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The Efficacy of a *cry1Ac9* Gene Conferring Insect Resistance Is Influenced by the Target Plant Species

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ABSTRACT

The efficacy of *cry* genes in different plant species is critical for the design and implementation of stewardship plans targeting resistance to polyphagous insects in multiple crops. Transgenic plants of three solanaceous species: *Nicotiana tabacum* L. (tobacco), *Solanum aethiopicum* L. (scarlet eggplant), and *Solanum tuberosum* L. (potato) were therefore transformed using the same binary vector with a *cry1Ac9* gene and assessed in bioassays against the same population of potato tuber moth (PTM; *Phthorimaea operculella*). The insect resistance conferred by the *cry1Ac9* gene differed markedly among the three plant species. The *cry1Ac9* gene was most effective in scarlet eggplant, with all four transgenic lines exhibiting high mortality of neonate PTM larvae (60%–100% mortality) and very low mean larval growth index. All 24 transgenic lines of tobacco produced significantly reduced mean larval growth indices, with 50% of the lines exhibiting 7%–33% mortality. Among the 119 transgenic potato lines from six cultivars the mean larval growth indices were substantially higher, with only 85% exhibiting significant growth inhibition. No larval mortality was observed on the transgenic potato lines. The findings have important implications for managing polyphagous insect pests when the same *cry* gene is deployed in multiple crops.

1 | Introduction

Insect-resistant transgenic crops expressing δ -endotoxin genes from *Bacillus thuringiensis* (*cry* genes) have made a significant beneficial impact on global agriculture by providing highly effective insect control and significantly reducing application of chemical pesticides (Gatehouse et al. 2011; Khabbazi et al. 2024). It is widely recognised that the deployment of transgenic crops with *cry* genes must be carefully implemented to avoid insects overcoming the resistance conferred by *cry* genes (Jurat-Fuentes et al. 2021; Gassmann and Reisig 2023). To increase the usefulness and effectiveness of insect-resistant transgenic crops, it is important to implement resistance management strategies simultaneously with the release of such crops. Appropriate stewardship plans can be further enhanced by incorporating the management strategy into a wider framework of integrated pest

management. This exposes the pest to a range of mortality mechanisms, which reduces the selection pressure on the most important mortality factor (Bates et al. 2005; Gassmann and Reisig 2023).

Insects commonly inflict damage on more than one crop and the deployment of the same *cry* gene in different crops adds additional complexity for the management of the technology during the deployment of *cry* genes (Xiao and Wu 2019; Gassmann and Reisig 2023). For example, the *cry1Ac* gene from the *Bacillus thuringiensis* Kurstaki strain HD73 has been commercially released for cultivation in maize, rice, sugarcane, cotton, eggplant, tomato, poplar, and soybean to control various lepidopteran pests (ISAAA 2025). For such polyphagous insects, it is therefore important to understand if the response to *cry* genes is equally effective in different transgenic crops.

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One example of a polyphagous insect is potato tuber moth (PTM, *Phthorimaea operculella* (Zeller)), with a known host range involving over 60 plant species, primarily in the Solanaceae family (Das and Rahman 1994). In this study we report a differing response of PTM in three solanaceous species (*Nicotiana tabacum*, *Solanum aethiopicum*, and *Solanum tuberosum*) transgenic for the same *cry1Ac9* gene. To ensure a valid comparison, the three plant species were transformed with the same vector in the same *Agrobacterium* strain and used the same *P. operculella* population for bioassays. The findings have important implications for the management of polyphagous insect pests when the same *cry* gene is deployed in multiple crops.

2 | Materials and Methods

2.1 | Plant Transformation

Agrobacterium tumefaciens strain LBA4404 (Hoekema et al. 1983) harbouring the binary vector pART27 $cry1Ac9^B$, with a codon-optimised *cry* gene under the control of a 35S promoter (Beuning et al. 2001), was incubated overnight in Luria-Bertani medium plus 300 mg·L⁻¹ spectinomycin at 23°C on a shaking table. Explants from in vitro plants of each species were excised, then dipped in the *Agrobacterium* suspension for approximately 30 s. They were then blotted dry on sterile filter paper (Whatman No. 1, 100 mm diameter) and cultured as described below.

Tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) plants were grown and transformed using the leaf disc transformation procedure (Horsch et al. 1985). Scarlet eggplant (*Solanum aethiopicum* L.) ecotype 'Cuzi' was grown *in vitro* and cotyledon explants (2 days after germination) were co-cultivated with *Agrobacterium* and cultured on MS salts, vitamins and sucrose, supplemented with 2 mg·L⁻¹ 6-benzylaminopurine and 0.5 mg·L⁻¹ indole-3-acetic acid (Zhang and Conner 2008). Potato (*Solanum tuberosum* L.) cultivars were transformed using leaf segments as previously described (Davidson, Takla et al. 2004). During co-cultivation with *Agrobacterium* and shoot regeneration the cultures were incubated at 26°C under cool white fluorescent lamps (30–40 μmol m⁻² per s⁻¹; 16 h photoperiod). The light level was raised to 80–100 μmol m⁻² per s⁻¹ after shoot regeneration.

Selection for regenerated transformed shoots was achieved with 50–100 mg·L⁻¹ kanamycin for potato (Davidson, Takla et al. 2004) and 100 mg·L⁻¹ kanamycin for scarlet eggplant and tobacco. Overgrowth of the *Agrobacterium* was controlled by the addition of 200 mg·L⁻¹ Timentin. To ensure independent transformation events, no more than one shoot was harvested from each explant. For all three species, excised shoots that rooted in the presence of 50 mg·L⁻¹ kanamycin were considered as putative transgenic plants.

2.2 | Screening of Putative Transformed Lines Using PCR

Genomic DNA was isolated from the first shoots regenerated from transformation events and from control non-transgenic plants based on the previously described method (Bernatzky and Tanksley 1986). DNA was amplified in a polymerase chain reaction (PCR) containing primers specific for each transgene in a multiplexed reaction. For the *cry1Ac9* gene the primers (5' to 3')

were GCCACAGAATAACAACGTGC (forward primer) and GCATACCGTACACGAACTCG (reverse primer) which produce an expected amplicon of 359 bp. The primers for the *nptII* gene (5' to 3') were ATGACTGGGCACAACAGACAATCGGCTGCT (forward primer) and CGGGTAGCCAACGCTATGTCCTGATA GCGG (reverse primer) which produce an expected amplicon of 612 bp. PCRs were carried out in a Mastercycler (Eppendorf, Hamburg, Germany). The reactions included 2.5 μl 10× buffer (750 mM Tris-HCl (pH 8.8), 200 mM (NH₄)₂SO₄, 0.1% (v/v) Tween 20), 1.5 μl 25 mM MgCl₂, 2.5 μl dNTP (at 2 mM each of dATP, dCTP, dGTP, dTTP), 0.25 μl Red Hot DNA polymerase at 5U per μl (Advanced Biotechnologies, Surrey, U.K.), 0.5 μl of each primer (at 10 μM), 1.0 μl of DNA (10–50 ng) and water to a total volume of 25 μl. The conditions for PCR were: 1 min at 93°C, followed by 35 cycles of 30 s 92°C, 30 s 60°C, 90 s 72°C, followed by a 6 min extension at 72°C. Amplified products were separated by electrophoresis in a TAE-buffered 2% agarose gel and visualised under UV light after staining with ethidium bromide.

2.3 | Southern Analysis

Digestions of genomic DNA were performed using *Hind*III (NEB, Beverly, M.A.) at 5 U per μg DNA. *Hind*III restricts once within the T-DNA of pART27 $cry1Ac9^B$. Approximately 10 μg of restricted DNA was loaded per lane on a TAE-buffered 0.8% agarose gel. Following separation of fragments, the DNA was transferred to Hybond-N+ membrane (Amersham, Little Chalfont, U.K.). Hybridisation was performed using a fragment from the *cry* gene that was radiolabelled using the Megaprime DNA labelling system (Amersham, Little Chalfont, U.K.). Membranes were placed on Kodak X-Omat X-ray film (Eastman Kodak Company, Rochester, N.Y.) for 1–5 days.

2.4 | Enzyme-Linked Immunosorbent Assays (ELISA)

A double-antibody sandwich quantitative enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of the Cry protein in leaf tissue of non-transgenic and transgenic plants. This involved the Cry1Ab/Cry1Ac Microwell Plate Kit (EnviroLogix Inc., Portland, Maine, United States), with Cry1Ac calibrators of 1.5, 10, and 25 μg·L⁻¹. The detection limit was 1.2 μg·L⁻¹ for Cry1Ac in extracts from samples. A ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA, United States) at 450 nm was used to determine spectrophotometric measurements. The levels of Cry1Ac protein in the leaf extracts were determined by extrapolation to a standard curve based on sample and standard absorbance values.

2.5 | Bioassays against PTM Larvae

Young, fully expanded leaves from transgenic and non-transgenic control lines were excised from plants grown in the greenhouse for 4 to 6 weeks for tobacco and 8 to 10 weeks for scarlet eggplant and potato. This ensured bioassays were conducted on plants of similar size and development stage for each species. The insects used in the bioassays were obtained from a laboratory colony maintained as previously described (Davidson et al. 2002). Excised leaf material was placed on top of dry filter paper (Whatman, No. 1, 50 mm diameter) in a 350 ml plastic container with a plastic screw lid.

Five neonate PTM larvae were collectively weighed and placed on the leaf material for each of three replicate plants for each independently transformed line. The containers were placed in a controlled temperature room at $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and under a photoperiod of 16 h light and 8 h dark. Surviving larvae were transferred to fresh leaf material after 4 days. After a total of 9 days surviving larvae were removed and weighed individually. A growth index (GI) for each larva was calculated as $\text{GI} = \log_e(\text{final weight}/\text{mean initial weight})$. For tobacco and potato, the bioassays were conducted in batches, each with a non-transgenic control and up to eight transgenic lines.

2.6 | Statistical Analysis

The data generated were analysed using ANOVA to explore treatment effects on the GI of PTM larvae. For each plant line tested, the GI data involving the five larvae from each of three replicate plants were treated as three biological replicates.

3 | Results

3.1 | Plant Transformation and Molecular Characterisation

Putative transgenic plants that developed roots in the presence of $50 \text{ mg} \cdot \text{L}^{-1}$ kanamycin were assessed by PCR to confirm their transgenic status for the *nptII* gene conferring kanamycin resistance and the *cry1Ac9* gene. This is illustrated for representative tobacco lines in Figure 1. Further molecular characterisation of the transgenic scarlet eggplant lines via Southern analysis established two intact insertions of the *cry* gene in line X1 and single intact insertions in the lines Y2, Y3, and Y4 (Figure 2). The different sizes of the hybridised bands in each line confirmed that the insertion events were at different loci and the lines were therefore of independent origin. The amount of Cry protein determined by ELISA in the transgenic plant lines was $<60 \text{ ng} \cdot \text{g}^{-1}$ of fresh leaf tissue in all lines tested. This approximates the detection limit of the ELISA method used.

For bioassays against PTM larvae, transgenic lines were identified with both the *nptII* gene and the *cry1Ac9* gene. This included

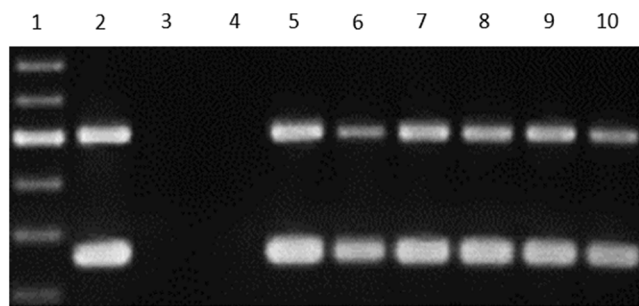


FIGURE 1 | PCR analysis of putative transgenic tobacco (*Nicotiana tabacum*) cv. Petit Havana SR1 lines. The multiplexed reaction used the *cry1Ac9* primers, producing an expected 359 bp amplicon and the *nptII* primers, producing an expected 612 bp amplicon. Lane 1, 100 bp molecular ruler 10380-012 (Invitrogen, Carlsbad, California) size marker; lane 2, binary vector pART27*cry1Ac9*^B control; lane 3, no DNA template control; lane 4, non-transgenic Petit Havana SR1 control; lanes 5–10, representative transgenic lines (Nt01, Nt06, Nt11, Nt16, Nt21, Nt24).

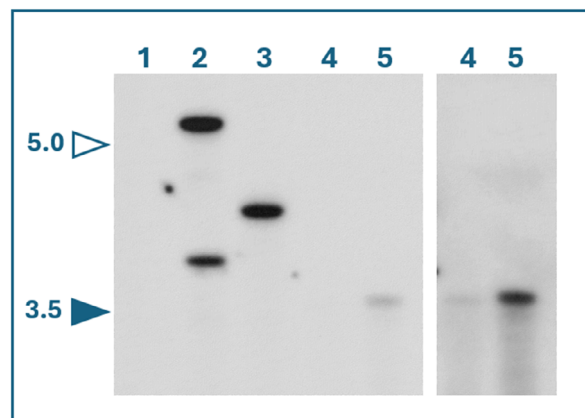


FIGURE 2 | Southern analysis of four lines of transgenic scarlet eggplant (*Solanum aethiopicum* L.) ecotype 'Cuzi'. DNA was restricted with *Hin*DIII and probed with the *cry* gene. A single band greater than 3.69 kb is expected for each intact insertion of the *cry* gene. The arrowheads point to the position of 5.0 kb (open) and 3.5 kb (solid) size markers respectively. Lane 1, non-transgenic 'Cuzi' control; lanes 2–5, 'Cuzi' lines X1 (two bands at approximately 4.5 and 8 kb), Y2 (one band at approximately 5.5 kb), Y3 (one very faint band at about 3.7 kb), and Y4 (one band at 4 kb). The right panel with a repeat of lanes 4 and 5 represents a more intense exposure of the autoradiograph so that the band in lane 4 is visible.

24 transgenic lines of tobacco (labelled Nt01 to Nt24) and four transgenic lines of scarlet eggplant (labelled X1, Y2, Y3 and Y4). The transgenic potato lines summarised in this study were derived from our previous studies (Davidson et al. 2002; Davidson, Takla et al. 2004), with the original data extracted from Davidson (2001) and Takla (2001). This involved data from 119 transgenic lines representing six potato cultivars with at least four transgenic lines per cultivar (40 from Ilam Hardy, 38 from Iwa, 11 from Karaka, 4 from Pacific, 17 from Red Rascal, and 9 from Russet Burbank).

3.2 | Tobacco Bioassays against PTM Larvae

The tobacco bioassays were conducted in a series of three batches, each with a non-transgenic control. ANOVA established no batch effects ($P > 0.05$) therefore data were pooled for subsequent analysis. Upon challenging the transgenic tobacco lines with neonate larvae of PTM, all 24 lines exhibited significantly reduced larval growth indices (Figure 3). The independently derived transgenic lines varied markedly in their efficacy of insect resistance, with the larval growth indices ranging from 0.4 to 3.7, compared to 4.1–4.2 for the non-transgenic controls. Some lines were highly resistant and only supported very low larval growth indices (Nt05, Nt09, Nt10, Nt16, Nt20), with these lines also exhibiting higher frequencies of larval mortality (7%–33%) (Figure 3).

3.3 | Scarlet Eggplant Bioassays against PTM Larvae

All the transgenic lines of scarlet eggplant exhibited a high level of resistance to neonate larvae of PTM (Figure 4). The larval growth indices on these lines ranged from 0.0 to 0.4, compared to 4.1 for the non-transgenic controls. The frequency of larval mortality on the transgenic lines was 60%–100% compared to only 13% for the non-transgenic controls (Figure 4).

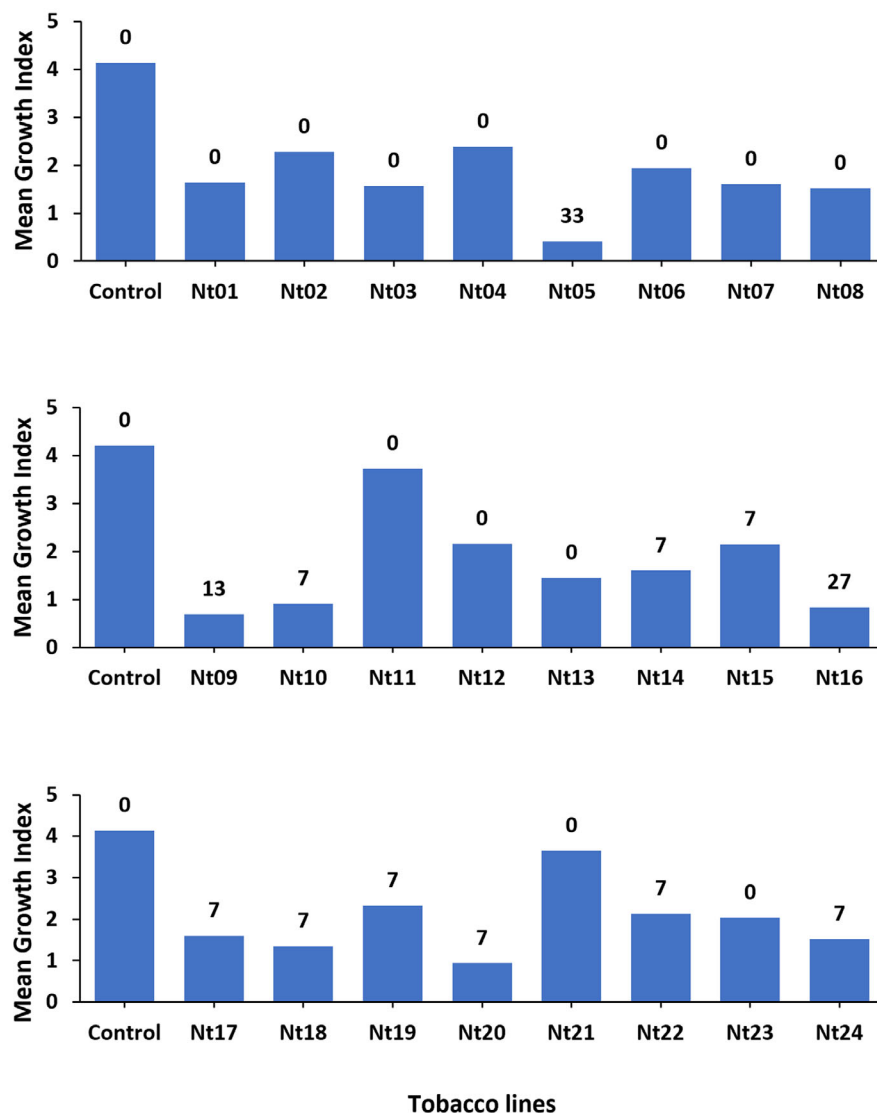


FIGURE 3 | The mean growth indices of potato tuber moth (PTM) larvae reared on leaves of tobacco (*Nicotiana tabacum*) cv. Petit Havana SR1 plants grown in a greenhouse. Growth index (GI) is calculated as $GI = \log_e(\text{final weight}/\text{mean initial weight})$. Analysis of variance established a highly significant difference among tobacco lines for the growth of PTM larvae ($F = 141$; d.f. = 26,54; $P < 0.001$). Least significant differences at the 5%, 1% and 0.1% probability levels are 0.26, 0.34 and 0.42, respectively. The number above the blue bar for each tobacco line represents the % mortality for PTM larvae feeding on that line.

3.4 | Potato Bioassays against PTM Larvae

A summary of the bioassay data from 119 lines of potato transgenic for the *cry1Ac9* gene encompassing six cultivars is presented in Table 1. Similar results were observed in all six potato cultivars. Growth indices of the PTM neonate larvae ranged from 2.1 to 6.5 on the transgenic potato lines, with only 85% of the transgenic lines exhibiting significantly lower growth indices. No larval mortality on the transgenic potato lines attributable to the *cry* gene expression was observed (Table 1).

3.5 | Bioassays of Non-Transgenic Species against PTM Larvae

The susceptibility of the non-transgenic plants of three solanaceous species to PTM larvae was compared to help understand the differing responses to *cry1Ac9* transgene expression (Figure 5).

The growth indices of neonate larvae on the six potato cultivars were similar and ranged from 6.1 to 6.3, with no mortality observed. In contrast, both tobacco and scarlet eggplant support growth indices of 4.1–4.2. While no mortality was observed for PTM larvae raised on tobacco leaves, a mortality of 20% was observed for scarlet eggplant (Figure 5). Since a log scale is used for calculating the mean growth indices, the difference between potato and the other two solanaceous species is quite substantial.

4 | Discussion

The implementation of stewardship plans with the deployment of transgenic crops expressing *cry* genes has been critical to minimise the opportunity of insects overcoming the resistance conferred by *cry* genes (Bates et al. 2005; Gassmann and Reisig 2023). Various strategies can be considered for managing such resistance in transgenic crops (McGaughey and Whalon 1992; Conner and

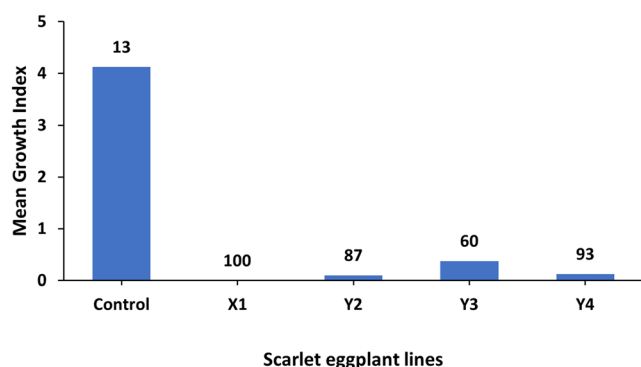


FIGURE 4 | The mean growth indices of PTM larvae reared on leaves of scarlet eggplant (*Solanum aethiopicum* L.) ecotype ‘Cuzi’ plants grown in a greenhouse. GI is calculated as $GI = \log_e(\text{final weight}/\text{mean initial weight})$. Analysis of variance established a highly significant difference among scarlet eggplant lines for the growth of PTM larvae ($F = 34.5$; d.f. = 4,10; $P < 0.001$). Least significant differences at the 5%, 1% and 0.1% probability levels are 0.96, 1.36 and 1.78, respectively. The number above the blue bar for each scarlet eggplant line represents the % mortality for PTM larvae feeding on that line.

Christey 1994). The predominant approach has involved the combination of a consistent and high expression level of the *cry* gene with the deployment of a refuge (Gould 2000). This has subsequently developed into patterns of pyramiding and/or stacking multiple *cry* genes with high expression, coupled with refuges (Gassmann and Reisig 2023). The purpose of the refuge is to maintain a population of the target insects with susceptible alleles to the Cry proteins and is enhanced by resistance alleles in insects being conferred by recessive mutations (Tabashnik and Carrière 2017). Polyphagous insects feed on a wide range of plant species and the use of the same *cry* gene in different crop species to target such insects poses additional complexity for the design and implementation of stewardship plans (Xiao and Wu 2019; Gassmann and Reisig 2023). It is therefore important to understand the efficacy of *cry* genes for conferring insect resistance in different plant species. This study reports the response of a PTM population to three

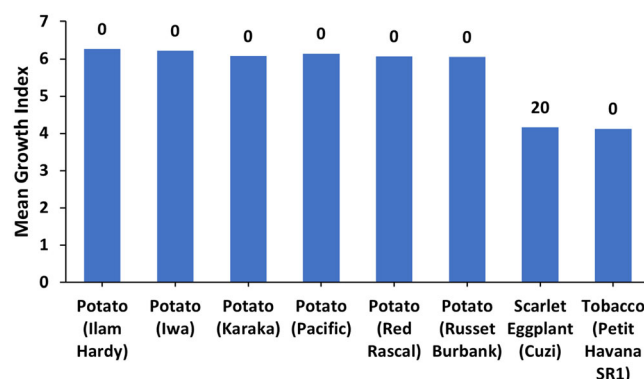


FIGURE 5 | The mean growth indices of PTM larvae reared on leaves of non-transgenic solanaceous plants grown in a greenhouse. GI is calculated as $GI = \log_e(\text{final weight}/\text{mean initial weight})$. Analysis of variance established a highly significant difference between plants of different treatments for the growth of PTM larvae ($F = 53.3$; d.f. = 7,15; $P < 0.001$). Least significant differences at the 5%, 1% and 0.1% probability levels are 0.46, 0.64 and 0.80, respectively. The number above the blue bar for each plant represents the % mortality for PTM larvae feeding on that line.

solanaceous species transgenic for the same *cry1Ac9* gene, using the same vector in the same *Agrobacterium* strain. The *cry1Ac9* gene had been codon optimised for expression in plants and shown in transgenic tobacco plants to be highly effective against PTM larvae (Beuning et al. 2001).

All the transgenic lines used in this study were confirmed by PCR to be transgenic for the *cry1Ac9* gene (e.g., Figure 1, Davidson et al. 2002; Davidson, Takla et al. 2004). Since we were unaware of any other reports of transformation in scarlet eggplant, we also validated the transgenic status of these plants using Southern analysis (Figure 2). The growth indices of PTM larvae on the transgenic plant lines differed markedly between the three solanaceous species. The growth indices on the transgenic potato lines ranged from 2.1 to 6.5 (Table 1), substantially higher than those observed for either tobacco (Figure 3) or scarlet eggplant (Figure 4). No larval mortality was observed on any of the

TABLE 1 | Summary of resistance to potato tuber moth (PTM) larvae among transgenic lines of potato (*S. tuberosum*) from six cultivars. Analysis of variance established significant differences among transgenic lines and the respective control for each potato cultivar ($P < 0.05$) (Davidson et al. 2002, Davidson, Takla et al. 2004).

Plant species, cultivar	Number of transgenic lines	PTM larval growth index (mean) ^a	PTM larval growth index (range) ^b	Number of lines with larval growth index < 3
<i>S. tuberosum</i> , Ilam Hardy	40	4.66	2.3 to 6.5	6
<i>S. tuberosum</i> , Iwa	38	3.38	2.1 to 4.9	12
<i>S. tuberosum</i> , Karaka	11	3.95	3.5 to 4.7	0
<i>S. tuberosum</i> , Pacific	4	4.38	3.3 to 5.1	0
<i>S. tuberosum</i> , Red Rascal	17	3.53	2.6 to 4.6	4
<i>S. tuberosum</i> , Russet Burbank	9	4.16	2.6 to 5.7	2
All cultivars	119	4.11	2.1 to 6.5	24

^aThe PTM larval growth index on all non-transgenic potato cultivars was over six (see Figure 5). For all potato cultivars, 101 (85%) of the transgenic lines exhibited significantly reduced ($P < 0.05$) larval growth index relative to the non-transgenic control (Davidson et al. 2002; Davidson, Takla et al. 2004).

^bNo mortality of PTM larvae was observed for any of the transgenic potato lines.

transgenic potato lines (Table 1), whereas in tobacco larval mortality ranged from 0% to 33% among the 24 transgenic lines. In scarlet eggplant larval mortality was much higher and ranged from 60% to 100% among the four transgenic lines. The range of larval growth indices and larval mortality obtained for tobacco (*N. tabacum* cv. Petit Havana SR1) (Figure 3) were very similar to the results reported by Beuning et al. (2001) who used the same *cry* gene and binary vector in a different tobacco cultivar (*N. tabacum* cv. Samsun).

In all three solanaceous species there was considerable variation for inhibition of larval GI and mortality among the independently derived transgenic lines with the *cry1Ac9* gene (Figures 3, 4, and Table 1). In potato this included some transgenic lines with no apparent resistance to PTM larvae, whereas other transgenic lines were highly effective (Table 1). This inconsistency in performance among populations of independently derived transgenic plants is commonly reported (Conner and Christey 1994). It can be attributed to unpredictable transgene expression resulting from the random integration of transgenes into different sites in plant genomes and/or differing number of insertion events in each line (Mlynárová et al. 1995).

The results from potato (Table 1), tobacco (Figure 3) and scarlet eggplant (Figure 4) clearly established that the recipient plant species influences the efficacy of the *cry1Ac9* gene in conferring resistance to PTM. Despite potato having the lowest efficacy of the three species, the better performing potato lines exhibited high levels of resistance to PTM when tested in the field (Davidson, Butler et al. 2004; Davidson et al. 2005, 2006). Although PTM larvae can remain alive during the 9-day bioassay, for the potato lines used in this study a larval GI under three generally results in failure to reach pupation (Davidson et al. 2002). This failure to reach maturity for further reproduction therefore provides very effective control of PTM. In this study a mean GI of less than three was observed for 22 of the 24 (92%) transgenic tobacco lines (Figure 3), all four (100%) of the transgenic scarlet eggplant lines (Figure 4), and 24 of 119 (20%) transgenic potato lines (Table 1).

The mean growth indices of PTM larvae were substantially lower on non-transgenic tobacco and scarlet eggplant relative to potato, with scarlet eggplant also inflicting 20% mortality (Figure 5). The higher effectiveness of the *cry1Ac9* gene against PTM larvae in tobacco and scarlet eggplant is a consequence of a synergistic interaction with inherent resistance in these two species that is probably attributable to leaf alkaloids. This concept is supported by potatoes transgenic for a *cry3A* gene being more effective against Colorado potato beetle when coupled with a natural resistance involving foliar leptine accumulation introgressed from *Solanum chacoense* (Cooper et al. 2004). Furthermore, the foliage of different *Solanum* species is known to support markedly different growth rates of PTM larvae, with field surveys finding very few larval mines on the foliage of weedy *Solanum* species growing alongside potato crops with high frequencies of larval mines (Davidson and Conner 2003). This establishes potato as a preferred host plant for PTM and is therefore likely to require higher expression of *cry* genes for effective insect resistance.

This study has established that the plant species into which the *cry1Ac9* gene is introduced influences the efficacy of insect resistance. For polyphagous insects such as PTM, this has important implications for the implementation of management strategies

when the same *cry* gene is deployed in multiple crops. The predominant management strategy for the deployment of *cry* genes has involved the combination of a consistent and high expression level of the *cry* gene with the planting of a refuge (Gould 2000; Gassmann and Reisig 2023). The purpose of the refuge is to maintain a population of the target insects with susceptible alleles to the Cry proteins. Any rare, isolated insect surviving on a plant expressing a *cry* gene due to a mutation for resistance is therefore likely to mate with a susceptible insect from the refuge. Since resistance to *cry* genes in insects is conferred by recessive mutations (Tabashnik and Carrière 2017), any resulting heterozygous progeny will therefore be susceptible. However, exposure to plants with a lower efficacy of *cry* genes may contribute to the survival of the heterozygous progeny, thereby allowing them to interbreed and produce homozygous individuals capable of overcoming the resistance in plants with higher efficacy of the *cry* gene. This is especially relevant for polyphagous insects that can be secondary pests on transgenic crops expressing *cry* genes to target a different insect. While there might be high efficacy against the target insect, there may be lower efficacy against a secondary pest. As a consequence, this can interfere with the management strategy in another crop where that secondary pest is the primary target.

Author Contributions

Jeanne M. E. Jacobs and Anthony J. Conner conceived the concept and designed the experiments. Sathiyamoorthy Meiyalaghan coordinated the transformation and propagation of tobacco and scarlet eggplant. Jeanne M. E. Jacobs and Sathiyamoorthy Meiyalaghan performed molecular analyses. Anthony J. Conner conducted the insect bioassays on scarlet eggplant and tobacco. Jeanne M. E. Jacobs and Anthony J. Conner analysed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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