds to

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### Table 2. Effects of imidacloprid on *H. variegate* egg hatching

| Treatment   | Dose (g/L) | N   | Number hatching | Hatching (%) | Hatching inhibition (%) |
|-------------|------------|-----|-----------------|--------------|-------------------------|
| Water       | 43         | 39  | 39              | 90.7$^a$     | —                       |
|             | 0.06       | 58  | 58              | 100.00       | —                       |
|             | 0.12       | 66  | 58              | 87.88$^a$    | 10.25                   |
| Imidacloprid| 0.24       | 31  | 22              | 70.97$^a$    | 21.75                   |
|             | 0.48       | 36  | 18              | 50.00$^b$    | 44.87                   |
|             | 0.72       | 35  | 18              | 51.43$^b$    | 43.30                   |

$^a$ $p<0.05$; N, number of insects tested. $^b$ $p<0.01$.

### Table 3. Effects of imidacloprid on eclosion of *H. variegata* pupae

| Treatment   | Dose (g/L) | N | Number emerging | Emergence rate (%) | Emergence inhibition (%) |
|-------------|------------|---|-----------------|---------------------|--------------------------|
| Water       | 30         | 29 | 29              | 96.67$^a$           | —                       |
|             | 0.06       | 30 | 29              | 96.67               | 0.00                     |
|             | 0.12       | 30 | 28              | 93.33               | 3.46                     |
| Imidacloprid| 0.24       | 30 | 21              | 70.00$^a$           | 27.59                    |
|             | 0.48       | 30 | 20              | 66.67$^a$           | 31.03                    |
|             | 0.72       | 30 | 10              | 33.33$^b$           | 65.52                    |

$^a$ $p<0.05$; N, number of insects tested. $^b$ $p<0.01$.

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Fig. 1. Feeding behavior of *H. variegata* adults on live and dead aphids treated with imidacloprid. * $p<0.05$. Dry dead aphids were not consumed.
imidacloprid directly, adult feeding on healthy aphids decreased from 100 (control) to 90%, 65%, and 56% at concentrations of 0.06 g/L, 0.24 g/L, and 0.72 g/L, respectively. After adult exposure by feeding on poisoned aphids (stomach toxicity), their feeding decreased to 82%, 53%, and 44% at concentrations of 0.06 g/L, 0.24 g/L, and 0.72 g/L, respectively. These data indicated that, regardless of whether the ladybirds had been exposed to the insecticide directly or via ingestion of poisoned aphids, they were significantly and adversely affected by imidacloprid. This was especially true as the exposure dose increased (Fig. 3).

Discussion

Biological control plays an important role in agriculture and ecological protection, and IPM allows for the development of long-term control without the extensive use of chemical agents.39–42 Pesticides alter the balance between the pest and natural enemies, and pesticides do not always distinguish between the two.33–36 Among the neonicotinoid insecticides, imidacloprid has been used as a primary chemical control agent against A. gossypii and has a reduced effect on natural control insects as compared with other insecticides.37,38 For instance, imidacloprid was not lethal to adults of the predator Coccinella septempunctata or to Harmonia axyridis (harlequin beetle) or Cyclotella sanguinea (spotless lady beetle) larvae.39,40 In contrast, our laboratory tests indicated that exposure to 0.72 g/L imidacloprid had significant lethal effects on H. variegata adults. The corrected mortality of ladybird adults was 86% at 0.72 g/L imidacloprid. Our results are consistent with those of Kar and with the lethal effect of imidacloprid on coccinellids in fields tests. This level of pesticide application led to a significant decrease in the predation rate of ladybirds.

The effects of treated aphids on ladybird feeding experiments also indicated that ladybird adults feed on poisoned aphids. Altering the exposure time of the prey to the pesticide also altered ladybird predation rates. An increase in the imidacloprid concentration and prolongation of the predation time decreased the number of aphids consumed. Direct exposure of the ladybird (the predator of aphids) to the insecticide affected aphid consumption less than when healthy ladybirds fed on poisoned aphids. The influence of pesticides on the natural enemies of pests is the basic theoretical problem of coordinating chemical and biological control and is the key to IPM.41–44 Therefore, scientific and rational treatment strategies for the use of chemical pesticides in an IPM setting can prevent or reduce the adverse effects of pesticides on natural predators.

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References

1) R. B. Shrestha and M. N. Parajulee: Insect Sci. 20, 778–788 (2013).
2) H. N. Koo, J. J. An, S. E. Park, J. I. Kim and G. H. Kim: Crop Prot. 55, 91–97 (2014).
3) K. Wang, T. Liu, X. Jiang and M. Yi: Phytoparasitica 29, 393–399 (2001).
4) C. Yizhou et al.: Pest Manag. Sci. 69, 938–948 (2013).
5) P. Jeschke and R. Nauen: Pest Manag. Sci. 64, 1084–1098 (2008).
6) P. Jeschke, R. Nauen, M. Schindler and A. Elbert: J. Agric. Food Chem. 59, 2897–2908 (2011).
7) H. E. Conway, T. J. Kring and R. Mcnew: Fla. Entomol. 86, 474–476 (2003).
8) A. Kar: J. Entomol. Zool. Stud. 5, 1064–1067 (2015).
9) J. Zhang, L. Cui, X. Xu and C. Rui: Pest. Biochem. Physiol. 125, 1–7 (2015).
10) İ. Döker, M. L. Pappas, K. Samaras, A. Triantafyllou, C. Kazak and G. D. Broufas: Pest Manag. Sci. 71, 1267–1273 (2015).
11) U. Philipp, B. Roman, R. B. Schäfer and M. H. Entling: Chemosphere 132, 152–158 (2015).
12) A. F. G. Dixon: Q. Rev. Biol. 82, 244 (2000).
13) A. O. Soares, E. C. Dan and H. Schanderl: J. Anim. Ecol. 73, 478–486 (2004).
14) Y. Gao and C. Liu: Plant. Protect. 32, 51–53 (2006).
15) F. F. Rain et al.: Int. J. Appl. Sci. Technol. 4, 408–416 (2016).
16) A. Golizadeh and V. Jafari-Behi: Appl. Entomol. Zool. (Jpn.) 47, 199–205 (2012).
17) L. Shu et al.: Acta Phytophylacica Sinica. 14, 699–704 (2014).
18) G. Vasquez, D. Orr and J. Baker: J. Econ. Entomol. 99, 1104–1111 (2006).
19) N. Desneux, A. Decourtye and J. Delpuech: Annu. Rev. Entomol. 52, 81–106 (2007).
20) A. A. Castro, M. C. Lacerda, T. V. Zanuncio, F. de S Ramalho, R. A. Polanczyk, J. E. Serrão and J. C. Zanuncio: Ecotoxicology 21, 96–103 (2012).
21) P. G. Cole, A. R. Cutler, A. J. Kobelt and P. A. Horne: Aust. J. Entomol. 49, 160–165 (2010).
22) Q. Y. Yang, C. Wei, X. L. Sun and C. Z. Liu: Praticultural Sci 33, 1418–1425 (2016).
23) M. A. Amin, A. Hameed, M. Rizwan and M. Akmal: Int. J. Scientific Res. Environ. Sci. 2, 340–345 (2014).
24) S. R. Singh, K. F. A. Walters, G. R. Port and P. Northing: Biol. Control 30, 127–133 (2004).
25) D. G. Thornham, C. Stamp, K. F. A. Walters, J. J. Mathers, M. Wakefield, A. Blackwell and K. A. Evans: Biocontrol Sci. Technol. 17, 983–994 (2007).
26) X. I. Ren, W. I. Jiang, Y. Ma and Y. Ma: China Cotton. 43, 24–26, 31 (2016).
27) X. Y. Wang and Z. R. Shen: Acta Ecol. Sin. 22, 2278–2284 (2002).
28) W. S. Abbott: J. Econ. Entomol. 18, 265–267 (1925).
29) I. Denholm and M. W. Rowland: Annu. Rev. Entomol. 37, 91–112 (1992).
30) Q. Shang, Y. Pan, K. Fang, J. Xi and J. A. Brennan: Crop Prot. 31, 15–20 (2012).
31) C. Bass, I. Denholm, M. S. Williamson and R. Nauen: Pestic. Biochem. Physiol. 121, 78–87 (2015).
32) A. Matsuura and M. Nakamura: Appl. Entomol. Zool. (Jpn.) 49, 535–540 (2014).
33) J.-H. Lee and T.-J. Kang: Biol. Control 31, 306–310 (2004).
34) L. I. Jing and Z. J. Han: J. Pestic. Sci. 9, 257–262 (2007).
35) J. Bradley: Ecol. Lett. 6, 857–865 (2010).
36) S. Moosa: Ecotoxicology 20, 1476–1484 (2011).
37) X. Jiang et al.: Plant. Protect. 41, 151–153+170 (2015).
38) Z. Liu et al.: Pest Manag. Sci. 59, 1355–1359 (2003).
39) J. P. Michaud: J. Entomol. Sci. 1, 83–93 (2002).
40) A. Bozsik: Pest Manag. Sci. 62, 651–654 (2006).
41) J. Gardner, M. P. Hoffmann, S. A. Pitcher and J. K. Harper: Biol. Control 56, 9–16 (2011).
42) A. D. F. Bueno, M. J. Batistela, J. D. B. França-Neto, M. A. N. Nishikawa and A. L. Filho: Crop Prot. 30, 937–945 (2011).
43) P. Nilima, S. J. Castle, S. E. Naranjo, N. C. Toscano and J. G. Morse: J. Econ. Entomol. 104, 773–781 (2011).
44) B. Leandro et al.: Pest Manag. Sci. 63, 699–706 (2010).