



Ultrasonic Exposure as a Potential Non-chemical Approach for Managing the Cotton Leafworm, *Spodoptera littoralis* (Boisduval): Developmental, Reproductive, and Digestive Physiological Responses

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Abstract: The cotton leafworm, *Spodoptera littoralis* (Boisduval), is a major agricultural pest causing substantial economic losses in many crops. The present study evaluated the biological and physiological effects of ultrasound exposure on second- and fourth-instar larvae of *S. littoralis* under laboratory conditions. Developmental parameters, survival, reproductive performance, and digestive enzyme activities were assessed to determine the susceptibility of different larval stages to ultrasound treatment. Ultrasound exposure significantly affected the development and fitness of *S. littoralis*. In second-instar larvae, larval and pupal durations increased significantly, while pupation success, adult emergence, egg hatchability, and fecundity decreased to 65.0%, 40.0%, 48.5%, and 52.1% of the control, respectively. Malformation incidence increased markedly to 36.8%. In fourth-instar larvae, pupal duration was also prolonged, whereas larval duration was shortened. Pupation success, adult emergence, egg hatchability, and fecundity were reduced to 80.0%, 48.0%, 61.2%, and 68.4% of the control, respectively, accompanied by a malformation rate of 22.5%. Comparative analysis indicated that second-instar larvae were more susceptible to ultrasound treatment than fourth-instar larvae. No significant effect on sex ratio was detected in either larval stage. Biochemical analyses revealed significant inhibition of digestive enzyme activities following ultrasound exposure. Amylase, invertase, trehalase, and lipase activities were reduced by 40.5%, 34.5%, 45.0%, and 29.6%, respectively, compared with untreated controls. These reductions suggest impairment of nutrient digestion and metabolic efficiency, which may contribute to the observed developmental and reproductive disruptions. Overall, ultrasound treatment exerted pronounced adverse effects on growth, survival, reproduction, and digestive physiology of *S. littoralis*, particularly in early larval instars. These findings highlight the potential of ultrasound technology as an environmentally friendly component of integrated pest management programs targeting this economically important pest.

Keywords: Ultrasonic waves, Digestive enzymes, latent effect, cotton leaf worm, IPM.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is among the most destructive polyphagous insect pests in Africa, the Middle East, and the Mediterranean region. The insect attacks a broad range of economically important crops, including cotton, maize, vegetables, legumes, and ornamental plants, causing substantial yield losses and significant economic damage (CABI, 2024). Its high reproductive potential, rapid developmental rate, and remarkable ability to adapt to different environmental conditions have contributed to its status as a persistent agricultural pest (EPPO, 2023).

For many years, management of *S. littoralis* has depended largely on the extensive use of synthetic insecticides. Although chemical control remains an effective tool for suppressing pest populations, the continuous and indiscriminate application of insecticides has led to the development of resistance, environmental contamination, disruption of natural enemy populations, and increasing concerns regarding pesticide residues in agricultural products (Desneux et al., 2007; Sparks & Nauen, 2015). Consequently, considerable research efforts have been directed toward the development of environmentally safer alternatives that can be integrated into sustainable pest management programs (El-Saghier et al., 2023).

In recent years, nanotechnology has emerged as a promising approach for enhancing pest management efficiency while reducing environmental risks. Nanomaterials have demonstrated unique physicochemical properties that improve biological activity, stability, and target specificity compared with conventional formulations. Several studies have reported significant insecticidal effects of nanoparticle-based formulations against lepidopteran pests, including *S. littoralis* (Abd Elnabi et al., 2021). Furthermore, previous investigations conducted by Shaker et al. (2017) demonstrated that titanium dioxide (TiO₂) nanoparticles adversely affected larval development, survival, and reproductive performance of *S. littoralis*, highlighting the potential of non-conventional technologies as alternatives to traditional insecticides. These findings encouraged further exploration of innovative approaches capable of disrupting insect physiology through mechanisms distinct from chemical toxicants (El-Ashry et al., 2022).

Alongside advances in nanotechnology, physical pest control methods have gained increasing attention as environmentally compatible components of integrated pest management (IPM) programs. Among these approaches, ultrasound technology has attracted particular interest due to its non-chemical nature and potential effects on insect behavior and physiology. Ultrasound consists of sound waves with frequencies exceeding 20 kHz and plays an important role in ecological interactions between insects and their natural predators, particularly echolocating bats (Miller & Surlykke, 2001). Exposure to ultrasonic waves has been reported to induce stress responses, alter feeding behavior, interfere with development, and reduce reproductive fitness in several insect species (Mankin, 2012; Benelli et al., 2018).

Despite the growing interest in acoustic technologies, information regarding the developmental and physiological effects of ultrasound on *S. littoralis* remains limited. In particular, little attention has been paid to the differential susceptibility of larval instars and the physiological mechanisms responsible for ultrasound-induced biological responses. Digestive enzymes such as amylase, invertase, trehalase, and lipase play fundamental roles in nutrient digestion, energy metabolism, and successful completion of insect development (Terra & Ferreira, 2012). Therefore, alterations in digestive enzyme activity may provide valuable insight into the physiological basis of growth inhibition, developmental abnormalities, and reproductive impairment observed following exposure to physical stressors (Dhaouafi et al., 2024).

Because susceptibility to environmental stress frequently varies according to developmental stage, understanding the responses of different larval instars is essential for optimizing the practical application of ultrasound-based control strategies. Early larval stages are generally characterized by elevated metabolic activity and greater sensitivity to

physiological disruption than older larvae (Nation, 2022; Senthil-Nathan, 2013). Therefore, the present study was undertaken to evaluate the effects of ultrasound exposure on the biological performance and digestive physiology of *S. littoralis*. Particular emphasis was placed on comparing the responses of second- and fourth-instar larvae through assessment of developmental duration, pupation success, adult emergence, fecundity, egg hatchability, malformation incidence, sex ratio, and digestive enzyme activities. The findings provide new insights into the potential use of ultrasound as an environmentally friendly tool that may complement existing integrated pest management programs for the sustainable control of this economically important pest.

MATERIALS AND METHODS

Insect Culture

The study was conducted at the Plant Protection Research Institute, Sids Agricultural Research Station, Agricultural Research Center, Beni-Suef Governorate, Egypt. A laboratory colony of the cotton leafworm, *Spodoptera littoralis* (Boisduval), was maintained under controlled laboratory conditions and reared for several generations without exposure to insecticides according to Ghoneim (1985). Larvae were fed daily with fresh castor bean leaves (*Ricinus communis* L.).

The insects were maintained in plastic rearing containers covered with fine white muslin mesh to provide adequate ventilation while preventing insect escape and contamination. Adult moths were transferred to separate oviposition cages and supplied continuously with cotton wool soaked in a 10% (w/v) sucrose solution as a carbohydrate source throughout their lifespan.

All experiments were carried out under controlled environmental conditions at [2± 27°C], RH%, [5% ±65].

Ultrasonic Device

Ultrasonic exposure was carried out using a commercial ultrasonic generator (Model DATA#SS65030, Germany), which operates within a frequency range of 30-65 kHz. The device was used as provided without modification, and operational conditions were maintained consistently throughout the experiment. The study evaluated the biological response to the ultrasonic device under standardized operating conditions. Therefore, the results should be interpreted as the biological effects produced under the tested exposure conditions.

Experimental Design

Second- and fourth-instar larvae of *S. littoralis* were selected for the experiments. For each larval stage, individuals were randomly assigned to treated and control groups. Each group consisted of five replicates, with five larvae per replicate (25 larvae per treatment).

The ultrasonic generator (30-65 kHz) was positioned at a fixed distance of 30 cm above the rearing containers. Treated larvae were exposed to ultrasonic waves for 9 h per day throughout the larval period, from the beginning of the designated larval instar until

pupation. Exposure was conducted daily at the same time to ensure consistency among replicates. During treatment, the ultrasonic generator did not make direct physical contact with the rearing containers, thereby minimizing mechanical vibration and ensuring that larvae were primarily exposed to airborne ultrasonic waves. Control groups were maintained under identical environmental and rearing conditions in a separate room sufficiently isolated from the ultrasonic source to prevent unintended exposure. (Shaker & Abeer, 2025).

Biological Parameters

Developmental duration was recorded as the time from the beginning of larval exposure until pupation and from pupation to adult emergence. Pupation success was calculated as the percentage of larvae that successfully reached the pupal stage. Adult emergence was recorded as the proportion of successfully emerged adults from the total pupae. Egg hatchability was determined as the percentage of eggs that successfully hatched. Fecundity was estimated by counting the number of eggs laid per female and expressed relative to the control. Malformation percentage was recorded based on visible abnormalities in pupae and adults. Sex ratio was calculated as the proportion of females among emerged adults.

Enzyme Extraction and Assays

Sixth-instar larvae originating from treated and control groups were collected and stored at $-17\pm1^{\circ}\text{C}$ until analysis. Samples were homogenized in ice-cold phosphate buffer (pH 7.0) and centrifuged at 8000 rpm for 15 min at 5°C . The supernatant was collected and used for biochemical analysis of digestive enzymes, including amylase, invertase, trehalase, and lipase, following standard procedures (Ishaaya & Swirski, 1976; Tatchell, 1958). Digestive enzyme activities were determined using standard spectrophotometric procedures commonly applied in insect physiological studies (Ghoneim et al., 2020). Enzyme activities were expressed as mean $\pm$ standard deviation.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA). Mean comparisons were performed using the Least Significant Difference (LSD) test at $P\leq 0.05$. Results are presented as mean $\pm$ standard error (SE) for biological parameters and mean $\pm$ standard deviation (SD) for biochemical data.

RESULTS AND DISCUSSION

Larval and Pupal Duration

Exposure of *Spodoptera littoralis* larvae to 30-65 kHz ultrasonic waves produced clear and statistically significant alterations in developmental timing, with responses strongly dependent on larval instar. Table (1) and Table (2). In second-instar larvae, larval duration increased significantly from 10.15 ± 0.29 days in controls to 11.30 ± 0.44 days in treated individuals ($F=4.70$, $p=0.0365$), indicating a delay in larval development under ultrasonic stress. In contrast, fourth-instar larvae exhibited an opposite trend, where larval duration decreased significantly from 8.20 ± 0.20 days to 6.80 ± 0.11 days ($F=36.51$, $p<0.0001$). This

divergence suggests that early instars are more susceptible to developmental suppression, whereas later instars may respond by accelerating development as a stress-avoidance strategy.

Such stage-dependent plasticity has been previously associated with adaptive responses in lepidopteran insects under environmental stress, where older larvae shorten their developmental period to reach pupation faster when conditions become unfavorable (Chapman, 2013). Despite the contrasting larval responses, pupal duration increased consistently in both treatments. In second instar-treated insects, pupal duration rose from 8.80 ± 0.32 days to 11.20 ± 0.25 days ($F=35.08$, $p<0.0001$). A more pronounced increase was observed in fourth instar-treated larvae, where pupal duration extended from 10.05 ± 0.17 days to 13.25 ± 0.16 days ($F=190.57$, $p<0.0001$).

This consistent prolongation of the pupal stage indicates that ultrasonic exposure disrupts metamorphic processes regardless of larval stage. The pupal period is highly sensitive to endocrine regulation, particularly the balance between ecdysteroids and juvenile hormone (Guedes et al., 2016; Gökçe et al., 2022). Disturbance of this hormonal system can delay tissue reorganization and adult differentiation (Sparks & Nauen, 2015; Smagghe & Degheele, 2020; Zheng et al., 2023).

Pupation and Adult Emergence

Ultrasonic exposure significantly reduced survival at key developmental transitions. In second-instar larvae, pupation success declined sharply from 100% to $65.0 \pm 10.94\%$, while adult emergence dropped further to $40.0 \pm 11.24\%$. In fourth-instar larvae, pupation success decreased to $80.0 \pm 9.18\%$, and adult emergence to $48.0 \pm 8.50\%$. The stronger reduction in adult emergence compared to pupation suggests that the pupal-to-adult transition is particularly vulnerable to ultrasonic stress. This may reflect cumulative physiological damage during earlier stages, resulting in incomplete metamorphosis and failure of adult tissue formation (Kumar et al., 2017; Benelli et al., 2018; Ali et al., 2021).

Egg Hatching and Fecundity

Marked impairment of reproductive traits was observed in both treatments, indicating that ultrasonic stress extends beyond individual development and affects population fitness. Egg hatchability declined from 100% in controls to $48.5 \pm 1.15\%$ in second instar-treated insects, and to $61.2 \pm 1.65\%$ in fourth instar-treated insects. This reduction suggests compromised embryonic viability in the F1 generation. Fecundity was also significantly reduced to $52.1 \pm 2.05\%$ and $68.4 \pm 2.80\%$ of control levels in the second and fourth instar treatments, respectively. This reduction in reproductive output may be linked to impaired gonadal development and disruption of vitellogenesis during adult maturation (Rostelato-Ferreira et al., 2016; Nasser & Zaki, 2019; Han et al., 2024).

Morphogenic Abnormalities and Sex Ratio

Adult deformities increased substantially, reaching $36.8 \pm 1.90\%$ in the second-instar treatment and $22.5 \pm 1.35\%$ in the fourth-instar treatment, compared with 0% in the control.

Adult moths emerging from larvae exposed to ultrasonic waves exhibited evident morphological abnormalities compared with the control. The treated adults showed marked variation in body size, with several individuals appearing noticeably smaller than normal. In addition, incomplete wing expansion, wrinkled and folded wings, body asymmetry, and irregular abdominal development were observed in some emerged adults (Figure 1). These deformities indicate that ultrasonic exposure during immature stages adversely affected normal metamorphosis and adult morphogenesis.

The observed morphological defects may be attributable to disruption of normal pupal development induced by ultrasonic stress. Exposure to ultrasonic waves may interfere with tissue remodeling, cellular differentiation, and cuticle formation during metamorphosis, resulting in impaired wing expansion and abnormal body development after adult emergence. Furthermore, physiological stress caused by ultrasonic treatment may alter hormonal regulation, particularly the balance of ecdysteroids and juvenile hormone, which are essential for successful metamorphosis. Consequently, adults emerging from treated larvae may exhibit reduced structural integrity and potentially lower flight ability, mating success, and reproductive fitness. (Zibae et al., 2015; Abdel-Gawad, 2019; Pavela et al., 2021). On the other hand, larvae exposed to ultrasonic waves exhibited conspicuous morphological abnormalities compared with untreated individuals. The treated larvae showed pronounced variation in body pigmentation, ranging from excessive melanization to abnormal pale discoloration. Considerable differences in body size and shape were also evident, together with irregular segmentation and deformities in the cephalic and thoracic regions. Some larvae appeared unable to complete normal emergence, displaying partially retained cuticle and abnormal body morphology (Figure 2). Therefore, the observed deformities may reflect disruption of normal developmental processes induced by ultrasonic exposure (Nation, 2022; Chapman, 2013; Truman & Riddiford, 2019).

Importantly, the sex ratio remained statistically unchanged in both treatments, indicating that ultrasonic exposure does not interfere with genetic sex determination pathways.

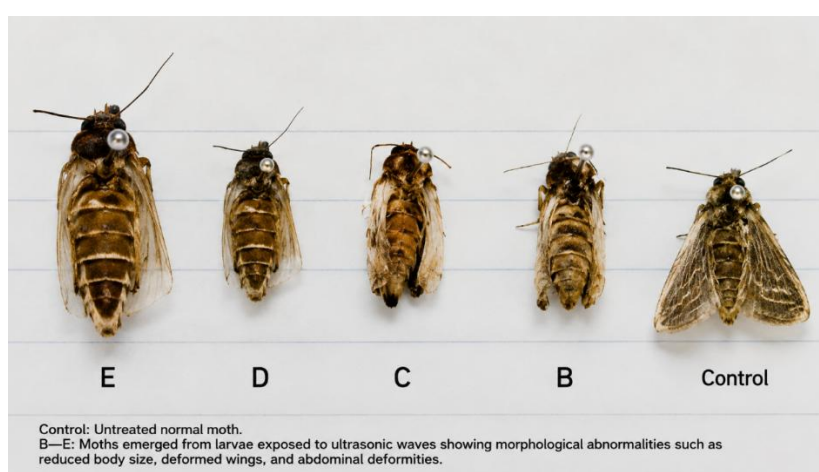


Figure 1: Representative adult *Spodoptera littoralis* moths emerging from larvae exposed to ultrasonic waves, showing variation in body size, incomplete wing expansion, wing deformation, and abnormal abdominal morphology compared with normal adult development.

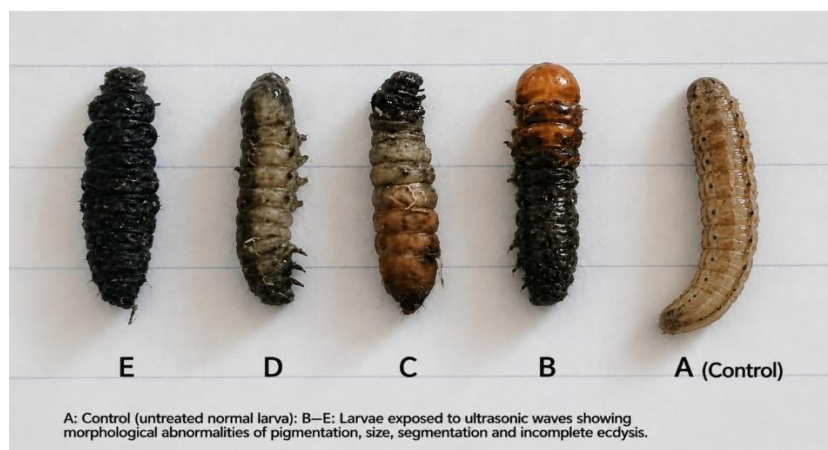


Figure 2: Representative larvae of *Spodoptera littoralis* following ultrasonic exposure, showing marked variation in pigmentation, body size, irregular segmentation, incomplete ecdysis, and deformities of the cephalic and thoracic regions compared with normal larval morphology.

Table 1: Biological parameters of second instar *Spodoptera littoralis* larvae following ultrasonic exposure

Biological Parameter	Control (Mean $\pm$ SE)	Treatment (Mean $\pm$ SE)	F-value	p-value	LSD _{0.05}	Significance
Larval duration (days)	10.15 $\pm$ 0.29	11.30 $\pm$ 0.44	4.70	0.0365	0.98	*
Pupal duration (days)	8.80 $\pm$ 0.32	11.20 $\pm$ 0.25	35.08	<0.0001	0.87	***
Pupation success (%)	100.00 $\pm$ 0.00	65.00 $\pm$ 10.94	10.45	0.0026	15.20	**
Adult emergence (%)	100.00 $\pm$ 0.00	40.00 $\pm$ 11.24	18.00	0.0001	21.80	***
Egg hatchability (%)	100.00 $\pm$ 0.00	48.50 $\pm$ 1.15	742.00	<0.0001	4.10	***
Malformation (%)	0.00 $\pm$ 0.00	36.80 $\pm$ 1.90	295.00	<0.0001	3.95	***
Fecundity (% of control)	100.00 $\pm$ 0.00	52.10 $\pm$ 2.05	485.00	<0.0001	6.85	***
Sex ratio (female %)	52.00 $\pm$ 5.02	53.50 $\pm$ 4.10	0.21	0.6480	11.50	ns

($P \leq 0.05$). ns = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 2: Biological parameters of fourth instar *Spodoptera littoralis* larvae following ultrasonic exposure

Biological Parameter	Control (Mean $\pm$ SE)	Treatment (Mean $\pm$ SE)	F-value	p-value	LSD _{0.05}	Significance
Larval duration (days)	8.20 $\pm$.20	6.80 $\pm$ 0.11	36.51	<0.0001	0.52	***
Pupal duration (days)	10.05 $\pm$ 0.17	13.25 $\pm$ 0.16	190.57	<0.0001	0.61	***
Pupation success (%)	100.00 $\pm$ 0.00	80.00 $\pm$ 9.18	4.75	0.0355	18.57	*
Adult emergence (%)	100.00 $\pm$ 0.00	48.00 $\pm$ 8.50	14.2	0.0008	24.3	***
Egg hatchability (%)	100.00 $\pm$ 0.00	61.20 $\pm$ 1.65	512	<0.0001	3.8	***
Malformation (%)	0.00 $\pm$ 0.00	22.50 $\pm$ 1.35	188	<0.0001	3.6	***
Fecundity (% of control)	100	68.40 $\pm$ 2.80	310	<0.0001	7.2	***
Sex ratio (female %)	51.50 $\pm$ 4.95	50.00 $\pm$ 3.85	0.08	0.779	10.9	ns

($P \leq 0.05$). ns = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Digestive Enzyme Inhibition and Metabolic Impairment

A clear and consistent suppression of midgut digestive enzymes was recorded following ultrasonic exposure Table (3). Amylase activity decreased by 40.50%, invertase by 34.50%, trehalase by 45.00%, and lipase by 29.60%. Trehalase showed the strongest inhibition, declining from 142.80 ± 10.30 to 78.45 ± 5.90 U/mg protein, highlighting a major disruption in carbohydrate energy metabolism (Wyatt & Mayer, 1957; Tang et al., 2017; Ghoneim et al., 2020).

Trehalase plays a central role in converting trehalose into glucose, which is the primary circulating energy source in insects. Its inhibition likely results in severe energy deficiency during energetically demanding processes such as molting and metamorphosis (Wyatt & Mayer, 2010). Combined suppression of carbohydrate- and lipid-digesting enzymes further intensifies nutritional stress, forcing larvae to rely on internal reserves rather than dietary assimilation (Abdel-Gawad, 2019; Dhaouafi et al., 2024).

Overall, the results demonstrate that 30-65 kHz ultrasonic waves induce systemic physiological stress in *S. littoralis*, affecting growth, metamorphosis, survival, reproduction, and metabolism in a coordinated manner (Tang et al., 2017). The observed developmental delays, reduced survival rates, increased malformations, and enzyme inhibition collectively suggest disruption of both endocrine regulation and midgut physiological integrity. Mechanical vibration and possible micro-level cellular disruption may impair epithelial function in the midgut, reducing enzyme secretion and nutrient absorption efficiency. In parallel, stress-induced hormonal imbalance may interfere with molting regulation and metamorphic progression (Chapman, 2013; Smagghe & Degheele, 2020).

From an applied perspective, these findings support the potential use of ultrasonic waves as a non-chemical pest management tool within integrated pest management (IPM) programs. However, field validation is necessary to evaluate effectiveness under natural conditions, as well as to assess energy requirements, delivery efficiency, and ecological safety (Metcalf & Luckmann, 2017).

Table 3: Midgut digestive enzyme activity (U/mg protein) changes in *Spodoptera littoralis* larvae under ultrasonic treatment

Enzyme	Control (Mean $\pm$ SD)	Treatment (Mean $\pm$ SD)	% Inhibition	F-value	p-value
Amylase	185.40 ± 12.50	110.25 ± 8.40	40.50%	31.62	<0.001
Invertase	95.15 ± 6.20	62.30 ± 4.15	34.50%	27.41	<0.01
Trehalase	142.80 ± 10.30	78.45 ± 5.90	45.00%	48.9	<0.001
Lipase	45.60 ± 3.15	32.10 ± 2.40	29.60%	14.85	<0.05

($P \leq 0.05$). ns = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

CONCLUSION

This study demonstrates that ultrasound exposure exerts potent entomotoxic and physiological disruptions on the cotton leafworm, *Spodoptera littoralis*. The treatment significantly impairs key fitness parameters, including prolonged developmental durations, elevated malformation rates, and stark reductions in pupation success, adult emergence, fecundity, and egg hatchability. Notably, the severity of these adverse effects is stage-

dependent, with the early (second-instar) larvae exhibiting a markedly higher susceptibility compared to the later (fourth-instar) developmental stage.

Mechanistically, the observed developmental stagnation and reproductive decline are strongly linked to the profound inhibition of vital digestive enzymes (amylase, invertase, trehalase, and lipase). This enzymatic suppression suggests a critical failure in nutrient assimilation and metabolic efficiency within the treated larvae.

In conclusion, these findings underscore the potential of ultrasound technology as a highly effective, eco-friendly tool. Incorporating ultrasound treatments into integrated pest management (IPM) programs could offer a sustainable alternative to conventional chemical insecticides for controlling *S. littoralis* populations, particularly when targeted at the more vulnerable early larval instars.

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