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Full Length Research Paper

Molt disruption and mortality of *Locusta migratoria* var. *manilensis* (Meyen) (Orthoptera: Acrididae) caused by insect growth regulators

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The acute toxicity effects of topical applications of five insect growth regulators (IGRs) on the oriental migratory locust *Locusta migratoria* var. *manilensis* were assessed. Under laboratory conditions, at the highest tested dose rate of 300 ppm, the percent mortality and molt inhibition after two weeks for the five tested compounds were: flufenoxuron (97 and 83%), swallowwort alkaloids (48 and 85%), azadirachtin (100 and 93%), compound RH-5849 (45 and 22%), and pyriproxyfen (48 and 37%) respectively but low doses caused higher molt inhibition for compound RH-5849. These IGRs affected the timing of the subsequent molt, the average timing of the molt (of survivors) was delayed 0-2 days after treatments with flufenoxuron at dose rates between 18.75-300ppm, RH-5849 at dose rates between 150-300 ppm, but occurred 0-2 days early following treatment with azadirachtin, swallowwort alkaloids or pyriproxyfen at dose rates between 18.75-300ppm, and RH-5849 at dose rates between 37.5-75 ppm. Initial studies of the insecticidal effects of these insect growth regulators on *L. migratoria manilensis* show them to be very potent locust control agents.

Key words: Insect growth regulators, *Locusta migratoria* var. *manilensis* (Meyen), biological control, sustainability.

INTRODUCTION

The oriental migratory locust, *Locusta migratoria manilensis* (Meyen), is an economically important pest that causes significant crop losses in China, Southeast Asia, and the Pacific Region. In northern China, there has been a pattern of increasingly serious locust outbreaks since 1985 (Feng, 2007). The control of the oriental migratory locust in China has been largely based on the use of broad-spectrum chemical pesticides, which can damage human health, agro-ecosystems (example, loss of beneficial insects and increase of insecticide-resistance), and the wider environment (example, effects on non-target species, groundwater, landscapes and communities) (Gregor and Michael, 2007). Biological control has not yet been widely applied for locust control (Dennis, 2008), and work is continuing to find sustainable methods of controlling locusts, including studies on physiology and behavior as mediated by insect growth regulators.

A number of insect growth regulators (IGRs) that have been tested and are used to control a wide variety of insect pests but only a few have been tested against locusts. IGRs are regarded as a third generation of insecticides or biorational pesticides because they differ in their mode of action from other insecticides and have low toxicity to non-target organisms (Zhou et al., 2003). These compounds can potentially affect any insect life stage, including ovicidal effects, inhibition of metamorphosis to the adult stage, or interference with molting of early instar larvae. Because of their desirable characteristics, such as low toxicity, less environmental pollution, high selectivity, and low impact on natural enemies and people, IGRs are used as low-toxicity control agents of various insect pests, and can assist in the development of sustainable agriculture (Wu, 2002; Cedric, 2005; Wang and Wang, 2007).

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Control of locusts requires the availability of safe methods, such as ecological, biological, or integrated controls (Wang, 2007), and the use of IGR pesticides is such an approach because of their low toxicity. However, only a small number of IGRs have been tested against locusts in the field, mainly just a few chitin inhibitors and plant-derived pesticides. Studies have shown that juvenile hormone analogues have a significant effect on the breeding and spawning of the adult oriental migratory locust (Roland and Uwe, 2004); however, there is a general lack of research on the effects of this class of compounds on locusts. Studies of five chitin-inhibiting IGRs in the laboratory showed that the LC_{50} of flufenoxuron and chlorbenzuron to 4th instar nymphs of the oriental migratory locust were 26.5 and 284.4 ppm, respectively, and their LT_{50} values were 4.95 and 5.23 d, respectively (Lei et al., 2002), while diflubenzuron, chlorbenzuron and triflumuron had only very low toxicity to this locust. In another case, 0.3% matrine (at 900 ml/ha) and 1% celangulins (at 300 ml/hm^2) killed more than 90% of test locusts (Cheng et al., 2007). As for the widely applied plant-derived pesticide azadirachtin (Dennis, 2008), research on its effects on the Oriental migratory locust has not been done.

This study focused on the effects of different types of IGRs, including the chitin synthesis inhibitors (flufenoxuron), plant-derived pesticides (swallowwort alkaloids and azadirachtin) (Xu, 2002; Cui et al., 2008), molting hormone analogues (RH-5849) and juvenile hormone analogues (pyriproxyfen), on the oriental migratory locust, aiming to find new techniques and methods for the sustainable management of this pest.

MATERIALS AND METHODS

Locusts

L. migratoria var. *manilensis* nymphs from the Key Biological Control Laboratory of the China Agricultural University in Beijing, China were used for the tests. A group of 150-180 individuals of both sexes were reared under crowded conditions in cages (15 ×15 × 30 cm^3), at 27-30°C and 60-85% RH, and were supplied with fresh maize seedlings for food. The third instar nymphs were selected for the experiment.

Pesticides

The sources of pesticides used in tests were as follows: (1) flufenoxuron: 95% TC, Manufacturer: Zhenjiang Agrosinic Co., Ltd. (2) azadirachtin: 98%, Manufacturers: Yunnan Zhongke Biology Industry, Ltd. and Yunnan Xinlian chemical factory. (3) RH-5849: 92% TC, Manufacturer: Mikania Biological Control Services Ltd. (4) pyriproxyfen: 95% TC, Manufacturer: Hangzhou Yilong Chemical Industry Co., Ltd. (5) swallowwort alkaloids: alkaloids extracted from *Cynanchum komarovii* AL. Iljinski using the following method: At room temperature (25 ± 2°C), 0.2 kg of the plant powder (screened with 40 mesh seives) was soaked in 2 L industrial alcohol (95.5%, Beijing Chemical Reagent Ltd =B.C.R. Ltd) containing 2 mL hydrochloric acid (37%, B.C.R.Ltd) for 72 h. The solution was then extracted through filter paper (20-30 µm, B.C.R. Ltd) using a vacuum pump (model SHB-III; Zhengzhou Great Wall Industrial and Trading Co.). The extracted solution was dissolved in 2% hydrochloric acid solution, and insoluble particles were removed by filtration and chloroform (RD1.4832 [20/4°C]) extraction. The solution was modified to pH 9-11 with sodium hydroxide (98%, B.C.R. Ltd) and the alkaloid solution was obtained through chloroform extraction and vacuum concentration, 3-5 times, with a rotary evaporator (RE52-98, Shanghai Yarong Biochemical Instrument Factory). Berberine chloride (National Institute for the Control of Pharmaceutical and Biological Products of China) was applied to a standard sample; 0.1% BTB (Committee of Chinese Pharmacopeia) was applied for acid dyes and the sample solution (at a pH of 5.4 in a citric acid-sodium citrate buffer) was analyzed with a Three-use Ultraviolet Analysis Instrument (UV-1) (Shanghai Gucun Electro-optical Instrument Factory). The absorbance value of the solution at 419 nm wave length was recorded and used to determine the alkaloid contents of the sample solution (Wang et al., 2006; Shi et al., unpublished data). All pesticides were diluted with distilled water into different concentrations for use in the experiment.

Toxicity tests

Third instar nymphs of *L. migratoria* var. *manilensis* were selected for the experiment. The wax on the surface of maize leaves was scraped off with knife, and then a 2 × 3 cm rectangle was punched out of the leaf. Onto these maize leaf sections we placed a 1 µL drop of the test concentration of a particular pesticide and then the leaf was air-dried. Distilled water was used to make up the pesticide concentrations that were used in the experiment: 0 (control, distilled water only), 18.75, 37.5, 75, 150 and 300 ppm. A third instar nymph and either a treated or control maize leaf section were placed in muslin-covered plastic container (8 cm diameter and 10 cm height). Each treatment consisted of 20 locusts (only those consuming the whole leaf disc were used in the experiment) and each treatment (by pesticide and concentration) was repeated 5 times. All subsequent incubation conditions are described as seen in the foregoing. Mortality and molt rates were recorded 24 h after the consumption of the leaf disc and every 24 h thereafter until all the insects died or became adults. A toxicity regression equation was calculated for each compound and an LC_{50} obtained using probit analysis.

Statistical analysis

All figures were made using EXCEL 2003 Windows (Microsoft Co., 2003). Statistical analyses were performed using SPSS 11.50 Windows (SPSS Inc., 2001). After arcsine transformation, the molt ratios of the nymphs of oriental migratory locust after treatment with IGRs were analyzed in One Way ANOVA.

RESULTS

Mortality of *Locusta migratoria manilensis* caused by IGRs

Acute lethal toxicity of IGRs against locust

The acute lethal toxicities of flufenoxuron and azadirachtin against the locusts were generally significantly (Figure 1 shows captions of statistical details and P values) higher than those of the swallowwort alkaloids, RH-5849, or pyriproxyfen. With the exception of RH-

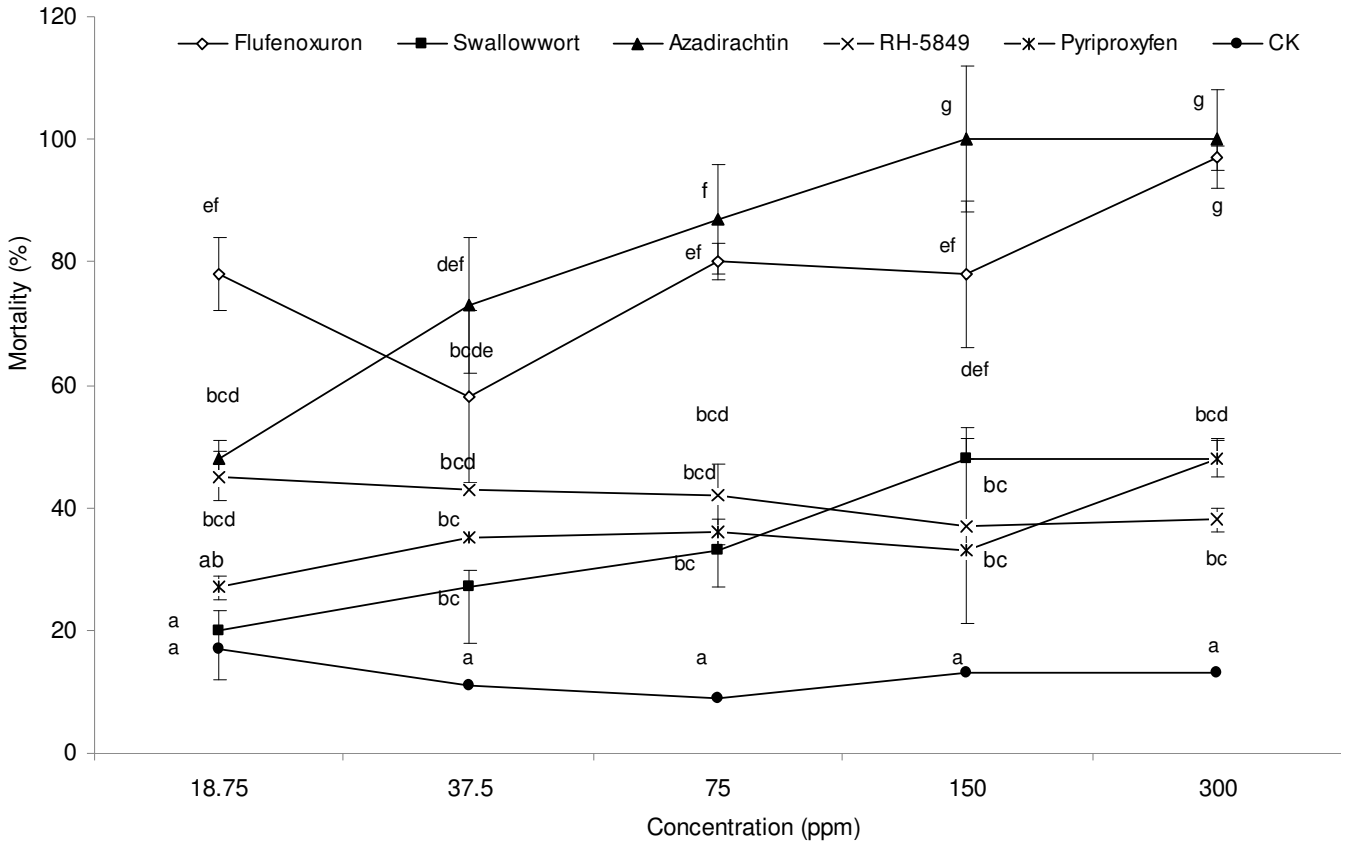

Figure 1. Mean percentage mortality (±SE) of locusts two weeks after treatment with different concentrations of various IGRs. Any two treatments with the same letter are not significantly different (α=0.05) [Student-Newman-Keuls].

5849, mortality generally increased with concentration. There was 100% mortality caused by treatments with high doses of azadirachtin (150 or 300 ppm) and flufenoxuron (300 ppm) (Figure 1).

With 300 ppm azadirachtin, 80% of locusts died within 2 days and 98% in 6 days and mortality was only slightly slower at a dose of 150 ppm. The acute lethal effect of flufenoxuron, at 4 and 8 days after treatment, reached around 80%. The swallowwort alkaloids had a mortality rate of 45% at 14 days post treatment. The lowest mortalities were caused by pyriproxyfen (48% after 14 days) and RH-5849 (45% after 14 days).

Insect toxicity of flufenoxuron and azadirachtin to locusts

The LD_{50} and LC_{50} of flufenoxuron on 3rd instar nymphs of *L. migratoria* var. *manilensis* were both lower than the values for azadirachtin, a toxicity index of flufenoxuron was index significantly higher than that of azadirachtin (Table 1). The mortalities caused by the swallowwort alkaloids, RH-5849, pyriproxyfen and in this experiment were below 50%, and therefore their LC_{50} and LD_{50} values could not be calculated.

Influence of IGRs on the molting of locust nymphs

Molt disruption of IGRs

While about 90% of untreated locusts molted, the percent molting was significantly lower when locusts were treated with flufenoxuron, swallowwort alkaloids, azadirachtin (at several concentrations) or RH-5849 (at the lower concentrations). RH-5849 at higher concentrations and pyriproxyfen had no significant effects on molting rates of the nymphs (Figure 2).

The Influence of IGRs on peak timing of molting by experimental populations

With untreated locusts the peak time of the subsequent molt was at 3 days but these IGRs affected molting by making it either earlier or later (Figure 3). Flufenoxuron led to a delay in molting that increased with dose. With swallowwort and azadirachtin at 18.75 ppm led to peak

Table 1. Toxicity of the alkaloids flufenoxuron and azadirachtin against *Locusta migratoria manilensis*, giving LC_{50} (ppm) and LD_{50} (mg Kg^{-1}) values for 3rd instar nymphs of *Locusta migratoria manilensis*.

IGRs	Value	TRE[a]	RTI	K^2	P
Flufenoxuron	LC_{50} 26.04	Y=-1.33811+0.94521x	136.39	5.171	0.160
	LD_{50} 0.90	Y=0.04416+0.94521x	136.39	5.171	0.160
Azadirachtin	LC_{50} 35.52	Y=-5.05125+3.25792x	100	0.783	0.853
	LD_{50} 1.22	Y=-0.28687+3.25789x	100	0.783	0.853

[a] In regression equations, X denotes the concentration and Y the probability of death. TRE, Toxicity regression equation; RTI, relative toxicity Index; K^2, Chi square.

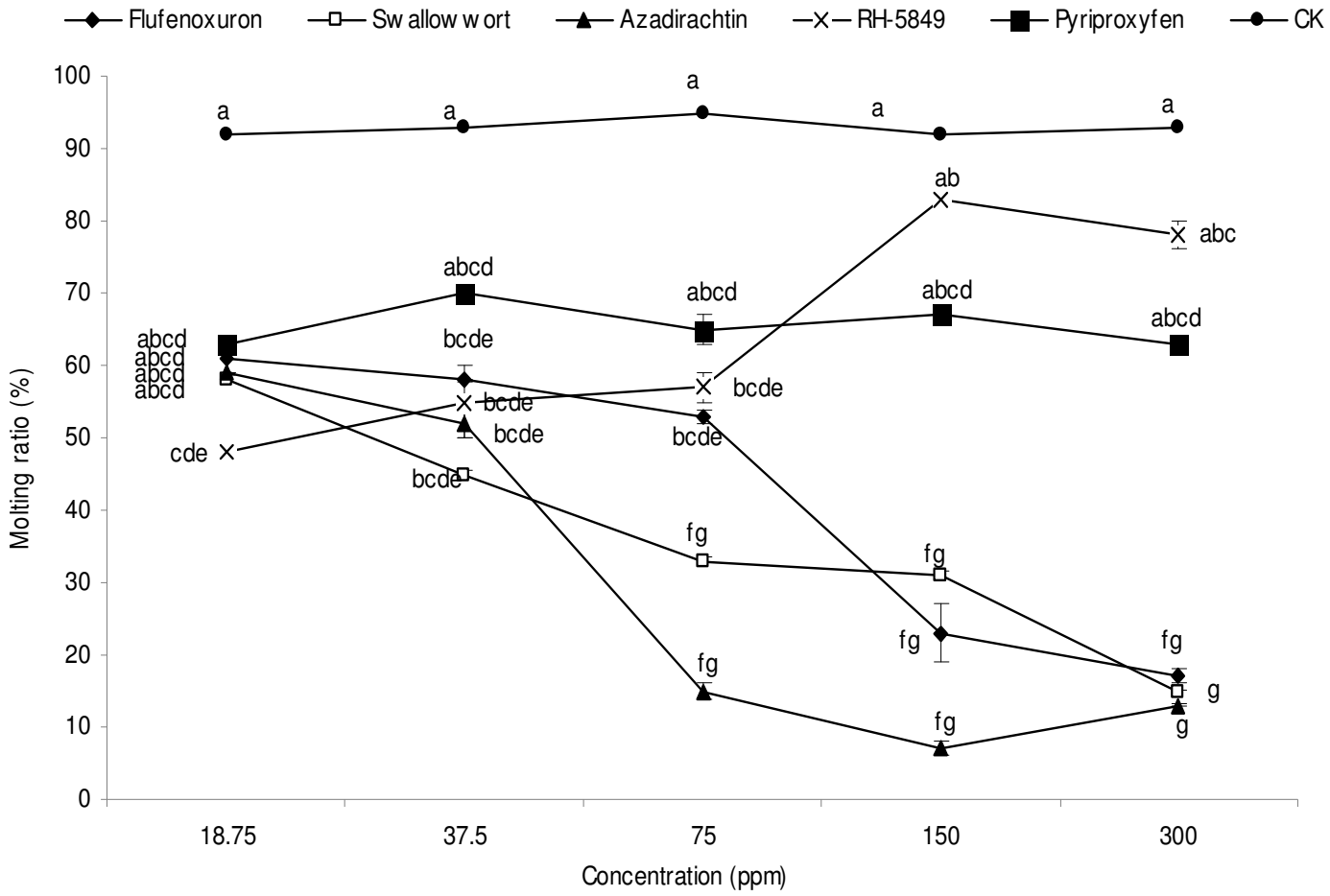

Figure 2. The effects of different concentrations of IGRs on the percentage of *Locusta migratoria.* var. *manilensis* that molted. Any two treatments with the same letters do not have significant difference at the level of α=0.05 [Student-Newman-Keuls (a, b)].

molting 2 days delay after treatment, while higher doses caused molting to be 1-2 days early (Figure 3). Azadirachtin at the highest dose (300 ppm) led to rapid mortality: only 18% survived to day 2, on the day of peak molting most, locusts died before they could molt. RH-5849 and pyripyroxyfen at low dose rates also shortened the time to peak molting but at higher doses, RH5849 delayed peak molting by 1 day (Figure 3).

DISCUSSION

Insect growth regulators can kill or debilitate treated insects by affecting physiological processes, inhibiting molting, preventing the formation of new integument, or reducing feeding (Bi et al., 2008). The acute lethality and molt-inhibiting effects of five types of IGRs on oriental migratory locust were evaluated. The results demonstra-

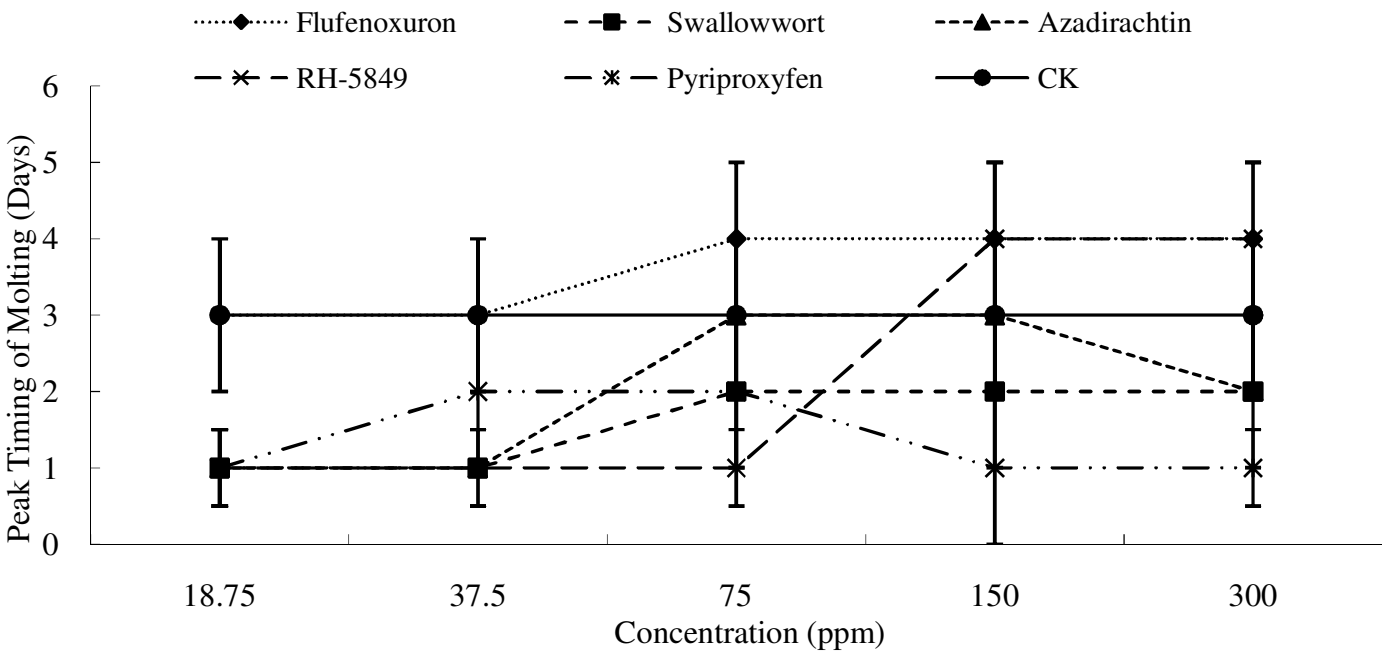

Figure 3. Peak timing of molting by experimental populations post-treatment with different concentrations of IGRs. These IGRs affected the timing of the subsequent molt, the average timing of the molt (of survivors) was delayed or forwarded 0-2 days after treatments with these pesticides at dose rates between 18.75-300 ppm respectively.

ted that at higher concentrations, azadirachtin caused high mortality of migratory locusts, producing 90-100% mortality 6-8 days after treatment. These findings are consistent with earlier results (Lei et al., 2002, Cheng et al., 2007). In addition, while lower doses caused less mortality, molting was disrupted and occurred much earlier in survivors. Azadirachtin is sometimes applied to control locusts and the latter sub-lethal effects indicate that lower doses could be used for effective control. Flufenoxuron required a higher dose (300 ppm) than azadirachtin to cause near complete mortality, but like the latter product, there were sub-lethal effects: with flufenoxuron, molting was delayed indicating that it could be an effective control product at a dose lower than that required for very high direct mortality.

With RH5849 and pyriproxyfen, there were only moderate levels of mortality. These products did disrupt molting somewhat by causing molt to occur early or later than normal, but it is uncertain whether these effects are sufficient to warrant further testing.

The swallowwort alkaloids caused only moderate mortality but had substantial effects on molting at higher doses consistent with the main mode of action of many IGRs in disrupting proper regulation of insect growth. Alkaloids from the swallowwort *C. komarovii* caused growth inhibition, malformation, abnormal developmental duration (from third instar to pupation), pupation inhibition, and emergence inhibition in the noctuid *Spodoptera litura* Fabricius (Sun et al., 2011). Further studies should focus on the impact of IGRs on physiological activities supporting locust growth, to gain a comprehensive evaluation of how these compounds might contribute to the control of locusts under field conditions. From the results of the experiments reported, it is clear that the impacts of these IGRs on locusts vary significantly because they have different mechanisms of action. Further studies should build on this understanding of how these products affect locust physiology and incorporate that understanding into assessments of how they affect locust populations in control projects in the field. This would promote the sustainable control of locusts.

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