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Specific Synergist for Neonicotinoid Insecticides: IPPA08, a *cis*-Neonicotinoid Compound with a Unique Oxabridged Substructure

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Supporting Information

ABSTRACT: Insecticide synergists are key components to increase the control efficacy and reduce active ingredient use. Here, we describe a novel insecticide synergist with activity specific for insecticidal neonicotinoids. The synergist IPPA08, a *cis* configuration neonicotinoid compound with a unique oxabridged substructure, could increase the toxicity of most neonicotinoid insecticides belonging to the Insecticide Resistance Action Committee (IRAC) 4A subgroup against a range of insect species, although IPPA08 itself was almost inactive to insects at synergistic concentrations. Unfortunately, similar effects were observed on the honey bee (*Apis mellifera*) and the brown planthopper (*Nilaparvata lugens*), resistant to imidacloprid. IPPA08 did not show any effects on toxicity of insecticides with different targets, which made us define it as a neonicotinoid-specific synergist. Unlike most insecticide synergists, by inhibition of activities of detoxification enzymes, IPPA08 showed no effects on enzyme activities. The results revealed that IPPA08 worked as a synergist through a distinct way. Although the modulating insect nicotinic acetylcholine receptors (nAChRs, targets of neonicotinoid insecticides) were supposed as a possible mode of action for IPPA08 as a neonicotinoid-specific synergist, direct evidence is needed in further studies. In insect pest control, IPPA08 acts as a target synergist to increase neonicotinoid toxicity and reduce the amount of neonicotinoid used. Combinations of IPPA08 and insecticidal neonicotinoids may be developed into new insecticide formulations. In summary, combining an active ingredient with a "custom" synergist appears to be a very promising approach for the development of effective new insecticide products.

KEYWORDS: *neonicotinoid, insecticide synergist, detoxification enzyme, nicotinic acetylcholine receptors*

INTRODUCTION

Insect pests threaten human welfare through food destruction and disease transmission. Insecticides are the primary means used to control most insect pests. Neonicotinoid is the largest insecticide now and has been playing an important role in crop protection and public health since the introduction of imidacloprid in the 1990s.[1,2] The emergence of imidacloprid initiated the splendid era of neonicotinoids, with six other neonicotinoids commercialized.[3] Currently, neonicotinoids have been used in more than 120 countries and areas,[3] sharing more than 24% of the total insecticide market in 2014.[4] However, the superiority of neonicotinoids is also challenged by the development of resistance, resulting from their frequent and irrational use.[5] Another major challenge for neonicotinoids is from the toxicity to honey bees, which has led to a re-evaluation of the overall biological safety of neonicotinoids in Europe and elsewhere.[6]

To deal with these challenges on neonicotinoids, the first strategy is insecticide structure modification. Neonicotinoid insecticides possess either an electron-withdrawing nitro ($-NO_2$) or cyano ($-CN$) group, which has been postulated to contribute directly to their selectivity.[7] The $-NO_2$ or $-CN$ group in all commercial neonicotinoids is in *trans* configuration. However, some *cis* configuration neonicotinoid compounds also show good insecticidal activities, which may provide substitutes for imidacloprid and other neonicotinoids, especially in the control of insect pests with high resistance to these *trans* configuration insecticides.[8−10] Cycloxaprid, discovered by our group, is a *cis*-neonicotinoid with a unique oxabridged substructure,[10,11] an outstanding activity,[12] and good safety profiles,[13,14] which has been newly registrated in China. Insecticide synergists may provide another important choice to deal with problems in insecticide applications, such as decreasing the application amount of insecticide active ingredient and lowering the resistant levels to insecticides. As a representative example, piperonyl butoxide (PBO) is the most common synergist of insecticides. Since its invention in the 1940s,[15] PBO makes a great contribution to public health and the control of household insect pests in the past several decades.[16] Triphenyl phosphate (TPP), diethyl maleate (DEM), and PBO are now the most important synergists on insecticides through inhibiting activities of detoxification enzymes.[17−19]

In the development of cycloxaprid, its eight-membered homologue, IPPA08 (Figure S1 of the Supporting Information), was synthesized as a comparison. IPPA08 is almost inactive to insects but has the unexpected synergistic effects on traditional *cis*-neonicotinoids. Here, we described the finding of IPPA08 as a specific synergist on neonicotinoids, which works

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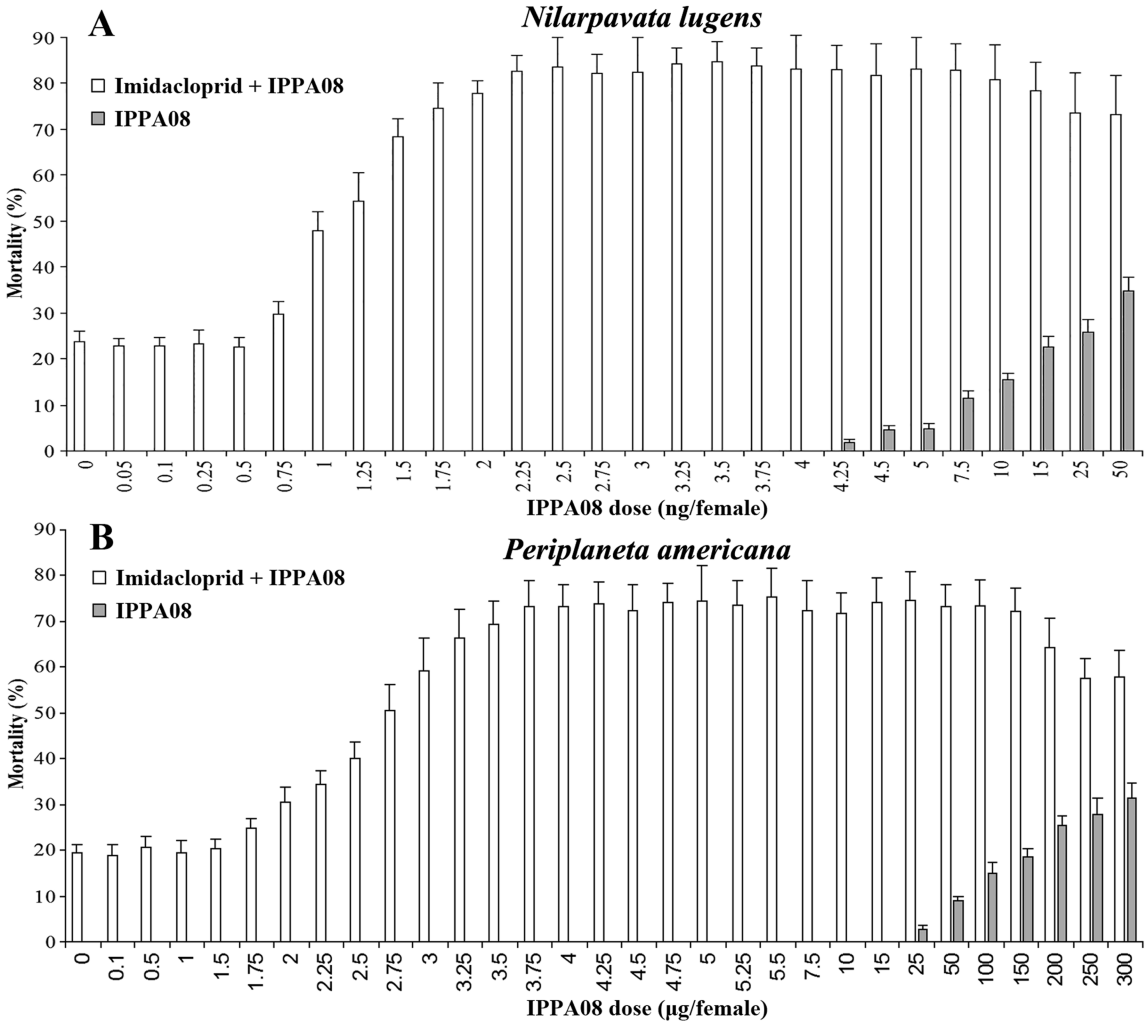

Figure 1. IPPA08 toxicity and its synergistic effects on imidacloprid toxicity. (A) IPPA08 toxicity and its effects on imidacloprid (2 ng/female) toxicity against *N. lugens*. (B) IPPA08 toxicity and its effects on imidacloprid (2 μg/female) toxicity against *P. americana*. Data are the means of at least six independent experiments ± standard error of the mean (SEM).

in a distinct mode from the synergists inhibiting activities of detoxification enzymes.

MATERIALS AND METHODS

Chemicals. PBO, TPP, and DEM were purchased from Sigma-Aldrich (St. Louis, MO). Dinotefuran was generously provided by Bayer CropSciences K.K. (Yuki, Ibaraki, Japan). Sulfoxaflor (97.0%) and flupyradifurone (92.5%) were generously provided by Wuhan JINGDING Chemicals Co., Ltd. (Wuhan, China). The representative oxabridged neonicotinoids, cycloxaprid and IPPA08, were synthesized and purified as previously described[11] (Figure S1 of the Supporting Information). Other insecticides were purchased from Sigma-Aldrich (St. Louis, MO). The solubility of IPPA08 at room temperature is greater than 1 mM (much higher if an emulsifier was added) in water and greater than 15 mM in acetone.

Insects and Bioassay. Three field populations of the brown planthopper (*Nilaparvata lugens*) were collected in Guilin (Guangxi, China), Nanjing (Jiangsu, China), and Chainat (Thailand) in September 2011. The bioassay was carried out using the topical application method.[20] If not specially noted, insects from the Nanjing population were used as the material source. The American cockroach (*Periplaneta americana*) was provided by Feitian Medicinal Animal Co., Ltd. (Danyang, Jiangsu, China) in September 2011, and a bioassay was performed using the topical application method.[21] *Aphis gossypii* was collected in Hangzhou (Zhejiang, China) in September 2013, and a bioassay was performed using the aphid-dip bioassay.[22] *Bemisia tabaci* was collected in Dezhou (Shandong, China) in September 2013, and a bioassay was performed using the leaf-dipping method.[23] *Musca domestica* was collected in Zhenjiang (Jiangsu, China) in September 2013, and a bioassay was performed using the artificial diet method.[24] *Apis mellifera* were purchased from Xishan apiary (Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China) in September 2014, and a bioassay was performed following the Organisation for Economic Co-operation and Development (OECD) method for the acute oral toxicity test on honey bees.[25] Because the insecticidal sensitivities of tested insect species are different, the concentration of IPPA08 was adjusted according to the insecticides used in the test. The mortality was recorded in 48 h. The data obtained were analyzed using Polo software (LeOra Software, Inc., Cary, NC) to determine LD_{50} or LC_{50} values based on the bioassay methods.

Determination of IPPA08 Stability in Phosphate-Buffered Saline (PBS) with Different pH. PBS was prepared with pH values of 4.0, 7.2, and 9.0. IPPA08 was dissolved in PBS buffer with the final concentrations of 0.1 and 1.0 mM and incubated at room temperature (28 ± 2 °C) for more than 48 h. Chromatographic analysis was performed using an Agilent 1260 series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a photodiode array detector. A Zobarx Extend-C18 column (250 × 4.6 mm; 5 μm particle size) was used with a column temperature of 30 °C. The ultraviolet (UV) detection wavelength was 345 nm. The mobile phase consisted

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Table 1. Insecticidal Activities of Three Compounds against Different Insect Species[a]

insect species	testing method	neonicotinoids	slope ± SE	LD_{50}/LC_{50} (95% FL)[b]
N. lugens	topical application (ng/female)	imidacloprid	1.742	7.545 (6.103−9.628)
		cycloxaprid	1.879	1.016 (0.905−1.143)
		IPPA08		>100
P. americana	topical application (μg/female)	imidacloprid	2.315	12.477 (10.514−15.302)
		cycloxaprid	1.898	17.003 (15.427−19.138)
		IPPA08		>250
A. gossypii	insect dipping (mg/L)	imidacloprid	1.821	1.272 (1.102−1.534)
		cycloxaprid	1.574	0.913 (0.797−1.068)
		IPPA08	1.328	65.160 (50.395−86.683)
B. tabaci	leaf dipping (mg/L)	imidacloprid	1.433	635.518 (488.361−827.046)
		cycloxaprid	1.690	94.557 (81.362−110.502)
		IPPA08		>10000
M. domestica	artificial diet (g/L)	imidacloprid	1.646	2.679 (2.375−3.214)
		cycloxaprid	1.596	1.133 (0.984−1.320)
		IPPA08		>100
A. mellifera	oral feeding ($\times10^{-2}$, μg/worker)	imidacloprid	2.513	1.542 (1.393−1.726)
		cycloxaprid	2.408	3.116 (2.872−3.423)
		IPPA08	1.704	36.227 (32.526−41.708)

[a]Data are the means of at least six independent experiments ± SEM. [b]LD_{50} values are provided for *N. lugens*, *P. americana*, and *A. mellifera*, and LC_{50} values are provided for the other insect species, which is the same in the following tables.

Table 2. Synergistic Effects of IPPA08 on Imidacloprid in Different Insect Species[a]

insect species	test method	IPPA08 concentration[b]	imidacloprid LD_{50}/LC_{50}	95% CI	slope	SR[c]
N. lugens	topical application (ng/female)	0	7.545	6.103−9.628	1.742	1.00
		1.25	1.696	1.344−2.157	1.622	4.45
		2.5	1.032	0.854−1.203	1.475	7.31
P. americana	topical application (μg/female)	0	12.477	10.514−15.302	2.315	1.00
		2.5	3.851	3.307−4.572	1.836	3.24
		3.75	2.244	1.956−2.718	1.724	5.56
A. gossypii	insect dipping (mg/L)	0	1.272	1.102−1.534	1.821	1.00
		1.5	0.361	0.310−0.418	1.633	3.52
		3.5	0.166	0.131−0.215	1.474	7.63
B. tabaci	leaf dipping (mg/L)	0	635.518	488.361−827.046	1.433	1.00
		200	164.642	125.271−209.832	1.276	3.86
		600	91.971	73.468−118.570	1.562	6.91
M. domestica	artificial diet (g/L)	0	2.679	2.375−3.214	1.646	1.00
		0.5	0.632	0.542−0.780	1.411	4.24
		1.25	0.445	0.376−0.529	1.358	6.02
A. mellifera	oral feeding ($\times10^{-2}$, μg/worker)	0	1.542	1.393−1.726	2.513	1.00
		0.1	0.366	0.323−0.444	1.972	4.21
		0.25	0.231	0.198−0.272	1.765	6.67

[a]Data are the means of at least six independent experiments ± SEM. [b]IPPA08 at the concentrations showed no insecticidal activities against the test insects. [c]SR = synergism ratio.

of methanol and H_2O with isocratic elution at a ratio of 50:50 (v/v). The flow rate was held constant (1.0 mL/min) throughout the process, and 5 μL of samples was injected. The analysis time was 6 min, and the retention time of IPPA08 was 3.8 min.

Activity Determination for Three Detoxification Enzymes. Females (50−56 mg) were homogenized in 500−560 μL of extraction buffer (0.1 M Tris−HCl and 0.5% Triton X-100 at pH 7.8), and the homogenate was centrifuged at 12000*g* for 15 min. The supernatant was used as the enzyme source for the determination of *O*-demethylase or *N*-demethylase activity of P450 monooxygenases and activities of esterases (ESTs) and glutathione *S*-transferases (GSTs). Protein concentrations were determined using the bicinchoninic acid (BCA) kit (St. Louis, MO), with bovine serum albumin (BSA) as the standard protein. Enzyme activities were then tested following methods reported before.[26,27]

To determine the influence of IPPA08 on enzyme activity, enzymes from female adults were treated in two different ways. In the first way, each female was treated by IPPA08 at a dose of 2.5 ng/female and the survival insects after 48 h were collected for the preparation of the enzyme source. Then, the enzyme activities were determined and compared to that of untreated insects. In the second way, the enzyme source was first prepared from untreated females and then incubated with IPPA08 at a final concentration of 0.5 mM for 2 h. Then, the enzyme activities were determined and compared to that of the enzyme source without incubation with IPPA08.

RESULTS

IPPA08 Toxicity and Its Effects on Imidacloprid Toxicities. IPPA08 toxicity was tested on several insect species and compared to imidacloprid and cycloxaprid, two neonicotinoids. IPPA08 had significantly lower toxicity to all tested insects and only caused mortality at relatively high doses, such as above 4.25 ng/female for *N. lugens* (Figure 1A) and 25 μg/

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Table 3. Synergistic Effects of IPPA08 on Imidacloprid in *N. lugens* with Different Resistance Levels to Imidacloprid[a]

population	IPPA08 concentration	imidacloprid LD_{50}	95% CI	slope	SR[b]
Guilin (RR[c] = 23.25)	0	3.209	2.854−3.706	2.227	1.00
	1.25	0.669	0.581−0.795	1.741	4.80
	2.5	0.425	0.374−0.480	1.790	7.55
Nanjing (RR = 54.67)	0	7.545	6.103−9.628	1.742	1.00
	1.25	1.696	1.344−2.157	1.622	4.45
	2.5	1.032	0.854−1.203	1.475	7.31
Chainat (RR = 115.38)	0	15.922	14.111−18.294	2.354	1.00
	1.25	3.951	3.326−4.817	1.663	4.03
	2.5	2.095	1.714−2.503	1.527	7.60

[a]The test method is the topical application, and the unit for LD_{50} values is ng/female. Data are the means of at least six independent experiments ± SEM. [b]SR = synergism ratio. [c]RR = resistance ratio.

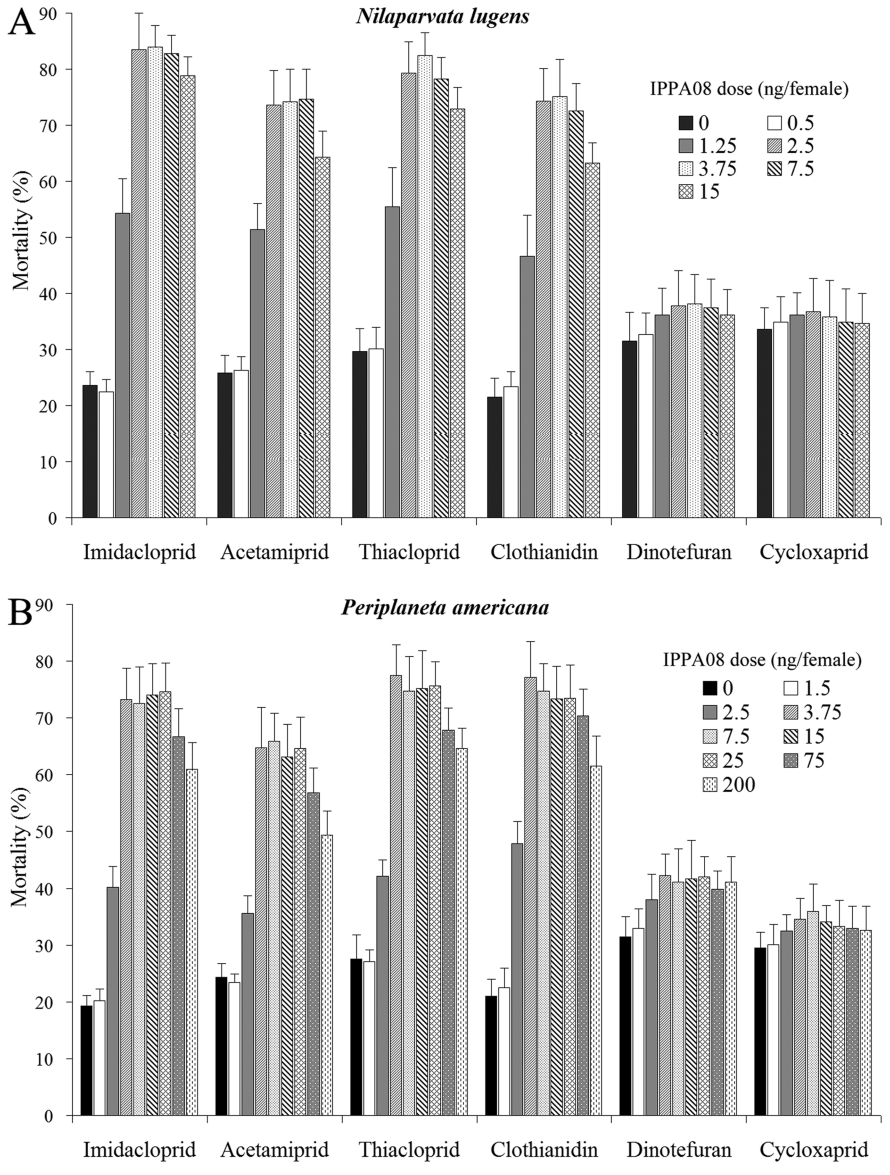

Figure 2. Effects of IPPA08 on the toxicity of neonicotinoid insecticides. (A) Effects in *N. lugens*. The dose for each neonicotinoid insecticide was 2 ng/female, and IPPA08 doses were listed in the figure. (B) Effects in *P. americana*. The dose for each neonicotinoid insecticide was 2 μg/female, and IPPA08 doses were listed in the figure. Data are means of at least six independent experiments ± SEM.

female for *P. americana* (Figure 1B). The calculated LD_{50}/LC_{50} values for IPPA08 against tested insect species were at least 10 times greater than those of imidacloprid and cycloxaprid (Table 1).

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Although IPPA08 did not show toxicity at low concentrations/doses, it increased imidacloprid toxicity. For *N. lugens*, 23% mortality by 2 ng/female imidacloprid was increased to 81% when co-applied with IPPA08 at low doses (1.0−4.0 ng/female), at which IPPA08 alone showed no insecticidal activity (Figure 1A). Similarly, in *P. americana*, 19% mortality by 2 μg/female imidacloprid was increased to 74% when co-applied with IPPA08 at low doses (3.0−15.0 ng/female), at which IPPA08 itself caused no mortality (Figure 1B).

The effects of IPPA08 on imidacloprid toxicities were also tested on other insect species, including *A. gossypii*, *B. tabaci*, *M. domestica*, and *A. mellifera*. Similar increases in imidacloprid insecticidal activity were also observed in these species, when imidacloprid was co-applied with IPPA08 at concentrations without direct toxicity (Table 2). Unfortunately, IPPA08 did not show selectivity in its effects on imidacloprid activity against the honey bee (*A. mellifera*).

When the influence of IPPA08 was tested on imidacloprid toxicities in *N. lugens* populations with different resistance levels to imidacloprid, it was found that IPPA08 had similar synergistic effects on imidacloprid toxicities (Table 3).

Influence of IPPA08 on Toxicities of Insecticides from Different Classes. The influence of IPPA08 on the toxicities of other neonicotinoids was tested, including acetamiprid, thiacloprid, clothianidin, dinotefuran, and cycloxaprid. For *N. lugens* (Figure 2A) and *P. americana* (Figure 2B), IPPA08 at low doses (causing no toxicity by itself) increased the mortalities caused by fixed doses (2 ng/female for *N. lugens*, and 2 μg/female for *P. americana*) of acetamiprid, thiacloprid, and clothianidin, which was similar to that on imidacloprid. However, IPPA08 showed little influence on toxicities of dinotefuran and cycloxaprid against both insect species.

On the basis of Insecticide Resistance Action Committee (IRAC) Mode of Action Classification Scheme (version 8.1, http://www.irac-online.org), the above tested insecticides belong to IRAC 4A (Group 4, nicotinic acetylcholine receptor competitive modulators). Recently, in Group 4, several insecticides were developed, belonging to different subgroups, such as sulfoxaflor in sulfoximines (4C), flupyradifurone in butenolides (4D), and triflumezopyrim in mesoionics (4E). Here, the influence of IPPA08 on the toxicities of sulfoxaflor and flupyradifurone was tested but not including triflumezopyrim, because it is difficult to obtain this compound within a short time. In three insect species, *N. lugens* (Figure 3A), *A. gossypii* (Figure 3B), and *B. tabaci* (Figure 3C), IPPA08 did not show obvious synergistic effects on sulfoxaflor and flupyradifurone, although the influence on imidacloprid toxicities was significant in these insects.

The influence of IPPA08 on the toxicities of insecticides from other classes was also evaluated. IPPA08 had no synergistic effects on insecticides with different targets, such as organophosphates and carbamates acting on insect acetylcholinesterases (AChEs), pyrethroids acting on sodium ion channels, and cyclopentadienes acting on γ-aminobutyric acid (GABA) receptors (Figure 4).

Key Properties of IPPA08 as a Specific Synergist. To exclude the possibility that the synergistic effects were caused by metabolites of IPPA08, such as glutaraldehyde or (nitromethylene)imidazole (NMI), its hydrolysis was studied. IPPA08 showed good stability at all test conditions, although a small proportion (1.85−2.10%) was degraded in 51 h at acidic conditions with pH of 4.0 (Table 4).

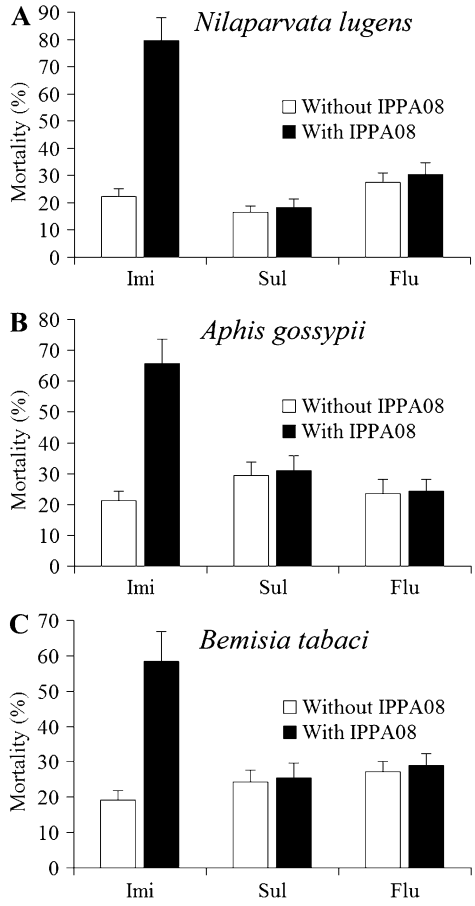

Figure 3. Effects of IPPA08 on the toxicity of sulfoxaflor (Sul) and flupyradifurone (Flu) compared to imidacloprid (Imi). (A) Effects in *N. lugens*. The doses for imidacloprid, sulfoxaflor, and flupyradifurone were 2.00, 0.25, and 0.25 ng/female, and the IPPA08 dose was 2.00 ng/female. (B) Effects in *A. gossypii*. The concenrations for three insecticides were 0.50, 0.50, and 0.25 mg/L, and the IPPA08 concenration was 0.50 mg/L. (C) Effects in *B. tabaci*. The concenrations for three insecticides were 100, 20, and 20 mg/L, and the IPPA08 concenration was 100 mg/L. Data are means of at least six independent experiments ± SEM.

Many synergists shows synergistic effects on insecticide toxicities, and the synergistic mode of most synergists is through inhibiting the activity of detoxification eznymes, such as PBO inhibiting activities of P450 monooxygenases, TPP inhibiting activities of ESTs, and DEM inhibiting activities of GSTs.[17−19] To find out whether IPPA08 had inhibition effects on the acitivities of three important detoxification enzymes, IPPA08 was either applied to insects or incubated with enzyme solutions and then the changes in enzyme activities were determined. On female adults treated with IPPA08 at a dose of 2.5 ng/female, no significant differences in the activities of P450s, ESTs, and GSTs were found when compared to the untreated control (Table 5). Incubation of enzyme solution with IPPA08 at a final concentration of 0.5 mM did not cause obvious changes in enzyme activities too. The results clearly revealed that IPPA08 had a distinct mode of action as a synergist.

DISCUSSION

There is an ongoing dilemma between the necessity for chemical insecticide use and the risks from potential adverse

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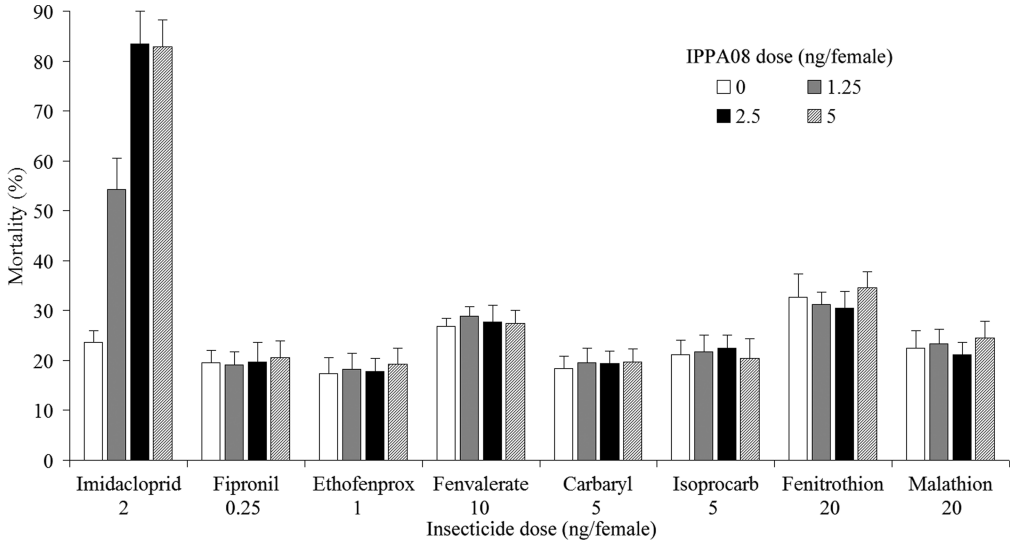

Figure 4. Effects of IPPA08 on the toxicity of insecticides from different classes against *N. lugens*. The doses for test insecticides are shown under the insecticide name. IPPA08 doses were listed in the figure. Data are means of at least six independent experiments ± SEM.

Table 4. Stability of IPPA08 in PBS Buffer at Different pH

treatment	pH	concentration (mM)	peak area I[a] (mAU s)	peak area II[b] (mAU s)	detection time	degradation rate (%)
T1	4.0	0.1	425.50 ± 4.59	417.61 ± 4.45	51 h	1.85
T2	4.0	1.0	3500.51 ± 37.55	3427.11 ± 36.72	51 h	2.10
T3	7.2	0.1	437.17 ± 4.63	431.43 ± 4.74	35 days	1.31
T4	7.2	1.0	4133.73 ± 44.54	4094.71 ± 44.51	35 days	0.94
T5	9.0	0.1	447.47 ± 5.03	447.35 ± 4.78	119 h	0.03
T6	9.0	1.0	3629.62 ± 39.24	3613.41 ± 39.17	118 h	0.45

[a]Detection at 0 h after the dissolution of IPPA08 in the buffer. [b]Detection of IPPA08 in the buffer at the time mentioned in the column of detection time.

Table 5. Influences of IPPA08 on the Activities of Detoxification Enzymes[a]

	insects treated with IPPA08		enzymes incubated with IPPA08	
enzyme	CK	treatment	CK	treatment
P450-OD (pmol min^{-1} mg^{-1})	7.33 ± 0.66	8.21 ± 1.02	6.84 ± 1.02	6.39 ± 0.86
P450-ND (pmol min^{-1} mg^{-1})	13.16 ± 1.65	12.74 ± 2.18	11.47 ± 1.37	11.23 ± 1.23
CarE (μmol min^{-1} mg^{-1})	1.83 ± 0.22	1.69 ± 0.17	1.66 ± 0.21	1.71 ± 0.28
GSTs (μmol min^{-1} mg^{-1})	104.47 ± 11.82	113.80 ± 13.05	92.15 ± 10.82	97.50 ± 18.35

[a]P450-OD/P450-ND, *O*-demethylase/*N*-demethylase activity of P450 monooxygenases; ESTs, esterases; and GSTs, glutathione *S*-transferases. Data are means of at least six independent experiments ± SEM.

effects. Ways to cope with this dilemma include reducing pesticide use, increasing unit activity and selectivity, and reducing the rate of insecticide resistance development.[28] An important strategy is the development of synergists to increase insecticide toxicity and reduce the amount of active ingredient. Here, we found that IPPA08, a *cis*-neonicotinoid with a unique oxabridged substructure, showed significantly synergistic effects on neonicotinoid insecticides, currently the most important class of insecticides. IPPA08 significantly increased toxicities of several neonicotinoid insecticides against a range of insect species, although IPPA08 itself was almost inactive to insects at synergistic concentrations. Unfortunately, the synergistic effects of IPPA08 showed little selectivity among insect species, and IPPA08 increased imidacloprid toxicity against honey bees at a level similar to other insect species. IPPA08 also produced similar effects in combination with imidacloprid on several *N. lugens* populations with different imidacloprid resistance levels, suggesting that IPPA08 cannot increase susceptibility to neonicotinoids if resistance has already developed.

IPPA08 only showed its synergistic effects on neonicotinoids specifically but was without any effects on the toxicity of insecticides from other classes, such as organophosphates, carbamates, pyrethroids, and cyclopentadienes. Until now, most successful synergists act as inhibitors of metabolic enzymes, such as P450 monooxygenases and other detoxification enzymes.[17,29] PBO is the most commonly used insecticide synergist,[15] which has made great contribution to the control of malaria mosquito vectors and insect vectors of other diseases. PBO is also commonly used in formulations made for the control of urban pest insects.[16] However, IPPA08 did not show any inhibition on the activity of three important detoxification enzymes, in either treating insects directly by IPPA08 or incubating enzymes with IPPA08. The results indicated that IPPA08 worked as a synergist in a way different from inhibiting activities of detoxification enzymes and did not obtain its

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specificity on neonicotinoids through inhibiting activity of some specific enzymes, which happens to only metabolize neonicotinoids. Detoxification enzymes play important roles in insecticide resistances, such as P450 monooxygenases in imidacloprid resistance in *N. lugens*.[30,31] In *N. lugens* with different imidacloprid resistance conferred by the enhanced activity of detoxification enzymes, IPPA08 showed similar effects to synergize imidacloprid, which also supported the supposal that IPPA08 did not exert its synergism by inhibiting enzyme activities.

Neonicotinoids act on insect nicotinic acetylcholine receptors (nAChRs), which play crucial roles in chemical-to-electrical transduction by mediating fast cholinergic synaptic transmission.[32] As a prototypical member of Cys-loop receptors, nAChRs mediate fast cholinergic synaptic transmission in both vertebrate and invertebrate nervous systems and have been investigated as targets of many clinical drugs and insecticides.[32] On mammalian nAChRs, two types of allosteric modulators, NS1738 as the representative member of the type I modulator and PNU-120596 as the type II modulator, can change the pharmacological properties through modulating the receptor protein, which could be recognized as synergists for the agonist drugs on mammalian nAChRs.[33,34] IPPA08 may also possess its synergistic effects through modulating insect nAChRs, which conferred its specificity for neonicotinoids acting on insect nAChRs. Of course, such supposal needed direct evidence, which will be in further studies, and the present study only provided some implicit cues for such supposal. IPPA08 likely only showed obvious effects on neonicotinoids belonging to the 4A subgroup in Group 4 of IRAC Mode of Action Classification Scheme (version 8.1, http://www.irac-online.org) but did not significantly synergize neonicotinoids belonging to other subgroups, such as 4C and 4D. Even among neonicotinoids in the 4A subgroup, IPPA08 showed little effects on the toxicity of dinotefuran, which may be because of the difference in chemical structures and acting sites on insect nAChRs. In contrast to dinotefuran, all of the other commercial neonicotinoid insecticides in the 4A subgroup contain a chlorinated heterocyclic (chloropyridyl or chlorothiazolyl) group,[35] and dinotefuran was also thought to act on the distinct site(s) from other neonicotinoids.[36] IPPA08 also showed little effects on the toxicity of cycloxaprid, whose mode of action is still unknown. The $-NO_2$ or $-CN$ group in all commercial neonicotinoids is in *trans* configuration, but cycloxaprid has the group in *cis* configuration and was with a unique oxabridged substructure, which may give it possibility to act on distinct site(s) of insect nAChRs.[10,11] If really through modulating insect nAChRs, IPPA08 may modify the microstructure of binding site(s) for most commercial neonicotinoids in the 4A subgroup, which consequently increase the binding affinity of these neonicotinoids on insect nAChRs. However, dinotefuran, cycloxaprid, and neonicotinoids belonging to non-4A subgroups may have the distinct site(s) from these neonicotinoids, and IPPA08 modulation may not change the microstructure of this distinct binding site(s).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b01512.

Chemical structures of imidacloprid, cycloxaprid, and IPPA08 (Figure S1) (PDF)

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Notes

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