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Mechanisms of Resistance to Beta-Cyfluthrin in the Cigarette Beetle, *Lasioderma serricorne*, and the Effectiveness of Imidacloprid Space Sprays

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ABSTRACT

Lasioderma serricorne (F.) (Coleoptera: Anobiidae) is widely recognized as the most harmful pest in the cigarette production industry. Beta-cyfluthrin, a synthetic pyrethroid, has long been used as an effective pesticide to control *L. serricorne* in cigarette and food processing facilities. This study aimed to evaluate the resistance of *L. serricorne* populations collected from the Hefei cigarette factory to beta-cyfluthrin and their response to imidacloprid, an alternative insecticide. The findings showed that the *L. serricorne* strain from Hefei exhibited significant resistance to beta-cyfluthrin but remained highly sensitive to imidacloprid, with a minimal cross-resistance ratio of 1.00-1.33. Further field trials showed that beta-cyfluthrin space sprays were ineffective in controlling the pests in the cigarette manufacturing area. In contrast, imidacloprid was highly effective, reducing the *L. serricorne* population by up to 100%. This study highlights the importance of early detection of insect resistance, the development of alternative pest control strategies, and the potential of imidacloprid as a viable option for the management of pyrethroid-resistant Coleoptera species. Furthermore, the results indicate that imidacloprid space sprays in confined spaces hold significant promise for the control of stored-product pests.

Keywords: Imidacloprid, Beta-cyfluthrin, Aerosol, Resistance

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Introduction

The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), is a widely distributed pest that infests a variety of stored products. It feeds on and inhabits many dried materials, including paper, cloth, furniture, coffee, grains, and tobacco [1]. As a polyphagous pest, cosmopolitan and *L. serricorne* are recognized as the most damaging insects in cigarette manufacturing and tobacco processing due to their ability to thrive on tobacco, a substance toxic to many other species [2-6]. The larvae's feeding habits during tobacco storage and processing result in weight loss, and if the beetles infest finished cigarette products, they can cause significant consumer dissatisfaction and even lead to the loss of a cigarette brand from the market, leading to severe economic consequences [3, 7].

Effective management of *L. serricorne* in cigarette manufacturing facilities has traditionally relied on stringent sanitation practices, including the prompt removal of waste and infected tobacco remnants [8]. However, cleaning alone is often insufficient to fully control the pest, making the application of space aerosol insecticides a commonly used supplementary measure [8, 9]. Aerosol insecticides, especially residual and contact types, have proven effective in preventing pest infestations in food processing environments due to their low cost, quick application, and compatibility with other management strategies such as sanitation [10, 11]. The use of aerosol insecticides to control stored-product pests has a long history, beginning with the application of dichlorvos

(DDVP) aerosol in tobacco warehouses during the 1950s to manage *L. serricorne* and other pests, such as black carpet beetles (*Attagenus megatoma*), red flour beetles (*Tribolium castaneum*), confused flour beetles (*Tribolium confusum*), and almond moths (*Cadra cautella*) [12]. While DDVP was highly effective in controlling these pests in both laboratory and field settings, its use was discontinued due to the development of pest resistance and concerns over its high toxicity to mammals and the environment. Today, pyrethroids and insect growth regulators are the primary insecticides used in such applications. Various aerosol insecticides, including prallethrin for *C. cautella*, cyfluthrin for *T. castaneum*, esfenvalerate for *Plodia interpunctella*, and pyrethrin for *T. castaneum* and *T. confusum*, have all been shown to be effective under laboratory conditions [13-22].

Beta-cyfluthrin, a synthetic pyrethroid, is widely recognized for its low environmental persistence, minimal toxicity to non-target organisms, and lack of bioaccumulation. As a broad-spectrum neurotoxin, it has proven effective in controlling *L. serricorne* [20, 23]. However, the number of chemical insecticides suitable for use in cigarette manufacturing facilities is limited, and the increasing resistance of *L. serricorne* to insecticides is a growing concern. Beta-cyfluthrin aerosol has long been used as a supplementary measure alongside sanitation practices to manage *L. serricorne* in the Hefei Cigarette Manufacturing Factory in Hefei City, Anhui Province, China. Therefore, it is crucial to assess the sensitivity of *L. serricorne* to beta-cyfluthrin to ensure continued control effectiveness and to address potential resistance development.

In this study, we evaluated the susceptibility of *L. serricorne* populations collected from the Hefei Cigarette Manufacturing Factory to beta-cyfluthrin. After conducting several rounds of bioassays with various insecticides (except imidacloprid), we selected imidacloprid as a different treatment. We then carried out field trials in the cigarette manufacturing facility to assess the practical effectiveness of space spraying with imidacloprid aerosol. Our findings aim to provide valuable insights into the early detection of insecticide resistance in *L. serricorne* and offer practical recommendations for effective pest resistance management in cigarette manufacturing settings.

Materials and Methods

Insecticides, atomizers, pheromone traps, and spray tower

The insecticides in this experiment were commercially available formulations: beta-cyfluthrin (12.5% active ingredient), suspension concentrate (SC), provided by Jiangsu Yangnong Chemical Co. LTD, and imidacloprid (100 g [a.i.] L⁻¹, SC) from Zhejiang Haizheng Chemical Co. LTD.

Pheromone traps, supplied by Japan Fuji Flavor Co. LTD. (Japan), were deployed to monitor the insect population in the cigarette production area of the Hefei Cigarette Manufacturing Factory. These traps featured a lure releasing sex pheromones onto sticky boards that captured beetles for counting.

For field trials, an electric atomizer from Nanjing Minghai Health Technology Development Co. LTD. (Nanjing, China) was employed to disperse aerosol particles sized $50 \pm 10 \mu\text{m}$, delivering spray at a rate of 500 mL/min. Additionally, a hand-held ultra-low volume atomizer, calibrated for misting, was utilized for the bioassay toxicity assessments.

A glass spray tower, designed by Beijing Wuyi Glass Instrument Factory was used to administer sprays in controlled conditions. The tower had a volume of 10 L, a height of 40 cm, and a base diameter of 20 cm, with a 3 cm diameter at the top. The tower was open at both ends, with only a glass cap covering the structure.

Insect strains and rearing conditions

Tobacco fragments and shorts were collected from the cigarette production machinery in the Hefei Cigarette Manufacturing Factory and brought to the lab. Using a stereoscopic microscope, *L. serricorne* larvae were found and extracted from the materials and reared to adulthood (LSH). This strain was maintained in the lab for more than 3 generations before bioassay testing. A susceptible reference strain (LSLAB) was kept in the lab, where it had not been exposed to pesticides for many years. Both strains were reared in dark incubators set at 28 °C and a humidity range of 65-75%. Wheat, frozen at -18 °C for over 7 days, was ground into small particles. The larvae were fed a mixture of wheat particles and active yeast at a ratio of 10:1 (w/w). Unsexed adults aged one to three days were used for bioassays.

Spray toxicity bioassay

The toxicity of beta-cyfluthrin and imidacloprid against *L. serricorne* was assessed using spray toxicity bioassays following standard protocols [24, 25]. Initial trials were conducted to establish appropriate concentration ranges. For the beta-cyfluthrin spray bioassay, the concentrations tested for the LSH strain were 156.25, 83.64, 41.82,

13.94, 6.35, and 3.17 mg/mL, while the LSLAB strain was tested with concentrations of 57.11, 19.04, 6.35, 3.17, 0.63, 0.13, and 0.03 mg/mL, with distilled water as the controller. For the imidacloprid spray bioassay, the concentrations for LSH and LSLAB strains were 1.75, 0.35, 0.035, 0.0035, 0.00035, and 0.000035 mg/mL, with distilled water used as the control.

In the laboratory (at 27 °C), a spray tower was placed on a flat marble surface to ensure proper alignment, preventing insect escape. About 20 adult insects were introduced to the tower base, and a volume of 0.5 mL of the insecticide solution was dispensed into the tower using a hand-held ultra-low volume atomizer, positioned 35 cm away. The tower's mouth was immediately covered with a glass cap (not sealed). Mortality was recorded at 2 and 24 hours post-exposure under dark conditions. An insect was deemed dead if no movement was observed upon prodding with a hairbrush [26]. Three replicates were conducted for each concentration.

Field trials

Field trials were done within different sections of the Hefei Cigarette Manufacturing Factory, located in Hefei, Anhui, China, including the shred-making, storage, blending, cigarette-making, and ripper sections. Before the trials, all five sections were cleaned following standard sanitation protocols. Pheromone traps were used to monitor insect populations across these areas [8, 11, 27-31]. The number of traps and their hanging height in each section were based on guidelines from CORESTA [8]. Each trap was positioned on steel nails fixed to walls or columns, and the location was marked for consistent placement. Weekly counts of captured *L. serricornis* were recorded, with traps replaced every month.

Details of the sections and the placement of traps are outlined in **Figure 1**. The shred-making section spans an area of 1600 m² (20 m x 80 m), with a height of 10 m, giving a volume of 16,000 m³. Nine pheromone traps were distributed across this area, with eight on the long walls at 1.5 m above the ground, and one positioned centrally on the column at a height of 5 m. The storage section, measuring 600 m² (20 m x 30 m), with a height of 5 m and a volume of 3000 m³, contained three traps evenly spaced on non-entrance walls, each 1.5 m above the ground. The blending section, also 600 m² with a volume of 3000 m³, had three traps arranged similarly. In the cigarette-making section, measuring 1200 m² (20 m x 60 m) with a height of 5 m, six traps were placed on the 60-meter-long walls at 1.5 m height. Finally, in the ripper section, with a 200 m² area and 5 m height (volume of 1000 m³), a single trap was placed on the central column at 1.5 m. The temperature in all sections, except for the ripper section, was maintained at 26 ± 2 °C with 60 ± 5% relative humidity.

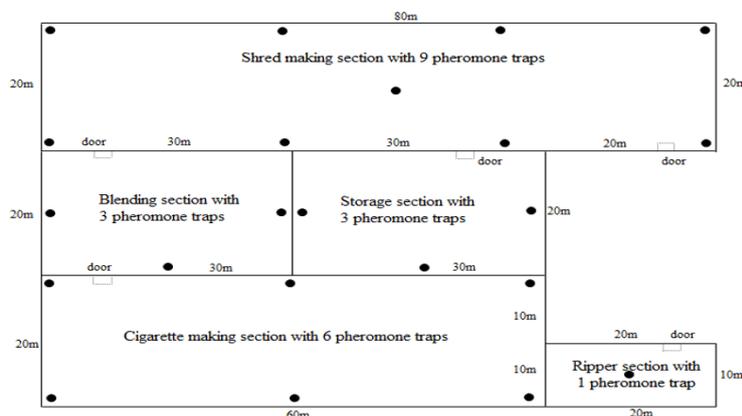


Figure 1. The layout of the cigarette manufacturing area at the Hefei Cigarette Manufacturing Factory highlights the locations of pheromone traps within each section (• indicates trap positions); note: Shred-making section: is the area where tobacco leaves are processed into tobacco shreds, the blending section: is the area where tobacco shreds of various grades are mixed, the storage section: is the location where tobacco shreds are stored, the cigarette-making section: is the area dedicated to producing cigarettes, the ripper section: is the area for handling defective cigarettes; together, these five sections constitute the cigarette manufacturing area of the Hefei Cigarette Manufacturing Factory.

Field trials

The application concentrations and doses of beta-cyfluthrin and imidacloprid were determined based on commercial pesticide guidelines and pre-experimentation results. Beta-cyfluthrin aerosols were applied across the

five sections at a dose of ten L per 3000 m³ (1 L of beta-cyfluthrin commercial formulation mixed with 9 L of water). After seven weeks, imidacloprid aerosols were sprayed at the same dose (0.5 L of imidacloprid commercial formulation in 9.5 L of water for 10 L/3000 m³). Both applications took place during factory downtime with all windows and doors closed. The following morning, windows were opened (equipped with insect-proof netting) to allow ventilation, followed by cleaning according to standard sanitation procedures. The personnel carrying out the spraying and monitoring were skilled workers at the factory.

Data analysis

Probit analysis (IBM SPSS® Statistics 25, IBM Corp., NY, USA) was used to process the bioassay data to calculate the lethal concentration required to kill 50% (LC50) of *L. serricornis*. The chi-square test was applied to verify the goodness-of-fit for the model. Data collected from the field trials regarding *L. serricornis* monitoring in the 5 sections were analyzed using graphing software (Origin 2018, OriginLab Corp., Northampton, Massachusetts, USA).

Decrease rate of detected *L. serricornis* in week

$$n = \frac{Nw0 - Nwn}{Nw0} \times 100\%, \quad (1)$$

NW0 represents the count of *L. serricornis* detected in the week before the application, while Nwn refers to the count of *L. serricornis* detected in week n following the application.

Results and Discussion

The findings from the spray are summarized in **Table 1**. Two hours following the beta-cyfluthrin application, the LC50 value for the LSH strain was 71.98 mg/mL, which was 7.48 times higher compared to the LSLAB strain. After 24 hours, the LC50 value for the LSH strain increased to 19.78 mg/mL, which was 15.10 times greater than that of the LSLAB strain. On the other hand, when imidacloprid was applied, the LC50 values for the LSH strain were 1.33 times and 1.00 times greater than those of the LSLAB strain at 2 hours and 24 hours, respectively.

Table 1. Insecticide toxicity to LSLAB and LSH strains of *L. serricornis* at 2 h and 24 h in laboratory bioassays (at 27 °C)

Exposure time	Insecticide	Strain	LC50 (95% fiducial limits) (mg/mL)	Slope ± SE	χ^2	df	P-value	Total insects (N)	Resistance ratio
2 h	Beta-cyfluthrin	LSLAB	9.62 (6.63-12.98)	-6.64 ± 1.07	12.17	18	0.84	800	-
		LSH	71.98 (34.81-122.24)	-5.73 ± 1.17	14.24	13	0.36	1400	7.48
	Imidacloprid	LSLAB	0.15 (0.04-0.40)	-1.13 ± 0.31	12.68	14	0.55	700	-
		LSH	0.20 (0.08-0.44)	-1.30 ± 0.27	8.17	14	0.88	600	1.33
24 h	Beta-cyfluthrin	LSLAB	1.31 (0.57-2.23)	-3.88 ± 0.72	12.68	18	0.81	800	-
		LSH	19.78 (0.41-76.26)	-4.63 ± 1.02	32.17	13	0.002	1400	15.10
	Imidacloprid	LSLAB	0.03 (0.01-0.06)	-0.95 ± 0.26	6.06	14	0.97	700	-
		LSH	0.03 (0.01-0.07)	-0.93 ± 0.27	8.67	14	0.85	600	1

LSLAB: Reference susceptible strain

LSH: *L. serricornis* collected from Hefei Cigarette Manufacturing Factory

Fiducial limits (FL): Confidence intervals for LC50 values

df: Degrees of freedom

P-value ≥ 0.05 indicates a significant fit between observed data and the expected linear regression model in Probit analysis

N: Total number of insects used

Resistance ratio (RR): LC50 of LSH strain / LC50 of LSLAB strain

Field trials

Quantitative change in *L. serricornis* detection in the shred-making section

As illustrated in A1 of **Figure 2**, the population of *L. serricornis* observed in the shred-making section initially increased from 17 beetles in week 0 to 116 beetles in week 5 after the beta-cyfluthrin application. However, this number began to decline in weeks 6 and 7. When compared to the count in week 0, the numbers increased by 0%, 41.18%, 82.35%, -282.35%, -582.35%, -341.18%, and -300.0% for weeks 1 through 7, respectively. As shown in A2 of **Figure 2**, a sharp decline was observed after application, with the number of beetles dropping from 68 in

week 0 to 7 in week 1, marking a decrease of 89.71%. The reduction rate continued as follows in the subsequent weeks: 95.58%, 94.12%, 95.58%, 91.18%, 83.82%, and 69.12%.

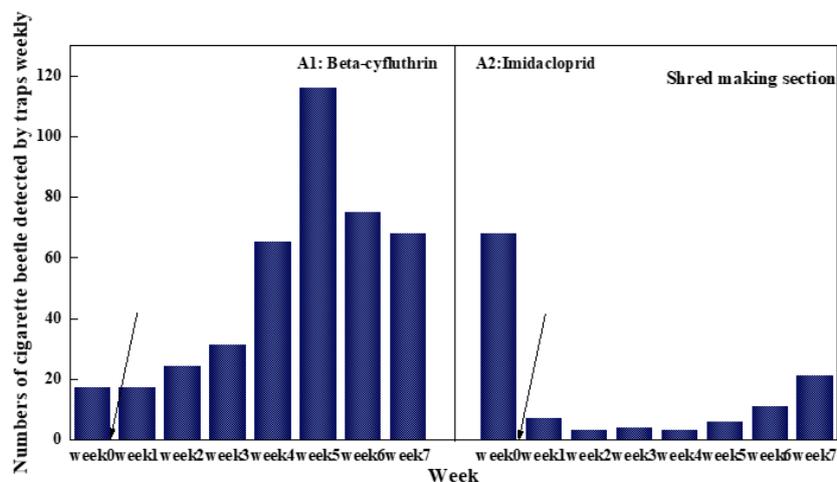


Figure 2. Weekly quantitative change in detected *L. serricorne* before and after insecticide application in the shred-making section; week 0 represents the week before the application, with an arrow indicating the application time (the last day of the week); A1) beta-cyfluthrin application, and A2) imidacloprid application.

Quantitative changes in detected L. serricorne in the storage section

The data presented in B1 (**Figure 3**) show the fluctuation in *L. serricorne* numbers in the storage section from weeks 1 to 7 following beta-cyfluthrin application. The observed decrease rates were -15.91%, -11.36%, 11.36%, 68.18%, -50.0%, -20.45%, and -9.1%, respectively. In contrast, B2 (**Figure 3**) illustrates a significant decline in *L. serricorne* after imidacloprid application, with decreased rates of 95.83%, 95.83%, 95.83%, 100.0%, 97.92%, 100.0%, and 97.92% in weeks 1 to 7. The storage section, which spans 600 m² with a volume of 3000 m³, saw a notable reduction in pest numbers post-treatment. After imidacloprid was applied, only up to two *L. serricorne* were detected per week. This section, dedicated to storing tobacco shreds, is considered a critical control point due to the potential risk of *L. serricorne* contaminating cigarette products. Hence, fewer *L. serricorne* in this area greatly reduce the likelihood of pest contamination in the final product. Additionally, the absence of complex machinery and the limited hiding spots for *L. serricorne* in the storage area contributed to the high efficacy of the pest control measures.

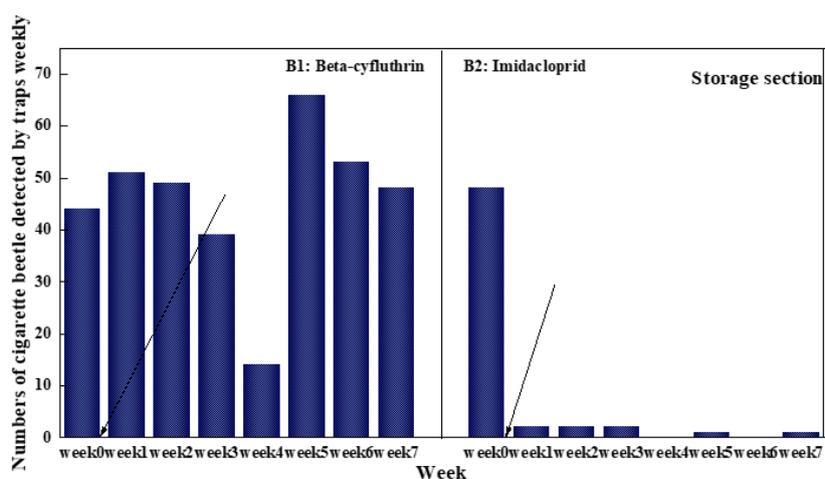


Figure 3. Weekly quantitative changes in detected *L. serricorne* before and after insecticide application in the storage section; the week before application is designated as week 0, with an arrow indicating the application time (the last day of the week); B1) application of beta-cyfluthrin, and B2) application of imidacloprid.

Quantitative changes in detected L. serricorne in the blending section

Following the application of beta-cyfluthrin, the reduction in *L. serricorne* numbers varied, except in week 4, where a significant decrease of 89.47% was recorded. In the other weeks, the decline rate ranged from 26.32% to 47.37% (Figure 4). In contrast, after applying imidacloprid, as shown in C2 (Figure 4), the reduction in *L. serricorne* remained consistently between 70.0% and 100.0% for the 7 weeks following the application. During this period, a maximum of three *L. serricorne* were detected per week.

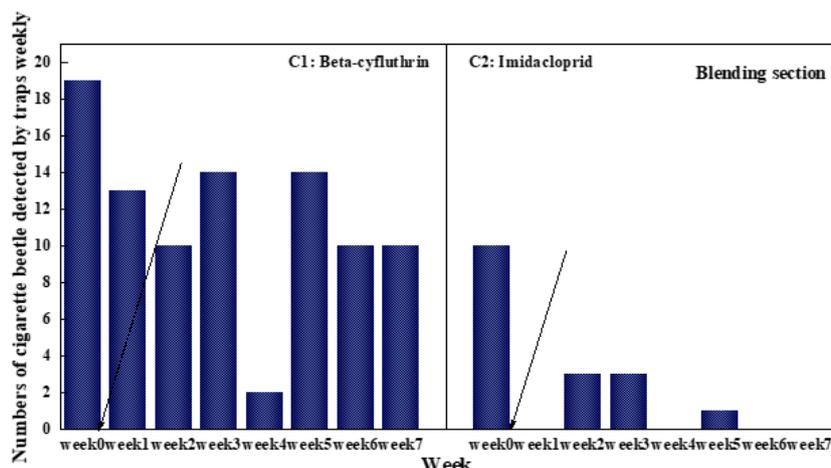


Figure 4. Weekly quantitative changes in detected *L. serricorne* before and after insecticide application in the blending section; the week preceding the application is labeled as week 0, with an arrow marking the time of application (the final day of the week); C1) beta-cyfluthrin application, and C2) imidacloprid application

Quantitative changes in detected L. serricorne in the cigarette-making section

This section features a complex design, large space, and high machine integration, representing the final risk area for *L. serricorne* to infest cigarette products. Given the stringent pest control and sanitation requirements within the tobacco industry, the captured data is crucial. Figure 5a, panel D1, illustrates the weekly variation in the number of *L. serricorne* detected before and after beta-cyfluthrin application. Overall, there were no significant fluctuations in the number of insects, with the reduction rates for *L. serricorne* in weeks 1-7 being -15.79%, 57.89%, 63.16%, 10.53%, 36.84%, -31.58%, and 31.58%, respectively. In contrast, Figure 5a, panel D2, shows that after the beta-cyfluthrin application, there was an 84.62% decrease in the first week, but the reduction slowed to 46.15% and 15.38% in the second and third weeks. Upon inspection, it was observed that the results differed significantly from those in other sections. Only one adult beetle was found in the pheromone trap, prompting a thorough check. The source of the infestation was traced to outdated equipment covered with plastic film, preventing the pesticide from reaching the insects. Only beetles that emerged through gaps in the film were attracted to the traps. The obsolete equipment was removed, and a second treatment with imidacloprid was applied. From weeks 4 to 7, the decrease in detected *L. serricorne* remained high, with a reduction rate of 84.62% to 100%, reaching the pest control threshold for the section (□ one beetle per week per trap).

Quantitative changes in detected L. serricorne in the ripper section

This section in the cigarette manufacturing area is physically separated from the other units, with distinct architectural and spatial isolation. Figure 5b, panel E1, shows that while the number of *L. serricorne* in this section was low, there was no significant reduction after the application of beta-cyfluthrin. However, Figure 5b, panel E2, reveals that following imidacloprid application, no *L. serricorne* was detected in the first five weeks, and only two were detected in weeks 6 and 7.

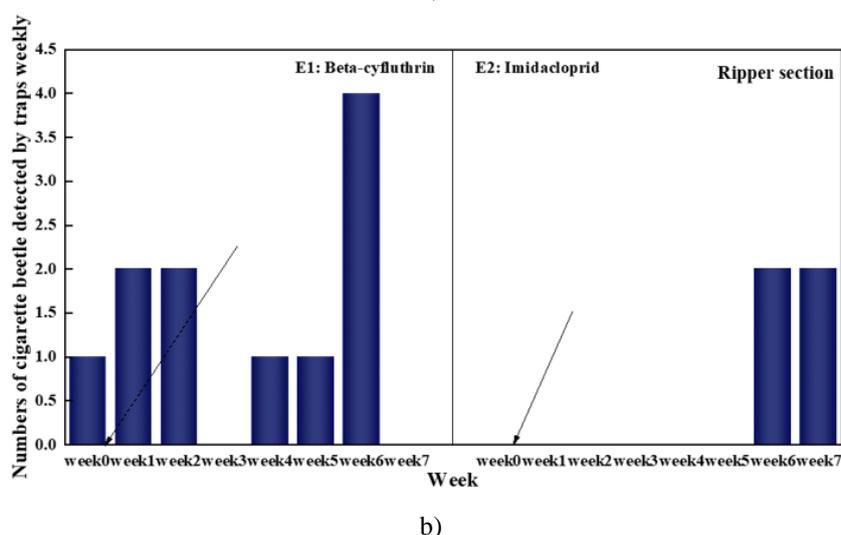
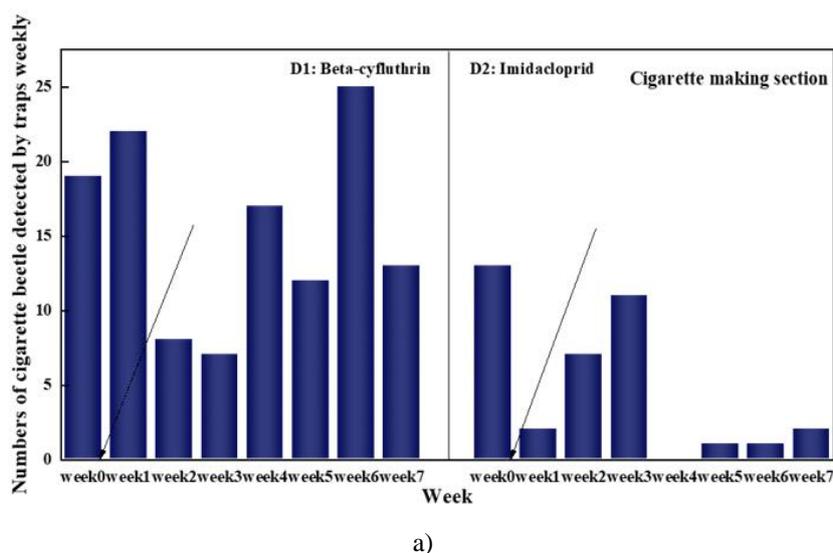


Figure 5. Weekly changes in the number of *L. serricorne* detected before and after insecticide application in the cigarette-making section (a) and ripper section, (b) the week before application is designated as week 0, with an arrow marking the application time (the final day of the week); D1) application of beta-cyfluthrin, and D2) application of imidacloprid.

Table 2 shows the weekly decrease rates of detected *L. serricorne* in the cigarette manufacturing area following the application of different insecticides. After space spraying with beta-cyfluthrin aerosol, the decrease rate of detected *L. serricorne* ranged from -109% to 9%, with an average decrease of -29%. In contrast, after imidacloprid application, the decrease rate ranged from 81.29% to 97.84%, with an average decrease of 89.93%.

Table 2. Decrease rate of detected *L. serricorne* weekly in cigarette manufacturing area (five sections overall) after application

Insecticide	A week after insecticide application							Average
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	
Beta-cyfluthrin	-5.00%	7.00%	9.00%	1.00%	-109.00%	-67.00%	-39.00%	-29%
Imidacloprid	92.09%	89.21%	85.61%	97.84%	93.53%	89.93%	81.29%	89.93%

Pyrethroids are commonly used in China to manage *L. serricorne* in cigarette manufacturing. However, due to their prolonged use, it is essential to evaluate their effectiveness in both laboratory and field conditions. Our study revealed that the LSH strain had developed significant resistance—up to 15.10 times greater than the LSLAB strain—to beta-cyfluthrin, making this insecticide ineffective in controlling the pests in both settings.

Consequently, pyrethroids cannot be relied upon for controlling *L. serricornis* for the time being. On the other hand, we found that the LSH strain remained highly susceptible to the neonicotinoid insecticide imidacloprid.

It is important to consider insecticide cross-resistance when selecting alternative insecticides in resistance management programs. Research has shown that metabolic mechanisms contribute to insect resistance to both pyrethroids and neonicotinoids. Specifically, the development of resistance to neonicotinoid insecticides is often linked to increased cytochrome P450 activity and gene overexpression [32]. Furthermore, enhanced cytochrome P450 activity has also been associated with pyrethroid resistance and is likely a key factor in its development. Another important mechanism of pyrethroid resistance is the reduced sensitivity of sodium channels [33-37].

Despite the similar metabolic resistance mechanisms for both insecticide classes, our study found no significant cross-resistance between beta-cyfluthrin and imidacloprid, with a cross-resistance ratio of only 1.00 to 1.33 (**Table 1**). This lack of cross-resistance is a key reason why imidacloprid proved effective in suppressing *L. serricornis* in the field. Previous studies have similarly found no cross-resistance between insecticides that share similar metabolic resistance pathways. For example, Chen *et al.* [38] observed that despite the overexpression of cytochrome P450 genes and enhanced cytochrome P450 activity contributing to metabolic resistance to dinotefuran in *Aphis gossypii*, the insects showed only minimal resistance to imidacloprid (1.1-fold), indicating no cross-resistance, while exhibiting much higher resistance to thiamethoxam (15.3-fold). Similarly, Zhang and Ling [39] found that *Nilaparvata lugens* exhibited resistance to bisultap, but only minimal resistance (1.02-fold) to imidacloprid. These findings suggest that although cytochrome P450 activity plays a central role in metabolic resistance, the overall insect resistance outcome may be determined by a combination of metabolic mechanisms, target site sensitivity, and other factors such as body surface penetration [35, 40].

Imidacloprid, a systemic insecticide from the neonicotinoid class, has been widely studied for its efficacy in controlling pests like thrips, aphids, and mosquitoes [41-43]. However, its use in managing stored-product pests has received less attention. Wakil and Schmitt [44] conducted field trials on farms and suggested that imidacloprid could offer effective protection for stored wheat against 4 major grain pests. Similarly, Nayak and Daglish [45] evaluated imidacloprid's control over 4 species of psocids, proposing its potential as a grain protectant against stored-product pests. Athanassiou *et al.* [46] found that combining imidacloprid with beta-cyfluthrin did not improve pest control over using beta-cyfluthrin alone, with the efficacy varying based on the pest species. In a separate study, Arthur and Fontenot [47] examined dinotefuran, another neonicotinoid, and found it can be incorporated into pest management strategies for stored products. These studies primarily used imidacloprid in grain treatments or surface sprays in storage areas.

In contrast, our approach involved spraying imidacloprid in aerosol form directly into confined spaces to control *L. serricornis*. The aerosol droplets, generated by an atomizer, remained suspended in the air for an extended period, allowing for effective pest control through knockdown and contact-killing effects.

Conclusion

In the food and tobacco industries, Integrated Pest Management (IPM) primarily targets source control, physical barriers, and sanitation to prevent pest infestations. Additionally, space spraying with insecticide aerosols serves as an effective supplementary control method. Our research demonstrated that when *L. serricornis* develops resistance to pyrethroids and the use of organophosphate and carbamate insecticides is restricted because of toxicity or odor concerns, the application of imidacloprid aerosol as a space spray in enclosed areas is a practical and dependable solution for pest management. These results suggest that imidacloprid can be highly effective not only for controlling cigarette beetles but also for managing other pyrethroid-resistant Coleoptera pests in processing and storage environments.

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Ethics Statement: None

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