Journal of Economic Entomology, 2026, **00**, 1–11
DOI: https://doi.org/10.1093/jee/toag187
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Field and Forage Crops

The effect of phenological stage and different plant structures of Bt cotton on the development and survival of *Spodoptera litura* (Lepidoptera: Noctuidae)

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Subject Editor: Nicholas Seiter

Variability in *Bacillus thuringiensis* (Bt) protein expression, during plant development and between different plant structures, may contribute to the survival of *Spodoptera litura* (Fabricius) in transgenic Bt crops. This study examined the survival of neonate and second instar *S. litura* larvae when fed leaf material from field-grown BG3, expressing Cry1Ac, Cry2Ab, and Vip3A, and non-Bt (NBT) cotton plants at different phenological stages (squaring, first flower and/or peak flowering, and cut-out) or different plant structures (leaves, buds, flowers, bolls, and bracts). The effect of alternating diets, where second instar larvae received different combinations of BG3 plant structures and/or NBT leaves was also tested. High mortality levels (>98%) were recorded at day 5 when neonates were fed BG3 plant material from plants of all phenological stages and on all plant structures. Similarly, mortality was high (>99%) when second instar larvae were fed plant material from different BG3 plant structures. However, when larvae were fed an alternating diet of BG3 and NBT leaves from second instar onward, the proportion surviving to adults significantly increased compared with larvae fed exclusively BG3 cotton, irrespective of plant structure combination. These findings indicate that access to a NBT food source, providing intermittent respite from BG3 cotton, can increase larval survival. Further research on NBT host plants, such as weeds or volunteer plants within BG3 fields, is needed to clarify their role in facilitating S. *litura* survival in BG3 cotton systems.

Keywords: dietary impact, larval survival, pest management, insect-plant interactions

Introduction

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a highly polyphagous pest species distributed widely across the Middle East, Asia, and Oceania (EFSA Panel on Plant Health et al. 2019). In tropical northern Australia, *S. litura* was a key pest of non-transgenic cotton during the 1960s and 1970s (Richards 1964, Yeates et al. 2013), where it was observed feeding on all plant structures (Michael and Woods 1980), although it is primarily considered to be a leaf feeder (EFSA Panel on Plant Health et al. 2019). Bollgard 3 (BG3) cotton, expressing Cry1Ac, Cry2Ab, and Vip3A insecticidal proteins derived from *Bacillus thuringiensis*, is registered to provide control of the key pests, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae); however, it has also been shown to provide effective control of *S. litura* (Holman et al. 2025). Glasshouse-grown BG3 cotton leaves caused very high levels of mortality (>99%) in *S. litura* larvae in laboratory bioassays (Holman et al. 2025). However, this contrasts with observations of larvae of all instars feeding on leaves and various reproductive structures in BG3 cotton fields in tropical northern Australia since its commercial release in 2017. Larvae have been recorded in BG3 cotton fields each season, although their occurrence has been sporadic and intermittent. Anecdotal observations indicate that densities are generally low, typically ranging from 0 to 3 larvae per meter, with occasional infestations of up to 8 larvae per meter observed under favourable conditions (Holman, personal observation). Despite these relatively low numbers, the factors influencing larval survival are poorly understood, raising concerns about their persistence in BG3 cotton systems.

Temporal and structure-specific variation in Bt protein expression within a plant can lead to inconsistent efficacy

Received: 7 April 2026. **Revised:** 4 June 2026. **Accepted:** 9 June 2026

against lepidopteran pests (Fitt et al. 1998, Pyke 2003, Kranthi et al. 2005, Olsen et al. 2005a, Dong and Li 2007, Llewellyn et al. 2007, Bommireddy and Leonard 2008, Bahar et al. 2019). Many studies report a positive relationship between Bt expression, measured using enzyme-linked immunosorbent assays (ELISA), and pest mortality (Kranthi et al. 2005, Olsen et al. 2005b, Yu et al. 2013). However, this relationship is not always consistent, as Bt expression does not always fully explain variation in control efficacy. In addition to Bt expression, secondary plant compounds, such as phenolics, terpenoids, and gossypol, can modify expected larval survival, contributing to variability in efficacy (Olsen et al. 1998, Olsen and Daly 2000, Gore et al. 2001, Li et al. 2016, Wang et al. 2021). Whether the variability in efficacy against lepidopteran pests is due to changes in Bt protein expression (Adamczyk et al. 2001b, Kranthi et al. 2005, Yu et al. 2013) or influenced by secondary plant compounds (Olsen and Daly 2000, Gore et al. 2001), the resulting larval survival poses challenges for pest management and insect resistance management in transgenic Bt crops.

Spodoptera litura is a pest of cotton, capable of causing substantial economic losses through defoliation and direct damage to reproductive structures (Srivastava et al. 2018). Despite its prominence as a pest of cotton (Richards 1964, Michael and Woods 1980, EFSA Panel on Plant Health et al. 2019), little is known about how feeding on different phenological stages of the plant or different plant structures affects the survival and development of *S. litura* larvae. Additionally, *S. litura* neonate larvae exhibit greater movement and dispersal on BG3 cotton plants compared with cotton plants that express no Bt proteins (Holman et al. 2026), potentially increasing their exposure to a range of plant structures. Understanding how these factors influence insect biology is important for informing pest management tactics in both non-transgenic and Bt cotton production systems. In this study, we investigated whether survival of *S. litura* larvae differed among cotton phenological stages and plant structures, testing whether patterns of survival were consistent with the hypothesis that within-plant and temporal variability in Bt efficacy may contribute to field survival.

Previous research suggests that Bt protein expression and the efficacy of Bt cotton declines as plants age, particularly during flowering (Fitt et al. 1998, Olsen et al. 2005a, Wan et al. 2005), and that Bt protein expression is low in flowers themselves (Stewart et al. 2001, Kranthi et al. 2005). Based on this, 3 hypotheses were tested. The first was that flowering BG3 cotton enables higher survival of *S. litura* neonate larvae compared to other phenological stages of the plant. This hypothesis was tested in laboratory bioassays to assess neonate survival (to day 5) on leaf material sourced from: (i) plants at the phenological stages of squaring, peak flowering, and cut-out, which were grown in sequentially staged plantings, and (ii) plants from commercial BG3 fields as the crop developed through the phenological stages of squaring, first flower, peak flowering, and cut-out over the course of normal crop cultivation. The second hypothesis tested was that BG3 cotton flowers enable higher survival of *S. litura* larvae compared to other BG3 plant structures. This hypothesis was tested using laboratory bioassays to investigate: (i) neonate survival (to day 5) on different BG3 plant structures, and (ii) *S. litura* survival from second instar through to adult eclosion when fed different BG3 plant structures. The third hypothesis tested was alternating feeding on BG3 plant structures, with expected lower or no Bt proteins, enables higher *S. litura* survival compared to continuous exposure to structures expressing high-doses. This hypothesis was tested in laboratory bioassays assessing *S. litura* survival from late second instar through to adult eclosion when larvae were provided with alternating diets of different BG3 plant structures, as well as a diet that alternated between BG3 and NBT leaves.

Materials and Methods

Plants

Laboratory bioassays were conducted using plant material collected from cotton grown at the Frank Wise Institute of Tropical Agriculture, Department of Primary Industries and Regional Development in Kununurra, Western Australia (–15.65, 128.70) or on commercial properties in the region. All cotton seed used in the study was supplied by Cotton Seed Distributors, Wee Waa, NSW.

Cotton was planted at the Frank Wise Institute of Tropical Agriculture in 2023 and 2024. In each year, seeds were sown on 3 dates to ensure that different phenological stages of plants were available simultaneously to provide the required plant material for bioassays. Two cotton types were planted: Bollgard 3 (BG3) cotton (Sicot 748B3F) and non-Bt (NBT) cotton (Sicot 711RRF), which didn't express Bt proteins but did express the Roundup Ready gene [CP4 EPSPS gene]. Each year, cotton plants were grown from seed sown on 1.2-m wide beds on vertosol soil (10 seeds m^{-1} of row, 2 rows to a bed) separated by 0.6-m irrigation furrow. Commercial irrigation, nutrition, and weed management practices were adopted. Seeds were planted in a randomized split-plot design, where phenological stage (sowing date) was assigned to the main plot, and cotton type (BG3 and NBT) was assigned to the split-plot. Four plots were prepared for each phenological stage. In 2023, the main plots (phenological stage) were 8 rows wide by 98-m long, and the split plots (cotton type) were 4 rows wide. In 2024, the main plots were 6 rows wide by 98-m long, and the split plots were 3 rows wide.

Commercial fields planted to Bollgard 3 cotton, in 2023 and 2024, were used to provide leaf material to assess the effect of phenological stage on the survival of *S. litura* larvae. Leaves were harvested from plants as they developed over the course of the season. Cotton varieties were selected by producers and were primarily Sicot 748B3F and Sicot 606B3F (Table 1). Producers implemented commercial agronomic practices aimed at achieving high yields including irrigation scheduling, weed management, and nutrition strategies. No insecticides that targeted lepidopteran species were applied to the commercial crops. The cotton varieties used in all experiments share a common genetic background and a similar growth habit (Stiller, personal communication).

Plant Material Collections and Measurements

To minimize potential waterlogging effects on the plant material collected for bioassays, only plants located at least 25-m from the field tail drain were sampled. All plants were selected without bias and a standardized method of collecting a main stem leaf from the sixth node below the terminal was adopted. Plant measurements including height, number of nodes, and nodes above white flower (NAWF) were recorded from a designated number of plants when collecting main stem leaves for experiments assessing the effect of phenological stage.

In some experiments, additional plant structures were collected from plants at peak flowering. These included large squares "buds" (pre-candle stage), bracts of large squares, newly opened flowers "flowers" (< 6 hours), small bolls (2–3-cm in diameter) (Fig. S1A), and subtending leaves which were collected from the sixth node below the terminal (Fig. S1B). The bracts from all reproductive structures were removed. Petals and anthers were used in the place of whole flowers in experiments as preliminary studies showed that it was difficult to locate and account for neonate larval survival when intact flowers wilted.

Insects

A laboratory culture of *S. litura* was established from insects collected from maize and NBT cotton refuge crops in Kununurra, Western Australia in 2020 and 2021. The original culture was supplemented with field-collected insects annually to minimize inbreeding and maintained as described previously in Holman et al. (2025).

Laboratory Bioassays

Neonates (< 24 hours old) and late second instar larvae from the laboratory culture were used in the experiments as specified. When required, neonate larvae were reared to late second instar on NBT leaves and maintained on this diet in food-grade rectangular plastic containers (17.5 × 12 × 6-cm, Reward Hospitality, Toowoomba, Queensland) for 7 days prior to use in bioassays. All rearing was done, and all experimental bioassays were maintained in an environmentally controlled room (28 ± 2 °C, 12: 12-h light: dark cycle, 60% relative humidity).

Bioassays were conducted using leaf material (3 × 3 cm) or plant structures as specified for each experiment. The plant material was placed in 70-ml round food-grade plastic containers (75 × 28 mm, Reward Hospitality, Toowoomba, Queensland) with a 2% water-agar base to maintain moisture within the tissues. In each bioassay, the required number of larvae of the appropriate instar was placed onto the plant material in each container using a paint brush, and the lid secured. Insects were considered dead if they failed to respond to prodding with a fine paint brush (Bird and Drynan 2023).

Table 1. In 2023, leaf material collected from 4 fields across 3 commercial farms in Kununurra, Western Australia, was used in bioassays, whereas in 2024, leaf material collected from 5 fields across 5 commercial farms was utilized

Year	Field (replicate)	Sowing date	Variety
2023	1	9 March	Sicot 748B3F
	2	11 March	Sicot 606B3F
	3	13 March	Sicot 746B3F
	4	14 March	Sicot 606B3F
2024	5	3 February	Sicot 606B3F
	6	5 February	Sicot 748B3F
	7	7 February	Sicot 606B3F
	8	26 February	Sicot 606B3F
	9	27 February	Sicot 748B3F

Producers selected different cotton varieties, with sowing dates ranging from 3 February to 14 March.

Effect of Plant Phenological Stage on Survival of *S. litura* Neonate Larvae

Bioassays Using Sequentially Planted Cotton

Leaf material was collected from cotton planted at different dates to produce cohorts of plants at different phenological stages; thus, samples from different phenological stages (squaring, peak flowering, and cut-out) could be collected and used in experiments simultaneously (Table 2).

Ten main stem leaves were collected from each treatment plot (10 leaves per plot, 40 leaves per treatment). Plant measurements of 6 plants, selected without bias, from each BG3 plot were recorded (Table S1). Bioassays were conducted, as previously described, using 10 neonate larvae per container (70 ml, 75 × 28 mm) and mortality was assessed after 5 days. The leaf was not changed during this period. Each container was considered a replicate, and 40 containers were prepared for each treatment.

Bioassays Using Commercially Grown BG3 Cotton

To test whether phenological stage influenced the survival of *S. litura* neonate larvae, leaves were collected sequentially from BG3 cotton plants in commercial fields in the 2023 and 2024 seasons (Table 1). Leaves were collected from each field at 4 phenological stages over the course of normal crop cultivation: squaring, first flower (~50% of plants per m had reach first flower), peak flowering (first flower assessment plus 3 weeks), and cut-out (peak flowering assessment plus 3 weeks). At the assessment of each plant phenological stage, 32 main stem leaves were collected from each field and plant measurements were recorded from 10 plants (Table S2).

Table 2. Cotton plant sowing dates at Frank Wise Institute of Tropical Agriculture, during the 2023 and 2024 season, that were used to ensure simultaneous assessment of cotton at cut-out, peak flowering, and squaring stages

	2023[a]		2024[a]	
Phenological stage	Sowing date	Accumulated day-degrees at collection	Sowing date	Accumulated day-degrees at collection
Cut-out	8 February	1175.6	27 February	1212.4
Peak flowering	9 March	812.6	1 April	772
Squaring	5 April	485.7	24 April	520.4

The accumulated day-degrees for each phenological stage, when the leaves were collected for bioassays at the start of the experiment, were calculated using the 15–32 model (Bange et al. 2022).
[a]In 2023, the experiment was conducted on 26 May, and in 2024, the experiment was conducted on 23 June.

Bioassays were conducted, as previously described, using 12 neonate larvae per leaf in each 70-ml container and mortality was assessed after 5 days. The leaf was not changed during this period. The 32 containers prepared per field at each phenological stage were considered subsamples since they originated from the same field and were therefore not independent of one another. Each field was considered a replicate. As the phenological assessments spanned 3 months and involved different generations of the laboratory culture, controls were run using NBT cotton. The leaves of NBT cotton at phenological stages corresponding to the test stages from the commercial crop were used, with 10 containers, each containing 12 neonate larvae, prepared during a bioassay and survival assessed after 5 days.

Effect of Plant Structures on Survival of Neonate Larvae

To examine the effect of plant structures on the survival of *S. litura* neonate larvae, different plant structures were collected from plants and fed to larvae in bioassays. The experiments were conducted in May 2023 using BG3 and NBT cotton plants grown at Frank Wise Institute of Tropical Agriculture. The plant structures assessed were: (i) main stem leaves, (ii) subtending leaves, (iii) buds, (iv) bracts, (v) petals and anthers from newly opened flowers, and (vi) small bolls.

Bioassays were conducted, as previously described, using 10 neonate larvae per 70-ml container and mortality was assessed after 5 days. The main stem leaves, subtending leaves, and bracts were not changed during this period. Flowers (= petals and anthers) were replaced daily, and buds replaced every other day to prevent desiccation. Each container was considered a replicate, and each treatment was replicated 12 times.

Effect of Plant Structures on Survival of Second Instar Larvae

To examine the effect of plant structures on the survival of *S. litura* late second instar larvae and the development of insects to adults, different plant structures were collected from plants and fed to larvae in a bioassay. The larger instar was chosen to initiate the experiment due to high neonate mortality recorded during the previous experiment using BG3 cotton. The experiment was conducted in May 2023 using BG3 and NBT cotton grown at Frank Wise Institute of Tropical Agriculture. The plant structures assessed were: (i) main stem leaves, (ii) buds, (iii) bracts, (iv) petal and anthers of newly opened flowers, and (v) small bolls.

Bioassays were conducted, as previously described, using 1 late second instar larvae in each 70-ml container with a given plant structure. For each structure, a single larva was considered a replicate. Forty replicates of each plant structure from NBT cotton were prepared and 55 replicates of each plant structure from BG3 cotton were prepared. Plant structures were replaced every second day, except for flowers which were replaced daily to prevent desiccation. Larval survival was recorded every other day. For each surviving larva, the larval period (neonate to pupa), full development period (neonate to adult), and pupal mass (measured 24 hours after pupation) were recorded. These measurements included the 7-day period to develop to late second instar on NBT cotton. This approach was intended to represent expected developmental periods of *S. litura* in NBT cotton production systems.

Effect of Alternating Plant Structures on Survival, Growth, and Development of Second Instar Larvae

Various diet combinations of BG3 plant structures were tested to examine their effects on *S. litura* survival, growth, and development. Diet combinations, primarily alternating BG3 leaves with different BG3 plant structures, were evaluated as *S. litura* larvae are predominantly considered to be leaf feeders (EFSA Panel on Plant Health et al. 2019). The diet combinations represented the structures that larvae could access by moving within and between plants. Six diet combinations assessed: (i) NBT main stem leaves alone [control], (ii) alternating (24 hours) BG3 and NBT main stem leaves, (iii) BG3 bracts alone, (iv) alternating (24 hours) BG3 main stem leaves and bracts, (v) alternating (24 hours) BG3 main stem leaves and flowers (petals and anthers), and (vi) BG3 main stem leaves alone (Fig. 1). The BG3 bract treatment was included as a larva in the previous experiment survived to adult eclosion on this structure. The alternating BG3 and NBT leaves treatment was included to test the effects of alternate feeding between a Bt protein and NBT food source.

The experiment was conducted in June 2023 with BG3 and NBT cotton grown in the field at Frank Wise Institute of Tropical Agriculture. Bioassays were conducted, as previously described, using 1 late second instar larvae per diet in each 70-ml container. Each larva was considered a replicate. Due to expected differences in survival when exposed to Bt proteins, 25 replicates were prepared for the NBT leaf [control] treatments, while 50 replicates were prepared for treatments incorporating BG3 cotton plant structures. All plant structures were replaced daily in both the alternating and uniform diets, with survival recorded every other day. For each larva, the larval period (neonate to pupa), full development period (neonate to adult) and pupal mass were calculated as previously described.

Statistical Analysis

All analyses were conducted in GenStat Version 24 (VSN International 2024). To determine the effect of phenological stage and cotton type on the survival of neonate larvae within the sequentially planted cotton, the proportion of neonate surviving on day 5 was analysed using a general analysis of variance with year, cotton type and phenological stage, and their interactions as fixed factors. Year, replicate, main plot, and subplot were included as random factors in the model. Prior to analysis, the proportional data were transformed by arcsine square root to meet the assumptions of normality, and results presented as back-transformed data and converted to percentages for reporting. To determine the effect of phenological stage on the survival of neonate larvae in commercial fields of BG3 cotton, the percentage of larvae surviving on day 5 was adjusted for mortality in the appropriate control group using Schneider–Orelli's formula (Püntener 1981). Mortality within the control groups ranged from 6% to 17%, which falls within the acceptable range for applying correction methods (Praulins et al. 2022). The percentage of corrected survival on day 5 was analysed using a one-way ANOVA with farm as a statistical block. Prior to analysis, data were square root transformed to meet the assumptions for normality; back-transformed data and standard errors of the means are reported. To determine the effect of plant structure and cotton type on the survival of neonate larvae, the proportion of neonate surviving on different NBT and BG3 plant structures on day 5 was analysed using a

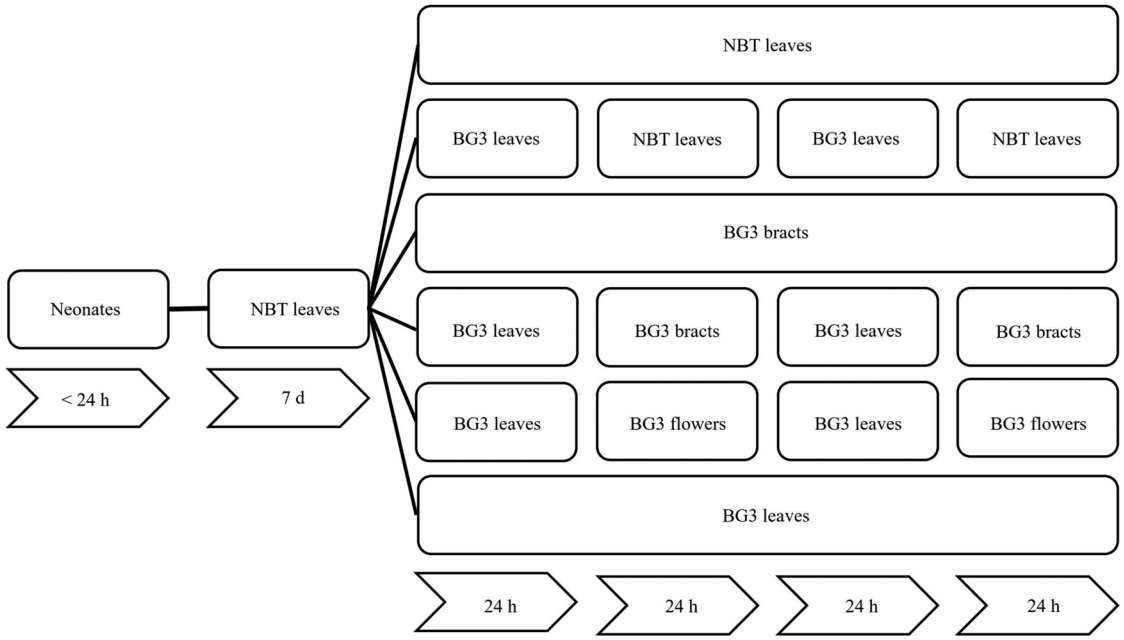

Fig. 1. Flow diagram summarizing the consistent and alternating diets provided to *S. litura* larvae in experiments to assess the effects of different diets on survival, growth, and development. Larvae were reared from neonate to late second instar on non-Bt (NBT) cotton leaves for 7 days. From this point, 6 different diets were tested: NBT leaves alone; Bollgard 3 (BG3) leaves alone; BG3 bracts alone; BG3 leaves (24 hours) followed by flowers (24 hours); BG3 leaves (24 hours) followed by BG3 bracts (24 hours); and BG3 leaves (24 hours) followed by NBT (24 hours). The experiment continues until larvae died or eclosed as adults. Plant material in uniform and alternating diets were changed every 24 hours.

two-way ANOVA. A Bonferroni test ($P < 0.05$) was then used for multiple means comparison. Prior to analysis, the data were transformed by arcsine square root to meet the assumptions of normality, and results presented as back-transformed data and converted to percentages.

Due to very low survival on BG3 cotton in the effect of plant structures on second instar bioassays, analyses were restricted to the NBT treatment. The proportion of individuals that successfully eclosed as adults on NBT plant structures was analysed using a binomial generalized linear model with a logit link, with plant structure as a fixed factor. Similarly, for the alternating plant structure experiment, the proportion of individuals that successfully pupated and eclosed as adults was analysed using a binomial generalized linear model with a logit link, with diet combination used as a fixed factor. Bonferroni post hoc comparisons among treatments were conducted on the logit scale ($P < 0.05$), with predicted means back-transformed and expressed as percentages for presentation. For adult eclosion in the alternating diet experiment, zero emergence in 1 treatment group resulted in complete separation in the model. Therefore, pairwise differences among treatments were assessed using separate binomial generalized linear models fitted to each treatment comparison to determine differences between treatments (statistical summaries are provided in Supplementary Tables 3–5).

A one-way ANOVA was used to analyse the effect of plant structures of NBT (due to low survival on BG3 cotton) and diet combinations on pupal mass and larval and neonate-to-adult development periods. Prior to analysis, development periods for the diet combination experiment were transformed by square root to meet the assumptions of normality, and results presented as back transformed data. Bonferroni tests ($P < 0.05$) were used for multiple mean comparisons.

Results

Effect of Plant Phenological Stage on Survival of *S. litura* Neonate Larvae

Bioassays Using Sequentially Planted Cotton

Neonate survival was unaffected by phenological stage of the cotton plants from which leaves were collected ($F = 0.3$; $df = 2, 12$; $P = 0.772$). Larval survival was extremely low on BG3 cotton, averaging 0.2% survival across all phenological stages, compared with an average of 97.3% survival on NBT cotton ($F = 5143.3$; $df = 1, 18$; $P < 0.001$). The interaction between cotton type and phenological stage had no effect on larval survival ($F = 2.4$; $df = 2, 18$; $P < 0.112$; Table 3). The interaction between year, cotton type, and phenological stage also had no effect on larval survival ($F = 0.1$; $df = 2, 18$; $P = 0.894$).

Bioassays Using Commercially Grown BG3 Cotton

When fed leaves from commercially grown BG3 cotton, neonate survival was very low (cut-out (2.1% ± 1.4%), squaring (0.5% ± 0.2%), peak flowering (0.1% ± 0.4%), and first flower (0.03% ± 0.2%)). Phenological stage of the plants from which the leaves were collected had no effect on the survival of neonate larvae ($F = 2.7$; $df = 3, 24$; $P = 0.072$).

Effect of Plant Structures on Survival of Neonate Larvae

Neonate survival was much lower on BG3 cotton than NBT cotton ($F = 1091.3$; $df = 1, 132$; $P < 0.001$; Fig. 2). The interaction between cotton type and plant structure affected larval survival ($F = 68$; $df = 5, 132$; $P < 0.001$; Fig. 2). Larval survival was higher on NBT main and subtending leaves compared with other NBT plant structures ($P < 0.05$; Fig. 2), whereas survival

Table 3. Mean percentage (±SE) survival of *S. litura* larvae after 5 days of feeding on leaves of different cotton types at key phenological stages

	2023			2024		
Cotton type	Cut-out	Peak flowering	Squaring	Cut-out	Peak flowering	Squaring
BG3	0.1 (±0%)	0 (±0%)	0 (±0%)	0.4 (±0.1%)	2 (±0.1%)	0.1 (±0%)
NBT	96.3 (±1.4%)	93.4 (±1.3%)	96.8 (±1.4%)	98.9 (±1.5%)	98.4 (±1.4%)	98.3 (±1.4%)

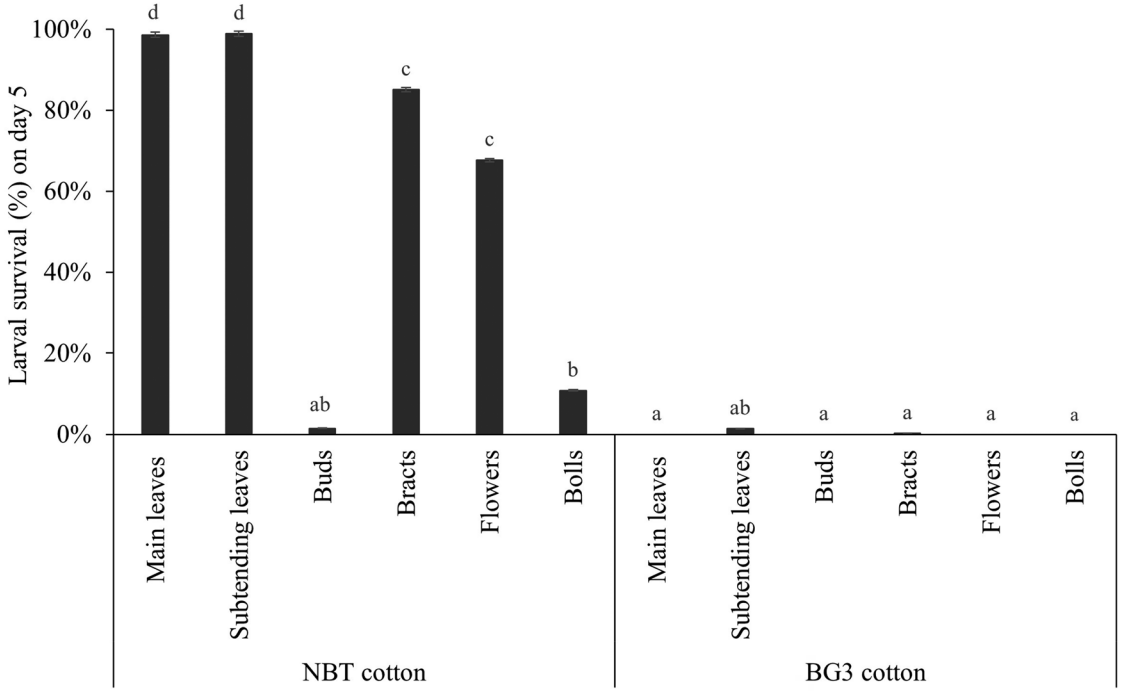

Fig. 2. Mean neonate survival (±SE) on different NBT and BG3 cotton plant structures. Larval survival varied between NBT plant structures, but it was very low across all BG3 plant structures. Different letters indicate significant differences (Bonferroni, $P < 0.05$).

did not differ between larvae reared on different BG3 cotton plant structures ($P > 0.05$; Fig. 2).

Effect of Plant Structures on Survival of Second Instar Larvae

Most larvae that fed on different BG3 cotton structures did not pupate (274/275 larvae = 99.6%). One larva that fed on BG3 cotton bracts successfully formed a pupa (0.187 g) after 28 days and eclosed as an adult. Sixty-three percent of larvae that fed on NBT cotton pupated (125/200 larvae fed NBT cotton, irrespective of plant structure) and 53% successfully emerged as adults (105/200 larvae fed NBT cotton, irrespective of plant structure).

The NBT cotton plant structures influenced the percentage of individuals that successfully developed to adults ($F = 23.5$; $df = 4, 195$; $P < 0.001$). Adult eclosion varied across plant structures, with higher survival on bracts, leaves, and flowers compared with buds and bolls ($P < 0.05$; Fig. 3). The NBT cotton plant structures also influenced developmental attributes (Table 4), including the larval development period ($F = 52.3$; $df = 4, 120$; $P < 0.001$), pupal mass ($F = 11.4$; $df = 4, 120$; $P < 0.001$), and period from neonate to adult eclosion ($F = 56.0$; $df = 4, 120$; $P < 0.00$). Larvae reared on bracts and leaves exhibited the shortest larval development period compared with flowers, buds, and bolls ($P < 0.05$; Table 4), while larvae reared on bolls and bracts developed into heavier pupae compared with buds and flowers ($P < 0.05$; Table 4).

Effect of Alternating Plant Structures on Survival, Growth, and Development of Second Instar Larvae

Diet combinations affected the percentage of larvae that could successfully pupate ($F = 64.2$; $df = 5, 269$; $P < 0.001$) and eclose into adults ($F = 85$; $df = 5, 269$; $P < 0.001$; Fig. 4). When larvae fed exclusively on plant structures from BG3 cotton, only those that fed on bracts survived to form pupae (3/50 = 6%), but none of these developed to adults. Larvae fed NBT leaves had higher pupation and adult eclosion rates (21/25 larvae = both 84%) than larvae fed diet combinations which included BG3 plant structures (Fig. 4). However, 44% of larvae (22/50 larvae) fed a diet alternating between NBT and BG3 leaves were still able to pupate and 28% (14/50 larvae) eclosed as adults, a higher survival rate than those that were exclusively fed BG3 plant structures ($P < 0.05$; Fig. 4).

Insects that developed on NBT cotton leaves [control] developed into pupae more rapidly (18.7 days) than those that developed on an alternating diet of NBT and BG3 leaves (28.3 days) or BG3 bracts (25.7 days) ($F = 105.1$; $df = 2, 43$; $P < 0.001$;

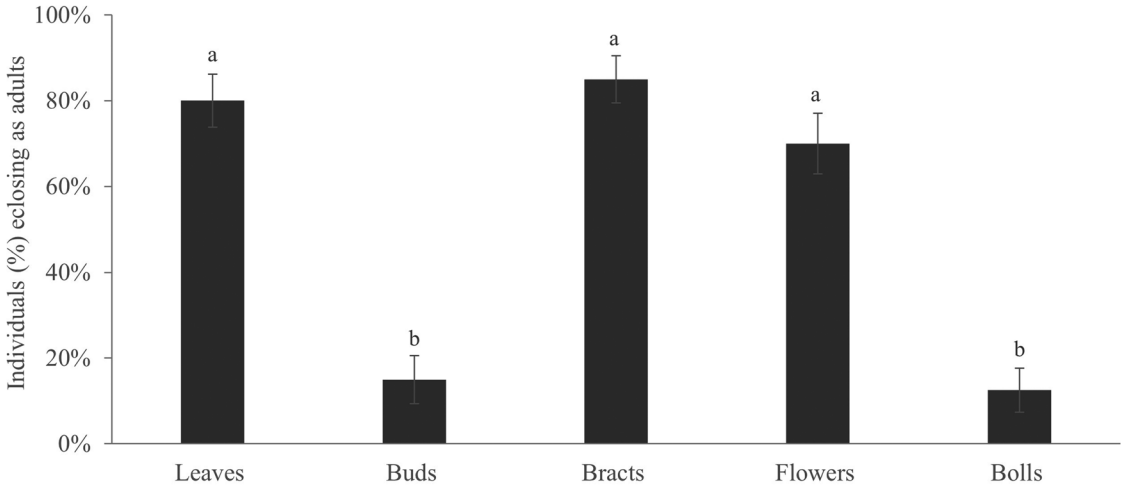

Fig. 3. The mean percentage of *S. litura* larvae (±SE) that eclosed as adults was highly variable when fed different NBT plant structures from late second instar larvae. Bars indicate the standard error, with different letters indicating significant differences (Bonferroni, $P < 0.05$).

Table 4. Mean (±SE) larval development period from neonate to pupation, pupal mass, and period between neonate and adult eclosion for NBT plant structure treatments

NBT plant structure	Larval development period (days)	Pupal mass (mg)	Period from neonate to adult eclosion (days)
Leaves	18.7 (±0.3) a	314 (±8) ac	26.5 (±0.3) ab
Buds	26.3 (±0.6) c	267 (±17) a	35.8 (±0.7) c
Bracts	18.7 (±0.3) a	334 (±7) c	26.3 (±0.2) a
Flowers	20.2 (±0.3) b	273 (±8) a	27.8 (±0.3) b
Bolls	24.8 (±0.6) c	345 (±16) c	33.6 (±0.8) c

Means within a column followed by the same letter are not significantly different (Bonferroni, $P > 0.05$).

Table 5). Similarly, pupae that developed from larvae fed on NBT cotton leaves (mean pupal mass = 362 mg) were heavier than those that alternated feeding between NBT cotton leaves and BG3 leaves (234 mg) and larvae that fed exclusively on BG3 bracts (177 mg) ($F = 38.8$; df = 2, 43; $P < 0.001$; Table 5). Diet also affected the period from neonate to adult eclosion ($F = 228.4$; df = 1, 30; $P < 0.001$; Table 5), and insects that alternated feeding between NBT and BG3 leaves took longer to eclose (36.0 days) compared with those that fed exclusively on NBT leaves (26.8 days).

Discussion

In bioassays, all phenological stages and plant structures of BG3 cotton demonstrated high efficacy against neonates or second instar *S. litura* larvae (Table 3, Figs 2 to 4). Nonetheless, a single individual that fed on BG3 bracts did complete development and successfully eclosed as an adult when fed exclusively on this plant structure. *Spodoptera litura* larvae have been observed feeding on all plant structures on NBT cotton (Michael and Woods 1980). However, the field implications of this specific structure for larval survival in BG3 cotton remain unclear and are likely limited. Consequently, variation in BG3 efficacy due to phenological stage or plant structure is unlikely to be a primary factor allowing the intermittent survival of *S. litura* larvae.

In this study, the effect of BG3 cotton phenological stages was assessed by both adjusting sowing dates to test different phenological stages simultaneously (Olsen and Daly 2000, Bernardi et al. 2014) and following a crop over its development (Adamczyk et al. 2001a, Olsen et al. 2005a, Greenberg et al. 2010, Knight et al. 2013, Yu et al. 2013). Each approach has strengths and limitations, particularly in accounting for weather-related influences on Bt expression and efficacy (Chen et al. 2005, Llewellyn et al. 2007, McCullough 2008, Girón-Calva et al. 2020). Despite these differences, results from both methods were consistent and indicated that phenological stage did not affect the survival of *S. litura* neonates on BG3 cotton.

Vip3A protein levels vary across plant structures in field-grown BG3 cotton, with the highest levels recorded in leaves (12–68 µg/g), followed by seeds (1.0–3.7 µg/g), and pollen (0.3–3.3 µg/g) (Office of the Gene Technology Regulator 2014). Despite these differences in protein expression, the poor survival of neonate larvae on BG3 plant structures in this experiment suggests that the Bt proteins are expressed at sufficient levels throughout the plant to provide effective control of neonates (Fig. 2). Similarly, Kumar and Prasad (2016) found there were no differences in the survival of *S. litura* neonates on the leaves, squares, or bolls of Bt cotton expressing Cry1Ac and Cry2Ab. In contrast, the larval survival of other lepidopteran species, such as *H. armigera* and *H. zea*, often differs across Bt cotton plant structures, with floral structures, particularly flower anthers, enabling higher larval survival in Cry1Ac (Kranthi et al. 2005), Cry1Ac and Cry2Ab (Gore et al. 2001) and Vip3A expressing (Bommireddy and Leonard 2008) cotton. While the floral structures (petals, anthers, etc.) were not fed separately to larvae in this study, as Gore et al. (2001) did, they were available for larvae to feed on within the flower treatment.

The susceptibility of larvae and their probability of survival was different for second instar larvae exposed to BG3 plant structures. Larvae fed exclusively BG3 bracts from second instar were able to pupate (Fig. 4), with one successfully eclosing as an adult. This may indicate lower Bt expression in bracts; however, the possibility that the surviving individual possessed some level of tolerance or resistance to Bt proteins cannot be

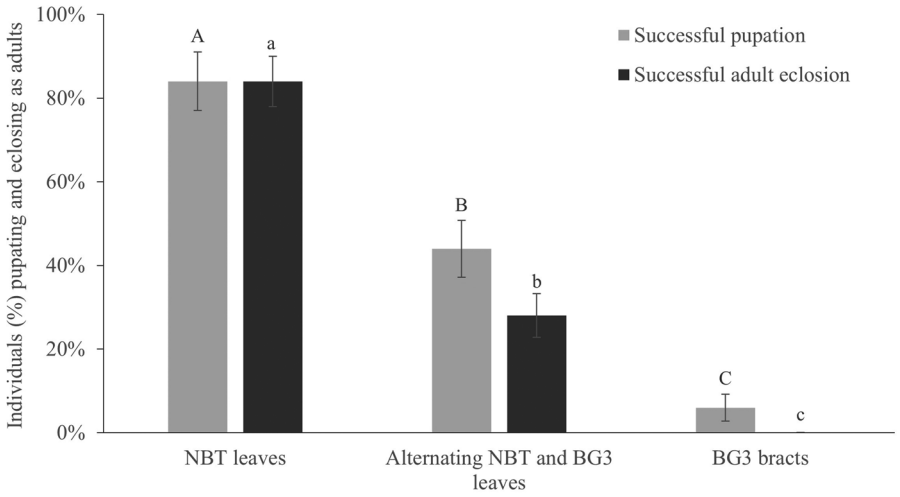

Fig. 4. The mean percentage of *S. litura* larvae (±SE) that successfully pupated and eclosed into adults (%) when fed NBT leaves (non-Bt diet) and/or different combinations of BG3 cotton plant structures. No larvae fed exclusively BG3 leaves, or alternating diets of BG3 leaves and flowers, or BG3 leaves and bracts pupated; these treatments were therefore removed from the analysis. Capital letters indicate significant differences among diet combination treatments for pupation [Bonferroni-adjusted (GLM), $P<0.05$], whereas lowercase letters indicate significant among diet combination treatments for adult eclosion (through paired GLMs, $P<0.05$).

Table 5. Mean (±SE) larval development period from neonate to pupation, pupal mass, and period between neonate and adult eclosion on different diet combinations

Diet combination[a]	Larval development period (days)	Pupal mass (mg)	Period from neonate to adult eclosion (days)
NBT leaves [control]	18.7 (±0.4) a	361 (±11) a	26.8 (±0.4) a
Alternating NBT and BG3 leaves	28.3 (±1.3) b	234 (±12) b	36.0 (±0.5) b
BG3 bracts	25.7 (±0.5) b	177 (±30) b	-

Means within a column followed by the same letter are not significantly different (Bonferroni, $P>0.05$).
[a]No larvae survived to pupation from the alternating BG3 leaves and bracts, alternating BG3 leaves and flower and exclusively BG3 leaves treatments.

excluded. Research indicates that the susceptibility of lepidopteran pests to Bt proteins decreases during larval development (Ashfaq et al. 2001, Sorgatto et al. 2015, Miraldo et al. 2016), which could allow for the survival of older instars on some plant structures, which have lower Bt expression, if they move into BG3 fields from nearby cultivated or non-cultivated plants. While evaluations of the Vip3A protein expression in some plant structures have been conducted (Llewellyn et al. 2007, Office of the Gene Technology Regulator 2014), bracts do not appear to have been tested. Alternatively, bracts may contain secondary plant compounds which interact with Bt proteins to reduce their potency, or these structures may be more nutritionally suitable for *S. litura* larvae, as larvae also had a high survival rate on NBT bracts (Fig. 3). However, the field implications of this specific structure for larval survival remain unclear and are likely limited. If bract-feeding were a major contributor to survival, consistent survival from squaring to post-cut stages would be expected across most Bollgard 3 cotton fields.

This study demonstrated the potential that a diet alternating between BG3 and NBT material could promote survival of *S. litura* larvae. Larvae are more likely to complete their development when BG3 leaves were alternated with NBT leaves, compared to those exclusively fed different combinations of BG3 plant structures (Fig. 4). *Spodoptera litura* is highly polyphagous and can switch host plants during the larval stage (EFSA Panel on Plant Health et al. 2019, Mitra et al. 2021). If *S. litura* larvae can move between BG3 and NBT food sources which are present within BG3 fields, such as non-cultivated weedy plants, larvae could persist for longer (and possibly complete development) compared to larvae that are exposed exclusively to BG3 cotton. The evidence of *S. litura* survival on a mixed diet of BG3 and NBT leaves (Fig. 4) warrants further investigation into their behavioural ecology. The use of non-cultivated plants, such as weeds, as host plants by *S. litura* larvae has been observed in northern Australia (Holman, personal observation) and recorded in other countries (Puja 2007, Ahmad et al. 2013). Additionally, other *Spodoptera* species, such as *S. frugiperda*, can use weeds as alternative hosts in the absence of their preferred plant hosts (Moraes et al. 2020, Adnan et al. 2024, Fortuna et al. 2024). Predominant weed species in farming systems across northern Australia have not been assessed as potential host plants for *S. litura* nor has the propensity for larvae to move to seek alternative food sources after feeding on Bt material. Mixed or diversified production systems may provide alternative food sources for mobile *S. litura* larvae. These larvae are known to feed on a range of crops, such as mungbeans (Bhati 2020), maize (Xue et al. 2010, Ahmad et al. 2013) and sorghum (EPPO 2015), which are commonly grown in association with cotton in northern Australia. The reduced pupal mass and slower development of insects fed on alternating NBT and BG3 cotton leaves (Table 4) could be a result of an aversion response, leading to a reduction or cessation of feeding on Bt leaf material (Binning et al. 2014, Li et al. 2023). It has been suggested that feeding cessation could reduce the amount of protein ingested and allow the larvae to repair damaged midgut epithelium (Castagnola and

Jurat-Fuentes 2016, Pinos et al. 2021). Food consumption was not measured as part of this experiment; however, observationally, larvae did consume some BG3 leaf material when it was provided. Further research may be warranted to investigate the amount of BG3 leaf material required to cause mortality either during a 24-hour period, or throughout the entire larval period, particularly if alternative NBT food sources are available.

The potential for *S. litura* to utilize NBT food sources may also have implications for resistance evolution in BG3 production systems. Although survival on BG3 cotton was very low in this study, the ability of some larvae to survive and complete development when provided with alternating BG3 and NBT plant material (Fig. 4) suggests that access to alternative hosts could reduce exposure to Bt proteins and allow a greater proportion of individuals to survive to adulthood. Survival alone does not indicate the presence of resistance (Downes et al. 2009, Whitburn and Downes 2009); however, repeated exposure of field populations to Bt proteins provides the selection pressure necessary for resistance alleles to increase in frequency within field populations (Storer et al. 2012, Tabashnik et al. 2013). Consequently, any factors that increase larval survival, including the oscillation of larvae between BG3 and NBT food sources such as weeds, may influence the rate at which resistance evolves (Tabashnik et al. 2013).

In conclusion, plant phenological stage is unlikely to be the primary factor contributing to the intermittent survival of *S. litura* larvae observed in BG3 cotton fields in tropical northern Australia. Nevertheless, the plant structure a larva feeds upon can influence its development in non-transgenic cotton production systems. When feeding on NBT cotton, larvae that fed on leaves and bracts developed more quickly, formed heavier pupae and had a higher proportion eclosing as adults than larvae feeing on other plant structures (Fig. 3, Table 4). Although survival on BG3 cotton was significantly lower, some larvae were able to successfully pupate and eclose as adults if fed bracts. Importantly, a higher proportion of larvae could survive and eclose as adults when they were fed a diet alternating NBT and BG3 leaves compared to larvae exclusively fed BG3 cotton (Fig. 4). Future research should focus on other factors that may contribute to the observed survival of larvae in BG3 cotton fields, such as the potential for weeds to act as alternative host plants for *S. litura* and provide respite from Bt proteins, and the effects of environmental stresses, which are common in tropical farming systems, on the efficacy of BG3 cotton against *S. litura*. This information will help better understand the circumstances under which *S. litura* larvae survive in BG3 cotton fields in tropical northern Australia.

Acknowledgements

Thanks to Cotton Seed Distributors for providing the cotton seed used in the study. Thanks to the cotton growers in Kununurra, Western Australia for providing access to their fields for the commercial field experiments. Thanks to the Kaylie Simpfendorfer, Nicole Bell, Gerard Morgan, Shayne Cullimore, and Kim Saligari from WA DPIRD (Frank Wise Institute of Tropical Agriculture) for their assistance in undertaking farming operations associated with the research site and helping to collect leaf material. Thanks to Clayton Forknall (formerly DPI) and Kerry Bell (DPI) for advice and assistance with statistical analysis.

Author Contributions

Sharna Holman (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [equal], Writing—original draft [lead], Writing—review & editing [equal]), Paul Grundy (Conceptualization [equal], Funding acquisition [lead], Methodology [equal], Writing—review & editing [equal]), Helen Spafford (Conceptualization [equal], Methodology [equal], Writing—review & editing [equal]), and Michael Furlong (Conceptualization [equal], Methodology [equal], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Journal of Economic Entomology* online.

Funding

This research was funded by the Cotton Research and Development Corporation (DAQ2201).

Conflicts of Interest

None declared.

References

Adamczyk J, Adams L, Hardee D. 2001a. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *J. Econ. Entomol.* 94:1589–1593. https://doi.org/10.1603/0022-0493-94.6.1589.

Adamczyk JJ, Jr., Hardee DD, Adams LC, et al. 2001b. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94:284–290. https://doi.org/10.1603/0022-0493-94.1.284.

Adnan SM, Cattermole H, Saligari K, et al. 2024. Pastoral grasses and legumes as potential host plants for fall armyworm *Spodoptera frugiperda* (J.E. Smith) development. *Int. J. Trop. Insect Sci.* 44:2339–2346. https://doi.org/10.1007/s42690-024-01331-4.

Ahmad M, Ghaffar A, Rafiq M. 2013. Host plants of leaf worm, *Spodoptera litura* (Fabricius)(Lepidoptera: Noctuidae) in Pakistan. *Asian J. Agric. Biol* 1:23–28.

Ashfaq M, Young SY, McNew RW. 2001. Larval mortality and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) reared on a transgenic *Bacillus thuringiensis* cotton cultivar expressing CryIAc insecticidal protein. *J. Econ. Entomol.* 94:1053–1058. https://doi.org/10.1603/0022-0493-94.5.1053.

Bahar MH, Stanley J, Backhouse D, et al. 2019. Survival of *Helicoverpa armigera* larvae on and Bt toxin expression in various parts of transgenic Bt cotton (Bollgard II) plants. *Entomol. Exp. Appl.* 167:415–423. https://doi.org/10.1111/eea.12792.

Bange M, Nunn C, Mahan J, et al. 2022. Improving temperature-based predictions of the timing of flowering in cotton. *Agron. J.* 114:2728–2742. https://doi.org/10.1002/agj2.21086

Bernardi O, Sorgatto RJ, Barbosa AD, et al. 2014. Low susceptibility of *Spodoptera cosmioides*, *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically-modified soybean expressing Cry1Ac protein. *Crop Prot.* 58:33–40. https://doi.org/10.1016/j.cropro.2014.01.001.

Bhati SS. 2020. Management of *Spodoptera litura* (Fab.) in green gram (*Vigna radiata* L.) through entomo-pathogenic nematode. In:

Soundararajan RP, Narayanasamy C, editors. *Trends in Integrated Insect Pest Management*. IntechOpen. https://doi.org/10.5772/intechopen.85645

Binning RR, Coats J, Kong X, et al. 2014. Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management. *J. Econ. Entomol.* 107:368–374. https://doi.org/10.1603/EC13352.

Bird LJ, Drynan L. 2023. Comparison of insecticide toxicity in adult and larval stages of *Spodoptera frugiperda* (JE Smith) and *Helicoverpa armigera* (Hünber) (Lepidoptera: Noctuidae). *Crop Prot.* 166:106185. https://doi.org/10.1016/j.cropro.2023.106185.

Bommireddy P, Leonard B. 2008. Survivorship of *Helicoverpa zea* and *Heliothis virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. *J. Econ. Entomol.* 101:1244–1252. https://doi.org/10.1093/jee/101.4.1244.

Castagnola A, Jurat-Fuentes JL. 2016. Intestinal regeneration as an insect resistance mechanism to entomopathogenic bacteria. *Curr. Opin. Insect Sci.* 15:104–110. https://doi.org/10.1016/j.cois.2016.04.008.

Chen D, Ye G, Yang C, et al. 2005. The effect of high temperature on the insecticidal properties of Bt cotton. *Environ. Exp. Bot.* 53:333–342. https://doi.org/10.1016/j.envexpbot.2004.04.004.

Dong H, Li W. 2007. Variability of endotoxin expression in Bt transgenic cotton. *J. Agron. Crop Sci.* 193:21–29. https://doi.org/10.1111/j.1439-037X.2006.00240.x.

Downes S, Parker TL, Mahon RJ. 2009. Frequency of alleles conferring resistance to the *Bacillus thuringiensis* toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa punctigera* (Lepidoptera: Noctuidae) from 2002 to 2006. *J. Econ. Entomol.* 102:733–742. https://doi.org/10.1603/029.102.0234.

EFSA Panel on Plant Health, Bragard C, Dehnen-Schmutz K, et al. 2019. Pest categorisation of *Spodoptera litura*. *EFSA J.* 17:e05765. https://doi.org/10.2903/j.efsa.2019.5765.

EPPO. 2015. PM 7/124 (1) *Spodoptera littoralis*, *Spodoptera litura*, *Spodoptera frugiperda*, *Spodoptera eridania*. *EPPO Bull.* 45:410–444. https://doi.org/10.1111/epp.12258.

Fitt G, Daly J, Mares C, et al. 1998. Changing efficacy of transgenic Bt cotton - patterns and consequences. *Pest Manag. Future Conseq.* 1:189–196.

Fortuna C, Godoy D, Reis A, et al. 2024. Survival and development of *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae) on *Conyza bonariensis* L. and *Amaranthus viridis* L. *J. Plant Dis. Prot.* 131:465–470. https://doi.org/10.1007/s41348-023-00859-w.

Girón-Calva PS, Twyman RM, Albajes R, et al. 2020. The impact of environmental stress on Bt crop performance. *Trends Plant Sci.* 25:264–278. https://doi.org/10.1016/j.tplants.2019.12.019.

Gore J, Leonard BR, Adamczyk JJ. 2001. Bollworm (Lepidoptera: Noctuidae) survival on 'Bollgard' and 'Bollgard II' cotton flower bud and flower components. *J. Econ. Entomol.* 94:1445–1451. https://doi.org/10.1603/0022-0493-94.6.1445.

Greenberg S, Li Y, Liu T. 2010. Effect of age of transgenic cotton on mortality of lepidopteran larvae. *Southwest. Entomol.* 35:261–268. https://doi.org/10.3958/059.035.0305.

Holman S, Grundy P, Spafford H, et al. 2025. Lethal and sublethal effects of cotton expressing single and pyramided proteins of Bacillus thuringiensis (Bt) on Helicoverpa armigera (Lepidoptera: Noctuidae), Spodoptera litura (Lepidoptera: Noctuidae), and Spodoptera frugiperda (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 118:1702–1710. https://doi.org/10.1093/jee/toaf089.

Holman S, Grundy P, Spafford H, et al. 2026. Movement and survival of Spodoptera litura (Lepidoptera: Noctuidae) neonate larvae on cotton expressing Bacillus thuringiensis proteins. *Entomol. Exp. Appl.* 174:549–558. https://doi.org/10.1111/eea.70086.

Knight K, Head G, Rogers J. 2013. Season-long expression of Cry1Ac and Cry2Ab proteins in Bollgard II cotton in Australia. *Crop Prot.* 44:50–58. https://doi.org/10.1016/j.cropro.2012.10.014.

Kranthi KR, Naidu S, Dhawad C, et al. 2005. Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). *Curr. Sci.* 291–298.

Kumar G, Prasad BR. 2016. Impact of Bt cotton expressing Cry1ac and Cry2ab on cotton leaf worm, *Spodoptera litura* (F.). *Ind. J. Entomol.* 78:239–245. https://doi.org/10.5958/0974-8172.2016.00065.1.

Li K, Yu S, Yang Y, et al. 2023. Mechanisms of feeding cessation in *Helicoverpa armigera* larvae exposed to *Bacillus thuringiensis* Cry1Ac toxin. *Pestic. Biochem. Physiol.* 195:105565. https://doi.org/10.1016/j.pestbp.2023.105565.

Li Z, Guan X, Michaud JP, et al. 2016. Quercetin interacts with Cry1Ac protein to affect larval growth and survival of *Helicoverpa armigera*. *Pest Manag. Sci.* 72:1359–1365. https://doi.org/10.1002/ps.4160.

Llewellyn DJ, Mares CL, Fitt GP. 2007. Field performance and seasonal changes in the efficacy against *Helicoverpa armigera* (Hübner) of transgenic cotton expressing the insecticidal protein Vip3A. *Agri. Forest Entomol.* 9:93–101. https://doi.org/10.1111/j.1461-9563.2007.00332.x.

McCullough ML. 2008. Environmental stress effects on the expression and efficacy of Bt toxins in WideStrike (R) and Bollgard II (R) brands of cotton [Masters of Biotechnology]. California State University.

Michael P, Woods W. 1980. Entomological review of cotton growing in the Ord River Area of Western Australia. Technical Bulletin: Western Australian Department of Agriculture.

Miraldo LL, Bernardi O, Horikoshi RJ, et al. 2016. Functional dominance of different aged larvae of Bt-resistant *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on transgenic maize expressing Vip3Aa20 protein. *Crop Prot.* 88:65–71. https://doi.org/10.1016/j.cropro.2016.06.004.

Mitra S, Firake D, Umesh K, et al. 2021. Polyphagous caterpillars of *Spodoptera litura* switch from a trap crop to the main crop, improve fitness, and shorten generation time. *J. Pest Sci.* 94:1091–1103. https://doi.org/10.1007/s10340-021-01351-w.

Moraes T, da Silva AF, Leite NA, et al. 2020. Survival and development of fall armyworm (Lepidoptera: Noctuidae) in weeds during the off-season. *Fla. Entomol.* 103:288–292. https://doi.org/10.1653/024.103.0221

Office of the Gene Technology Regulator. 2014. Risk assessment and risk management plan for DIR 124: commercial release of cotton genetically modified for insect resistance and herbicide tolerance. https://www.ogtr.gov.au/sites/default/files/2021-06/dir124-full_risk_assessment_and_risk_management_plan.pdf

Olsen K, Daly J. 2000. Plant-toxin interactions in transgenic Bt cotton and their effect on mortality of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 93:1293–1299. https://doi.org/10.1603/0022-0493-93.4.1293.

Olsen KM, Daly JC, Finnegan EJ, et al. 2005b. Changes in Cry1Ac Bt transgenic cotton in response to two environmental factors: temperature and insect damage. *J. Econ. Entomol.* 98:1382–1390. https://doi.org/10.1603/0022-0493-98.4.1382.

Olsen KM, Daly JC, Holt HE, et al. 2005a. Season-long variation in expression of Cry1Ac gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 98:1007–1017. https://doi.org/10.1603/0022-0493-98.3.1007.

Olsen KM, Daly JC, Tanner GJ. 1998. The effect of cotton condensed tannin on the efficacy of the Cry1Ac δ-endotoxin of *Bacillus thuringiensis*. 9th Australian Cotton Conference; 12–14 August 1998; Wee Waa, Australia.

Pinos D, Andrés-Garrido A, Ferré J, et al. 2021. Response mechanisms of invertebrates to Bacillus thuringiensis and its pesticidal proteins. *Microbiol. Mol. Biol. Rev.* 85:32. https://doi.org/10.1128/mmbr.00007-20.

Praulins G, McDermott DP, Spiers A, et al. 2022. Reviewing the WHO tube bioassay methodology: accurate method reporting and numbers of mosquitoes are key to producing robust results. *Insects* 13:544. https://doi.org/10.3390/insects13060544.

Puja R. 2007. Biology of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on some of its major weed hosts. *Entomon* 32:287–290.

Püntener W. 1981. *Manual for field trials in plant protection*. Ciba-Geigy.
Pyke B. 2003. The performance of Bt transgenic (INGARD®) cotton in Australia over six seasons. World Cotton Conference: cotton production for the new millennium; 9–13 March 2003; Cape Town, South Africa.
Richards K. 1964. Insect pests of cotton in the Ord River Irrigation Area. *J. Dep. Agric. West. Aust.* 5:79–86.
Sorgatto RJ, Bernardi O, Omoto C. 2015. Survival and development of *Spodoptera frugiperda* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) on Bt cotton and implications for resistance management strategies in Brazil. *Environ. Entomol.* 44:186–192. https://doi.org/10.1093/ee/nvu018.
Srivastava K, Sharma D, Anal A, et al. 2018. Integrated Management of *Spodoptera litura*: a review. *Int. J. Life-Sci. Sci. Res.* 4:1536–1538.
Stewart SD, Adamczyk , JJJr., Knighten KS, et al. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of Noctuid (Lepidoptera) larvae. *J. Econ. Entomol.* 94:752–760. https://doi.org/10.1603/0022-0493-94.3.752.
Storer NP, Kubiszak ME, King JE, et al. 2012. Status of resistance to Bt maize in Spodoptera frugiperda: lessons from Puerto Rico. *J. Invertebr. Pathol.* 110:294–300. https://doi.org/10.1016/j.jip.2012.04.007.
Tabashnik BE, Brévault T, Carrière Y. 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* 31:510–521. https://doi.org/10.1038/nbt.2597.
VSN International. 2024. Genstat reference manual (Release 24), part 1 summary.
Wan P, Zhang Y, Wu K, et al. 2005. Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for Bt cotton in the Yangtze River valley of China. *J. Econ. Entomol.* 98:195–201. https://doi.org/10.1093/jee/98.1.195.
Wang S, Zhang M, Huang J, et al. 2021. Inductive and synergistic interactions between plant allelochemical flavone and Bt toxin Cry1Ac in *Helicoverpa armigera*. *Insect Sci.* 28:1756–1765. https://doi.org/10.1111/1744-7917.12897.
Whitburn G, Downes S. 2009. Surviving Helicoverpa larvae in Bollgard II: survey results. *The Aust. Cottongrower Mag.* 30:12–16.
Xue M, Pang Y, Wang H, et al. 2010. Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. *J. Insect Sci.* 10:22. https://doi.org/10.1673/031.010.2201.
Yeates SJ, Strickland GR, Grundy PR. 2013. Can sustainable cotton production systems be developed for tropical northern Australia? *Crop Pasture Sci.* 64:1127–1140. https://doi.org/10.1071/CP13220.
Yu H, Li Y, Li X, et al. 2013. Expression of Cry1Ac in transgenic Bt soybean lines and their efficiency in controlling lepidopteran pests. *Pest Manag. Sci.* 69:1326–1333. https://doi.org/10.1002/ps.3508.

 Journal of Economic Entomology 2026 00(00) 1–11
https://doi.org/10.1093/jee/toag187
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