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Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance

A. Nicholas E. Birch[1,*], Irene E. Geoghegan[1], Michael E.N. Majerus[2], James W. McNicol[3], Christine A. Hackett[3], Angharad M.R. Gatehouse[4] and John A. Gatehouse[4]

[1]*Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK (*author for correspondence; e-mail n.birch@scri.sari.ac.uk);* [2]*Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK;* [3]*Biomathematics and Statistics Scotland, Dundee Unit, Invergowrie, Dundee, DD2 5DA, UK;* [4]*Department of Biological Sciences, University of Durham, Durham DH1 3LE, UK*

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Abstract

Transgenic crops genetically engineered for enhanced insect resistance should be compatible with other components of IPM for the pest resistance to be durable and effective. An experimental potato line was genetically engineered to express an anti-aphid plant protein (snowdrop lectin, GNA), and assessed for possible interactions of the insect resistance gene with a beneficial pest predator. These extended laboratory studies are the first to demonstrate adverse tri-trophic interactions involving a lectin-expressing transgenic crop, a target pest aphid and a beneficial aphidophagous predator. When adult 2-spot ladybirds (*Adalia bipunctata* [L.]) were fed for 12 days on peach-potato aphids (*Myzus persicae* Sulzer) colonising transgenic potatoes expressing GNA in leaves, ladybird fecundity, egg viability and longevity significantly decreased over the following 2–3 weeks. No acute toxicity due to the transgenic plants was observed, although female ladybird longevity was reduced by up to 51%. Adverse effects on ladybird reproduction, caused by eating peach-potato aphids from transgenic potatoes, were reversed after switching ladybirds to feeding on pea aphids from non-transgenic bean plants. These results demonstrate that expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a predatory ladybird via aphids in its food chain. The significance of these potential ecological risks under field conditions need to be further evaluated.

Abbreviations: Bt, *Bacillus thuringiensis*; CaMV, cauliflower mosaic virus; 'control' aphid diet, *M. persicae* aphids reared on non-transgenic potatoes; GNA, *Galanthus nivalis* agglutinin; 'GNA' aphid diet, *M. persicae* reared on GNA-expressing transgenic potatoes; 'optimal' aphid diet, *Acyrthosiphon pisum* reared on non-transgenic beans; IPM, integrated pest management; MP1, *Myzus persicae* laboratory clone 1; SE, standard error; SCRI, Scottish Crop Research Institute; GM, genetically modified

Introduction

Plant-derived defence genes have particular potential in transgenic pest control of target insect species or groups for which *Bacillus thuringiensis* toxin genes are currently unavailable, such as sap-sucking Homoptera including aphids. However, many currently available plant gene products are less acutely toxic and have a broader spectrum of anti-insect activities, compared with insecticidal Bt toxins. Plant lectins are considered to play a role in broad spectrum plant defence against pests and pathogens [1, 12]. Some plant lectins have been shown to be toxic towards insects, including sap-sucking hemipteran species [13, 14, 15]. The snowdrop lectin, *Galanthus nivalis* agglutinin (GNA), is a mannose-binding lectin which is effective against several aphid species both *in vitro* [3, 17]

and when expressed in transgenic tobacco [9, 18] and potato [3, 6], reducing aphid fecundity and retarding development. This type of partial resistance will impact on direct aphid feeding damage and on secondary spread of some aphid-borne viruses, but is unlikely to be sufficient on its own to reduce primary virus spread in potatoes. GNA also affects lepidopteran [5] and coleopteran [7] larvae when fed in artificial diet or delivered via a transgenic plant. Thus GNA expressed in transgenic potato as a means of aphid resistance can also provide some protection against lepidopteran tomato moth larvae [5]. Lectins are therefore considered a possible target for genetic engineering into crops for broader pest resistance. However, this broad-range protection may also have potential drawbacks. The GNA lectin is not strongly aphicidal nor does it prevent aphid feeding at levels expressed in leaves of currently tested transgenic plants. Rather, GNA retards the rate of aphid development and reproduction in subsequent aphid generations [3, 6]. Lectins are also known to bind to insect gut cells, including those of aphids [1, 12] and GNA can be present in aphids after feeding on GNA-expressing transgenic plants [16, 18]. Thus, an anti-pest lectin expressed in leaves of a transgenic plant could enter the food chain of aphidophagous insects, such as ladybirds, raising the potential for adverse effects on non-target beneficial insects if they are sensitive to the lectin. To test this possibility, experiments were set up to compare the performance of ladybirds fed peach-potato aphids reared on transgenic potato plants expressing GNA, to ladybirds fed peach-potato aphids reared on control (non-transgenic) potato plants and to a third control group, reared on pea aphids from non-transgenic *Vicia faba* beans.

Materials and methods

Transgenic plant material, gene construct and expression, insect cultures

GNA-expressing transgenic potato (*Solanum tuberosum* L., cv. Désirée) lines were produced as previously described [5], for experimental purposes only. Plants contained a gene construct where the GNA coding sequence from clone LECGNA2 [20] was expressed using the CaMV 35S promoter. The transgenic potato line GNA2#28 was selected in preliminary experiments at University of Durham and SCRI for the highest levels of resistance to several insect pests, including the peach-potato aphid, *M. persicae*. Expression levels of GNA were estimated as 0.4–0.6% of total protein in leaves of transformed potato plants grown from tissue culture then under glasshouse conditions [5]. Tissue-cultured control (non-transgenic) and GNA-expressing potato plants were grown to produce tubers for field tunnel (controlled release) planting. Assay of tuber-grown plants for the ladybird feeding experiments showed lower leaf GNA expression levels, in the range 0.02–0.1% of total leaf protein. The transgenic plants exhibited partial aphid resistance, reducing aphid numbers by 50% compared with control potato lines grown under identical conditions, as previously demonstrated [6]. 180 each of control and GNA tubers were planted in a randomised block design in four 12 m long tunnels covered in Nicofence aphid-proof mesh. Plants were fertilised and irrigated according to standard regimes. After 8 weeks growth, individually netted potato plants were infested with 100 virus-free adult *M. persicae* (clone *MP1* [22], previously cultured on control plants of potato cv. Désirée). These adult aphids were allowed to reproduce and their nymphs to develop for a further 14 days on the test potato plants, prior to daily sampling for feeding to laboratory-reared ladybirds during phase 1 of the ladybird feeding trial.

Progeny from twelve singly mated isofemale F_1 2-spot ladybirds, *Adalia bipunctata* (L.), collected from Glasgow, UK, were reared under controlled laboratory conditions. Eight males of progeny from each of six families and eight virgin female progeny from each of the other six families were fed separately, on one of three test diets (see below) in phase 1 (12 days) of the feeding trial and then used for replicated matings in six reciprocal crosses. After the first successful mating (>45 min of observed coupling) the individual females were then monitored in phase 2 for a further 28 days, or until natural death. Aphids were sampled daily from the netted control and GNA potatoes in the field tunnels, counted and weighed. Sibling ladybird groups were fed one of three different aphid diets (each offered to excess daily and consumption monitored) during phase 1 of the feeding trial (12 days).

Ladybird diet regimes during feeding trial phase 1

Diet 1: *M. persicae* from control potatoes ('control' aphid diet)

Diet 2: *M. persicae* from GNA potatoes ('GNA' aphid diet)

Diet 3: *Acyrthosiphon pisum* (Harris) from non-transgenic *V. faba* bean plants ('optimal' aphid diet, also used to feed all groups of ladybirds in phase 2, after diet switch).

Ladybird sibling groups fed with diets 1 and 2 in phase 1 were each given 140 mg aliquots (excess to daily requirement) of live *M. persicae* per day and aphid consumption was monitored daily for each ladybird group throughout phase 1. Ladybirds on diet 3 were fed *A. pisum* during phase 1, again offered in excess and changed daily. In phase 2 (day 13 of feeding trial until natural adult death) all ladybirds (regardless of their diet during phase 1) were fed on 'optimal' aphid diet (diet 3), offered in excess to daily requirement. This was done to simulate the change of diet likely to be experienced by actively flying and foraging ladybirds under field (choice) conditions, and also to measure the duration of any transient effect(s) caused by eating aphids from GNA-expressing transgenic potatoes during phase 1 (first 12 days) of the ladybird feeding trial.

Experimental design for ladybird reciprocal crosses

After phase 1, individual males and female ladybirds from each sibling group, previously fed on one of the three tested aphid diets, were mated in a full-sib reciprocal cross design. The following crossing regime and codings were used to partition out effects due to inter-family genetic variance, diet and ladybird sex on several ladybird fitness parameters (see below).
'GNA' diet male × 'optimal' diet female (**cross type 1**) and reciprocal cross (**cross type 2**)
'GNA' diet male × 'control' diet female (**cross type 3**) and reciprocal cross (**cross type 4**)
'Control' diet male × 'optimal' diet female (**cross type 5**) and reciprocal cross (**cross type 6**).
(Replication: cross types 1–4, n=24/cross; cross types 5–6, n=12/cross).

Two of 120 total ladybird matings failed, due to a high prevalence of early death in one isofemale line. These matings were excluded from the analysis. Each of the successfully (>45 min observed coupling) mated 118 female ladybirds were monitored daily until natural death. Males were mated with individual females then removed after each mating session. Matings using individual females were repeated every 7 days for 28 days to ensure maximal egg fertilisation, using males from the same sibling/diet group each time.

Assessment of impact on ladybirds using ecological fitness indicators

Several fitness parameters were monitored for each mated ladybird, to assess the impact of the aphid diet consumed during phase 1 on subsequent adult ladybird ecological fitness in phase 2 (when all ladybirds were switched to optimal diet).
Aphid consumption during phase 1 on initial test diets (monitored daily and analysed as total consumption after 12 days on phase 1 diets).
Egg production/female during phase 2 on 'optimal' diet (monitored daily for 28 days, analysed in weekly blocks for a time-course assessment of any transient effects).
Egg viability/female (fertility and hatch) during phase 2 on 'optimal' diet (monitored daily for 28 days, analysed in weekly blocks for a time-course assessment).
Adult longevity during phases 1 and 2 and until natural death (monitored and recorded daily).

Statistical methods and analysis

Matings producing only 0 or 1 hatched or fertilised eggs in total were ignored, as these were rare (ca. 3% of total matings) and always generated large residuals. Generalised linear models ('glms', Genstat) were used to estimate the effects of the six diet/sex combinations on all ladybird fitness parameters listed above except for aphid consumption (ANOVA, Genstat), considering crosses as complete replications. Egg totals were analysed as normally distributed with identity link, and the numbers of eggs hatched and fertilised as binomially distributed with logit link. Egg production, egg fertility and egg hatch were analysed in weekly blocks over 28 days in phase 2, so that any effects of diet switch (aphids ex GNA potato to pea aphid optimal diet) could be estimated over time, in terms of persistence of any GNA lectin effect in the food chain of ladybirds. The complete change in ladybirds' response pattern to 'GNA' aphid diet detected in phase 2, after two weeks on 'optimal' diet, made it advisable to analyse each week's data separately. Because of the observed change in ladybirds' responses to phase 1 diets over time (in phase 2), the total eggs laid in the 28 day assessment period did not give a meaningful reflection of the impact of 'GNA' aphids consumed

during phase 1, so was not included as a fitness parameter for analysis. Longevity of male and female ladybirds (days to natural death) was analysed using a generalised linear model with log link and Poisson error (glm, Genstat).

Pairwise comparisons between diets were by *t*-tests. The main diet comparisons of interest, selected *a priori*, were those keeping one ladybird sex constant (i.e. on 'optimal' diet), crossed with a partner of the opposite sex fed either the 'control' or 'GNA' aphid diets (i.e. cross 1 compared with cross 5 for effect of 'GNA' diet on males; cross 2 compared with cross 6, for 'GNA' diet effect on females). As the number of ladybird crosses made was small ($n = 6$), comparisons were carried out on the basis of least significant differences (on transformed data), rather than using multiple comparison tests.

Results

Aphid consumption

The weights of aphids eaten by each male or female ladybird dietary group (fed to excess each day) was recorded daily over 12 days, in order to assess whether aphids feeding on GNA-expressing transgenic potatoes were less palatable to ladybirds and so affected food intake during phase 1 of feeding trial. Under the 'no choice' conditions tested, there was no significant difference (ANOVA) between the cumulative weight of aphids eaten during 12 days by ladybirds fed the 'control' aphid diet and those fed 'GNA' aphid diet (diets 1 and 2, Materials and methods). Because aphids reared on GNA potatoes are smaller and less heavy than those on control potatoes [3, 17], more 'GNA' diet aphids (numbers of aphids eaten/adult ladybird) were consumed/ladybird than of 'control' aphids, although the total aphid biomasses of control and GNA aphids consumed did not differ significantly.

Total ladybird eggs laid

After phase 1 (aphids offered excess food daily, of one of the three diet treatments), individual ladybirds were mated with a sibling of the opposite sex, using the reciprocal cross design outlined (Materials and methods). Egg production of individual, mated females from each of the six crosses was monitored daily for a further 28 days during phase 2, fed on excess 'optimal' aphid diet **(Figure 1)**. By the second week of phase 2 there was a significant effect of the initial (phase 1) aphid diet on ladybird fecundity (where diet designation refers to that consumed during phase 1 of the experiment). For example, (means with standard errors for all 6 crosses are shown in Figure 1 for complete comparisons), in week 2 female ladybirds fed 'GNA' diet (cross type 2) produced a mean of 85.9 eggs/week, compared with 104.8 eggs/week for 'control' diet fed females from cross type 6 ($P<0.05$), when both female sets were crossed with 'optimal' diet males. Greater aphid diet effects were seen on female ladybirds in week 3, where 'GNA' aphid diet fed females (cross type 2) produced a mean of 64.1 eggs/week, compared with 100.8 eggs/week for 'control' aphid diet fed females from cross type 6 ($P<0.001$). The effect of GNA aphid diet on male ladybirds was also detrimental to mating (assessed by female fecundity) over the first 2 weeks and in week 4 (Figure 1, crosses 1 and 5). However, in week 3 the effect of 'GNA' aphid diet on male ladybirds used in crosses with 'optimal diet' females was less pronounced and not significant. By week 4 the diet effect on female ladybird fecundity was not statistically significant across the 6 cross types, although the overall trend was similar to that observed in weeks 2 and 3.

Ladybird egg fertility

The fertility of ladybird eggs laid by females from the 6 cross types was also recorded for 28 days in phase 2. For all cross types involving either the male or female ladybird fed on 'GNA' aphid diets in phase 1 (crosses 1–4), mean egg fertility (eggs showing embryonic development, as a percentage of total eggs) was significantly reduced, compared with crosses (types 5–6) involving ladybirds fed 'control' or 'optimal' aphid diets **(Figure 2)**. The effect of 'GNA' aphid diet was most evident during the first week of egg production. Egg fertility was reduced from 95% in crosses involving 'control'- and 'optimal' diet-fed insects (types 5 and 6), to 71–85% for crosses involving insects fed on 'GNA' aphid diet (crosses 1–4). This effect of the 'GNA' diet was significant for both females ($P<0.001$) and males ($P<0.001$) used in crosses. The 'GNA' diet effect on decreased egg fertility was also detected in the second week of phase 2, for both male ($P<0.001$) and female ($P<0.005$) ladybirds fed 'GNA' aphid diet and crossed with 'optimal' aphid diet partners (crosses 1–2), compared with crosses 5 and 6. However, by the third and fourth weeks of phase 2 the effect of 'GNA' diet on egg fertility of crosses 1–4 was not significantly different to that of crosses 5 and 6. This showed

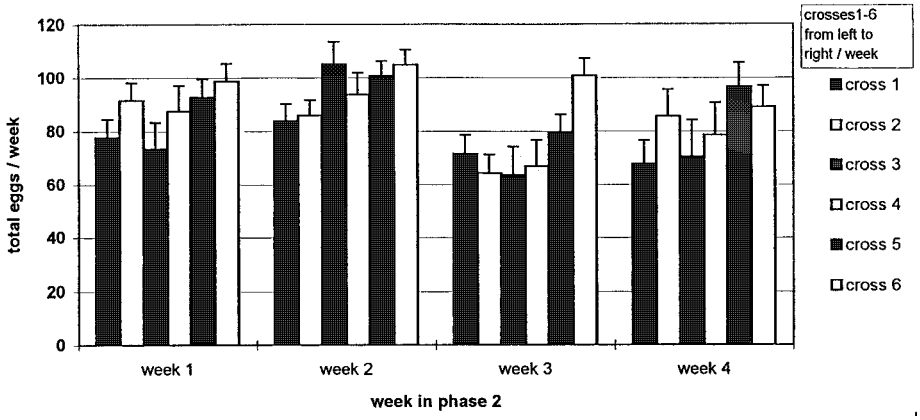

Figure 1. Effect of initial aphid diets (phase 1) on egg production by female ladybirds from the 6 cross types. Cross types 1–4 involve either male or female ladybirds fed initially on GNA aphid diet; crosses 5–6 were fed 'control' or 'optimal' aphid diets. Comparison of crosses 2 and 6 shows the effect on ladybird females of eating aphids from GNA potato ('GNA' diet), when mated with 'optimal' diet males. Comparison of crosses 1 and 5 shows the effect of 'GNA' diet on males when mated with 'optimal' diet females. Columns show mean values for each ladybird cross type, with standard error (SE) bars attached.

a transient effect of initial (phase 1) 'GNA' aphid diet on ladybird egg fertility, which was later reversed after switching to the 'optimal' aphid diet during phase 2.

Ladybird egg hatch

After egg fertilisation, the hatch rate of ladybird eggs from the 6 ladybird cross types was observed. Over the first 2 weeks of phase 2, all crosses involving a ladybird of either sex fed 'GNA' aphid diet produced eggs with significantly lower mean hatch rates than 'control' × 'optimal' diet crosses 5 and 6 **(Figure 3)**. In the first week, mean % hatch for females initially fed 'GNA' aphid diet (cross 2) was reduced to 69%, compared with 87% for the 'control' aphid diet females of cross 6 ($P < 0.001$). Males were also affected by the initial 'GNA' aphid diet, but to a lesser extent (74% hatch for cross 1, compared with 86% hatch for 'control' aphid diet males of cross 5, $P<0.001$). In the second week, the phase 1 'GNA' aphid diet effect was similar but less marked for both males (75% hatch after 'GNA' diet compared with 89% after 'control' diet, $P<0.001$) and females (79% hatch after 'GNA' diet, 88% after 'control' diet, $P<0.005$). After 2 weeks feeding on 'optimal' aphid diet during phase 2, the effect of the initial 'GNA' aphid diet on either sex was not significant when compared with 'control' diet-fed insects. This indicated that the switch to 'optimal' diet during phase 2 reversed the adverse effects on egg hatch of the 'GNA' aphid diet eaten by ladybirds during the initial 12 days (phase 1).

Ladybird adult longevity

The effect of aphid diet on adult ladybird survival was determined by monitoring the longevity of each mated ladybird pair. Female ladybirds fed on 'GNA' aphid diet during phase 1 died significantly sooner (mean female longevities for cross types 2 and 4 were 36 and 39 days respectively) than females fed on 'control' or 'optimal' aphid diets (mean longevities of cross type 5 and 6 females were 55 and 74 days respectively), despite all six ladybird cross types switching to the 'optimal' aphid diet in phase 2. This difference in female longevities was most marked ($P<0.001$) when comparing females fed 'GNA' aphid diet (cross 2) with those fed 'control' aphid diet (cross 6), resulting in a 51% reduction in longevity. The 'GNA' and 'control' aphid diet females compared using crosses 2 and 6 were mated with 'optimal' diet males in both cases. The effect of 'GNA' aphid diet on male ladybird longevity was less marked, but also significant; 'GNA' diet males (cross 1) lived for an average of 46 days, compared with an average lifespan of 51 days for 'control' diet fed males of cross 5 (10% reduction in lifespan; $P<0.05$). Both types of males in these comparisons were mated with 'optimal' aphid diet females, again to simplify the examination of the 'GNA' diet effect to one sex at a time. Time-mortality curves were not considered to be useful in representing survival responses to the 'GNA' aphid diet in a meaningful way; the experimental design incorporated a switch in diets to 'optimal' aphid diet in phase 2, after the initial 12 days of feeding on the three test

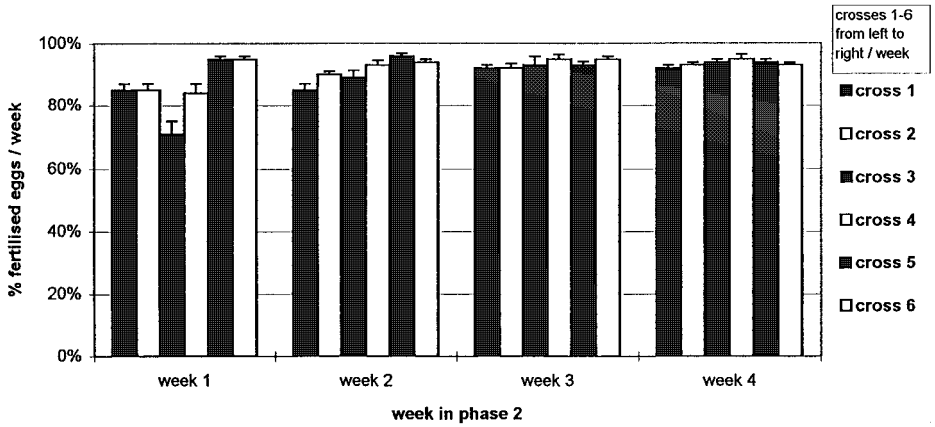

Figure 2. Effect of initial aphid diets eaten (phase 1) on the percent of total ladybird eggs laid in phase 2 which were fertile. The negative effect of the 'GNA' diet eaten in phase 1 on egg fertility of crosses 1–4 diminished after the first 2 weeks on 'optimal' diet (phase 2) and was not significant thereafter.

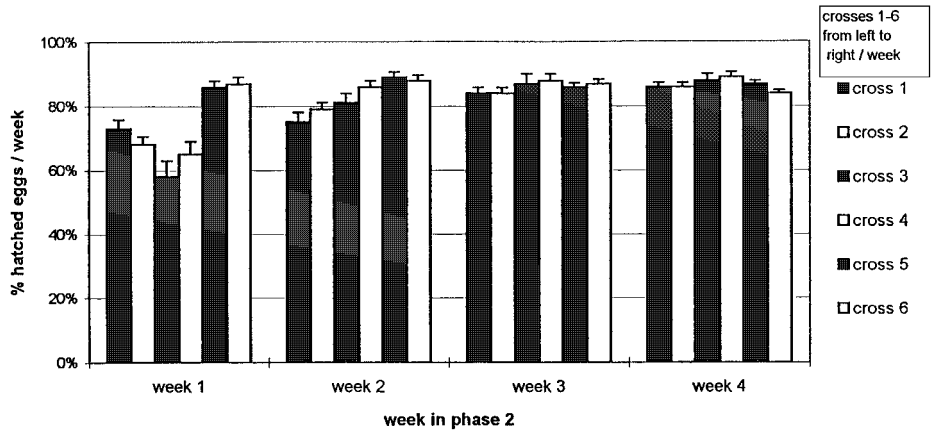

Figure 3. Effect of initial aphid diets eaten (phase 1) on the percent of ladybird eggs which successfully hatched in phase 2. Reduction in egg hatch for crosses 1–4 due, to feeding on the 'GNA' aphid diet in phase 1, was significant only during the first 2 weeks in phase 2, after switching to the 'optimal' diet.

diets. It was evident from our results that there was a time lag involved in the impact of the 'GNA' aphid diet consumed (phase 1), which was later expressed as premature female ladybird death in phase 2 of the experiment, even after consuming the 'optimal' aphid diet for several days after the diet switch.

Discussion

Our results from ladybird feeding trials under controlled environment laboratory conditions show that transgenic potatoes genetically engineered to express the anti-aphid lectin GNA can adversely affect the longevity and reproductive biology of an important natural enemy, via aphids in its food chain. GNA lectin has been shown to be taken up from transgenic tobacco plants by plant-feeding aphids, being detected in the honeydew of *M. persicae* by immunological assay [18]. Plant lectins have also been shown to bind to insect gut cells *in vivo* [1, 12] including those of aphids [16]. It is therefore likely that lectins are taken up and accumulate in aphid gut tissues and are thus efficiently delivered in increased concentration to the predatory ladybird. This food chain phenomenon involving ladybirds, aphids and their host plants has previously been observed in non-cultivated (non-transgenic) plants producing several other types of plant defence chemicals. Several types of natural plant defence chemicals in uncultivated plants can be sequestered or metabolised by aphids, making them

toxic or distasteful to predatory ladybirds via similar host plant food chain effects [10].

It is still to be established whether GNA is having a direct (sub-lethal toxicity) effect on ladybirds or if the effect of the transgenic host plant is indirect, via decreased nutritional value of the aphids reared on GNA-expressing plants, or a combination of direct and indirect effects. The decreased longevity of female ladybird adults fed aphids from transgenic plants (up to 51% reduction, compared with 'control' diet females in comparable crosses) suggests the observed decrease in longevity is due to more than just lowered nutritional quality of aphids consumed from the transgenic potatoes (equal aphid biomass consumed as from control plants). It is not yet known what the impact of reduced female ladybird longevity will be on the population dynamics of the ladybirds and aphids under agricultural conditions. Two-spot ladybirds may lay up to 2000 eggs over a period of 9–12 weeks after mating, as long as suitable aphid populations are available nearby. Reduced female ladybird longevity will reduce total fecundity, even if premature death occurs after the normal peak of egg laying. The reduced ladybird longevity detected in our experiments is also likely to impact via reduced total aphid consumption over a shorter ladybird's lifespan. A single ladybird can consume 5000–6000 aphids in its normal lifespan, so a 50% reduction in female ladybird longevity is likely to significantly impact on the predator's effectiveness as a biological control agent. Female 2-spot ladybirds consume 10–15 aphids/day, so over an adult ladybird's lifespan this consumption will impact on the reproductive capacity of the pest aphid population.

In contrast to our study, a recent risk assessment study using Bt endotoxin-expressing potatoes, genetically-engineered for resistance to Colorado beetles, found no adverse effects on the aphidophagous ladybird *Hippodamia convergens* (Guérin-Ménéville) after feeding on *M. persicae* from transgenic potato plants expressing Bt [2], although in that case the plants were not protected from aphid attack by the Bt toxin expressed. However, a further study, on Bt-expressing corn genetically engineered for resistance to a target lepidopteran pest (European cornborer), has shown the potential for Bt toxicity to affect predatory lacewing larvae via prey (lepidopteran larvae) in its food chain [8]. Our results on ladybirds, together with those on lacewings [8], suggest that tri-trophic effects of anti-pest transgenes on non-target predators may be more widespread than previously assumed. Such effects should be carefully tested using toxin-expressing transgenic plants (not just the purified toxin *in vitro*) prior to commercial release of insect-resistant GM crops. Ideally, the transgenic crops genetically engineered for pest resistance should also be monitored closely after commercial release, to check for possible longer term, sublethal impacts on the agro-environment.

Our experiments involved a change in ladybirds' diet after 12 days, from *Myzus persicae* aphids colonising transgenic or control potato plants (phase 1) to optimally nutritious *A. pisum* from normal faba bean plants (phase 2). After the switch from 'GNA' aphid diet to 'optimal' aphid diet, adult ladybird fitness parameters involving egg fertility and hatch improved, particularly after feeding on the 'optimal' aphid diet for 14 days. The reversible nature of the adverse effects of a lectin-expressing transgenic crop on ladybird reproduction observed in our extended laboratory tests suggests that under field conditions (as yet untested), it may be possible to reduce potentially harmful effects of lectin-expressing crops on aphid predators. This could be achieved by growing non-transgenic (susceptible host plants) 'refuges' (e.g. plantings of aphid-susceptible non-transgenic and aphid-resistant transgenic plants in a structured spatial arrangement), providing unaffected aphid populations within adult ladybirds' feeding range. Similar field management strategies using pest-susceptible 'refuges' are currently being implemented with insect-resistant transgenic crops, to reduce the rate of selection of resistance-breaking pest populations [4]. More field research is needed to optimise the structure and management of both the transgenic crop and non-transgenic 'refuges'. Natural enemies (reproducing adults) could be encouraged to move between the transgenic crop (i.e. exposed to the toxin) and the 'refuge' (i.e. not exposed to toxin), thus feeding on alternative food sources, and to have the opportunity to inter-breed between populations from the two locations.

The experimental design we have used should not be considered a transgenic crop 'worst case scenario' for three reasons. Firstly, the transgenic potato line used was selected as being low expressing for the GNA lectin in foliar tissue. This contrasts with a current strategy for high toxin (Bt) expression, widely used in current transgenic crops to combat the development of Bt resistance in populations of the target pest. Secondly, ladybirds were only exposed to the 'GNA' aphid diet for 12 days, then all ladybirds were switched to 'optimal' aphid diet. In a large-scale

field monoculture situation (lacking suitable refuges or alternative non-transgenic host plants for aphids) ladybirds could be exposed to aphids from toxin-containing plants for much longer periods of time. Thirdly, due to size limitations on our experimental design, we were unable to make crosses between male and female ladybirds which had both been fed on the 'GNA' aphid diet. Since we show that females and, to a lesser extent, males are affected by the 'GNA' aphid diet it is possible that the effects of GNA on both ladybird parents could then be compounded in such a cross. Again, this may reflect the situation in a large monoculture lacking non-transgenic refuges or alternative host plants for the pest and natural enemy, but requires testing under field conditions.

The combined effect of partial resistance (transgenic and/or natural) with pest-regulating natural enemies (predators, parasitoids) potentially offers a more diversified and potentially durable approach to crop protection through IPM [21]. Such an integrated approach is particularly important for pest resistance based on a single gene (vertical resistance), where selection pressure on the target pest can lead to the breakdown of the plants' genetic resistance. This phenomenon has already been observed following the widespread use of Bt toxin sprays on non-transgenic crops, which were attacked by Bt resistance-breaking populations of the diamondback moth, *Plutella xylostella* [L.] in several countries [19]. Further investigations under field or simulated conditions are needed to determine whether interactions between pests' sublethal exposure to plant-produced toxins (as in a resistant transgenic plant expressing GNA) and natural enemies are additive, synergistic or antagonistic, but some initial studies using Bt-expressing crops appear promising [11].

Further studies using artificial diets to feed known concentrations of labelled lectins to the aphids (i.e. food chain intake) compared with feeding lectins directly to the ladybirds are now in progress, to elucidate the mechanism(s) involved. Field trials will also need to be carried out, to assess the possible impact of such tri-trophic effects detected under laboratory conditions and 'no-choice' feeding regimes to those under different agricultural conditions. Studies are also needed to assess whether the impact of anti-pest gene products on natural enemies (via their food chain) are more harmful than effects caused by currently used insecticides for controlling aphids. For such comparisons to be most useful as a benchmark for future ecologically sensitive IPM, each new transgenic plant/insecticidal gene product will need to be compared in individual 'case-by-case' studies with the latest generation of insecticides, now developed for minimal impact on non-target organisms including beneficial natural enemies.

The careful choice of anti-pest genes, the use of targeted gene expression systems and the inclusion of tri-trophic interaction studies (involving transgenic plants, target pests and natural enemies under both laboratory and field conditions) will be important to the development of safe and durable pest-resistant transgenic crops for IPM.

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References

1. Chrispeels M, Raikhel NV: Lectins, lectin genes and their role in plant defence. Plant Cell 3: 1–9 (1991).
2. Dogan EB, Berry RE, Rossignol PA: Biological parameters of convergent lady beetle feeding on aphids on transgenic potato. J Econ Ent 89: 1105–1108 (1996).
3. Down RE, Gatehouse AMR, Hamilton WDO, Gatehouse JA: Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulocorthum solani*) when administered *in vitro* and via transgenic plants both in laboratory and glasshouse trials. J Insect Physiol 42: 1035–1045 (1996).
4. Estruch JJ, Carozzi NB, Desai N, Duck NB, Warren GW, Koziel MG: Transgenic plants: An emerging approach to pest control. Nature Biotechnol 15: 137–141 (1997).
5. Gatehouse AMR, Davison GM, Newell CA, Merryweather A, Hamilton WDO, Burgess EPJ, Gilbert RJC, Gatehouse JA: Transgenic potato plants with enhanced resistance to the potato moth, *Laconobia oleracea*: growth room trials. Mol Breed 3: 49–63 (1997).
6. Gatehouse AMR, Down RE, Powell KS, Sauvion N, Rahbé Y, Newell CA, Merryweather A, Hamilton WDO, Gatehouse JA: Transgenic potato plants with enhanced resistance to the peach-potato aphid *Myzus persicae*. Entomol Exp Appl 79: 295–307 (1996).

7. Gatehouse AMR, Powell KS, Van Damme EJM, Peumans WJ, Gatehouse JA: Insecticidal properties of lectins: their potential in plant protection. In: Pusztai AJ, & Bardocz S (eds) Lectins: Biomedical Perspectives, pp. 35–57. Taylor & Francis, Hants, UK (1995).
8. Hilbeck A, Baumgartner M, Fried PM, Bigler F: Effects of transgenic *Bacillus thuringiensis*-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). J Environ Entomol 27 (2): 480–487 (1998).
9. Hilder VA, Powell KS, Gatehouse AMR, Gatehouse JA, Shi Y, Hamilton WDO, Merryweather A, Newell CA, Timans JC, Peumans WJ, Van Damme E, Boulter D: Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids. Transgen Res 4: 18–25 (1995).
10. Hodek I: Habitat and food specificity in aphidophagous predators. Biocontrol Sci Technol 3: 91–100 (1993).
11. Mascarenhas VJ, Luttrell RG: Combined effect of sublethal exposure to cotton expressing the endotoxin protein of *Bacillus thuringiensis* and natural enemies on survival of bollworm (Lepidoptera: Noctuidae) larvae. Environ Entomol 26: 939–945 (1997).
12. Peumans WJ, Van Damme EJM: Lectins as plant defense proteins. Plant Physiol 109: 347–352 (1995).
13. Powell KS, Gatehouse AMR, Hilder VA, Gatehouse JA: Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*. Entomol Exp Appl 66: 119–126 (1993).
14. Rahbé Y, Febvay G: Protein toxicity to aphids: an *in vitro* test on *Acyrthosiphon pisum*. Entomol Exp Appl 67: 149–160 (1993).
15. Rahbé Y, Sauvion N, Febvay G, Peumans WJ, Gatehouse AMG: Toxicity of lectins and processing of ingested proteins in the pea aphid *Acyrthosiphon pisum.* Entomol Exp Appl 76: 143–155 (1995).
16. Sauvion N: Effects and mechanisms of toxicity of two lectins of the glucose/ mannose group towards the pea aphid, *Acyrthosiphon pisum* (Harris) Ph.D. thesis, University of Lyon, 196 pp. (1995).
17. Sauvion N, Rahbé Y, Peumans WJ, Van Damme EJM, Gatehouse JA, Gatehouse AMR: Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. Entomol Exp Appl 79: 285–293 (1996).
18. Shi Y, Wang MB, Powell KS, Van Damme E, Hilder VA, Gatehouse AMR, Boulter D, Gatehouse JA: Use of rice sucrose synthase-1 promoter to direct phloem-specific expression of ß-glucuronidase and snowdrop lectin genes in transgenic tobacco plants. J Expt Bot 45: 623–631 (1994).
19. Tang J, Gilboa S, Roush RT, Shelton AM: Inheritance, stability and lack of fitness costs of selected resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae) from Florida. J Econ Entomol 90: 732–741 (1997).
20. Van Damme EJM, De Clerq N, Claessens F, Hemschoote K, Peeters B, Peumans WJ: Molecular cloning and characterisation of multiple isoforms of the snowdrop (*Galanthus nivalis*) lectin. Planta 186: 35–43 (1991).
21. Waage J: What does biotechnology bring to integrated pest management? Biotech Dev Monitor 32: 19–21 (1997).
22. Woodford JAT, Gordon SC, Foster GN: Side-band application of systemic granular pesticides for the control of aphids and potato leafroll virus. Crop Prot 7: 96–105 (1988).