Transgenic potato plants with enhanced resistance to the peach-potato aphid
*Myzus persicae*

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Abstract

Potato plants (*Solanum tuberosum*) cv. Desireé were transformed with the genes encoding the proteins bean chitinase (BCH), snowdrop lectin (GNA) and wheat α-amylase inhibitor (WAI) under the control of the constitutive CaMV 35S promoter. Transgenic plants with detectable levels of foreign RNA were then selected for further characterisation with respect to protein expression levels by immunodot blot analysis using polyclonal antibodies raised against the respective protein. With the exception of WAI, plants expressing high levels of RNA, expressed correspondingly high levels of the foreign protein (1.5–2.0% of the total soluble protein). Although high levels of WAI mRNA were detected in some of the transformants, the protein could not be detected. On the bases of expression levels, two lines, designated PWG6#85 (transformed with the double construct WAI/GNA) and PBG6#47 (transformed with the double construct BCH/GNA), were selected for testing in aphid trials for enhanced levels of resistance.

Both transgenic lines had a marked and significant effect on fecundity. The number of nymphs produced per female per day peaked at 4.1 and 4.2 for lines PBG6#47 and PWG6#85 respectively, compared to a value of 5.4 on control plants. Total nymphal production was also significantly lower on either of the transgenic lines compared to control plants (P<0.001) with the differences between the lines being only just significant (P=0.058). On line PBG6#47 there was a delay in nymphal production of 1.6 days, representing a delay of 15%, and on line PWG6#85 this was 3.2 days, representing a delay of ca. 30%. The intrinsic rates of increase (rₙₜ) were also significantly lower on both of the transgenic lines in comparison to that on control plants (P<0.001), however the differences between the lines were not significant. The potential of using such genes as part of an over all strategy for the control of aphid populations is discussed.

Introduction

The world-wide distribution, polyphagous nature, and demonstrated ability of the peach-potato aphid, *Myzus persicae* (Sulzer), to transmit over a hundred different plant viruses makes this insect one of the most agriculturally important aphids, particularly in terms of viral transmission (Hill, 1987). In the UK *M. persicae* is a particularly serious problem on potatoes, sugar beet and brassicas as a virus vector, and in glasshouses where its presence on ornamental crops can adversely affect their marketability.

As a consequence of the extensive and widespread use of chemical insecticides in order to control aphids, there has been a rapid development of insecticide resistance throughout the world, and although there are reports of many resistant aphid species, the problem is most persistent and widespread in a few, of which *M. persicae* is one (Devonshire, 1989). In the UK, resistance was first detected in glasshouses (Needham...
& Sawicki, 1971) and subsequently in field crops on sugar beet in the early 1970's (Needham & Devonshire, 1974). Attention is now being focused on integrated control schemes in which host plant resistance must have a central role.

Provided that suitable genes can be identified and isolated, recombinant DNA technology offers a means of increasing inherent resistance of a crop to its target pests. There are now many such examples where plants have been genetically engineered for increased resistance to insect pests. In the main the engineered resistance has been directed against members of the Lepidoptera (Perlak et al. 1990; Peferoen, 1992), with a few examples that exhibit resistance to coleopteran species (McPherson et al. 1988). This is probably more a reflection of the ease of identifying suitable gene candidates, rather than the relative economic importance of the different insect orders. Most of these genetically transformed crops exhibiting enhanced levels of resistance express genes that encode *Bacillus thuringiensis* endotoxins (Fischhoff et al., 1987; Vaeck et al., 1987; Barton et al., 1987). Extensive field trials have now been carried out on many of these transgenic crops, in particular those expressing *B. t.* genes (Peferoen, 1992; Barton & Miller, 1993).

In addition to the well-studied and useful *Bt* toxin genes, there are a number of reports of other plant-derived genes that, when expressed in crops, afford significant levels of protection against lepidopteran (Gatehouse et al., 1993; Gatehouse & Hilder, 1994) and coleopteran pests (Shade et al., 1994; Leplé et al., 1995). Most notable among these genes are those that encode protease inhibitors (Hilder et al., 1987; Johnson et al., 1989; McManus et al, 1994). Genes encoding lectins constitute another class of plant-derived genes of potential utility for increasing insect resistance in transgenic plants, and in recent years screening programmes have been carried out to identify lectins which are toxic towards target insect pests (for review see Gatehouse et al., 1995). Lectins have been identified which are toxic towards members of the Coleoptera (Gatehouse et al., 1984, 1989; Czapla & Lang, 1990; Murdock et al., 1990), Lepidoptera (Shukle & Murdoc 1983; Czapla & Lang, 1990; Gatehouse et al., 1992), Diptera (Eisemann et al., 1994) and Homoptera (Powell et al., 1993, 1995a, 1995b; Habibi et al., 1993). Such studies have been extended to include aphids (Rahbè et al., 1995). The present authors have demonstrated that several mannose-specific lectins were toxic to *M. persicae* when tested in artificial diets; of these the lectin from snowdrops (*Galanthus nivalis*; GNA) was found to be the most effective, for it not only affected development of the insect, but also had a significant effect upon female fecundity when fed at low to moderate concentrations.

The present paper describes the production of transgenic potato plants constitutively expressing GNA and demonstrates that the effects of the lectin obtained in artificial diet studies can be reproduced on transgenic plants grown under environmentally controlled conditions. In both cases, the presence of GNA has a significant effect upon the calculated *r*ₘ (intrinsic rate of population increase) and fecundity of the aphid. Effects on *M. persicae* of transgenic potato plants expressing two transgenes, GNA and BCH (bean chitinase), upon these same parameters is also described. The implications for using such plants as part of an insect control strategy are discussed.

### Materials and methods

#### Plant material

Virus-free, sterile plantlets of *Solanum tuberosum* L. cv. Desireé were obtained from the Scottish Office, Agriculture and Fisheries Department, Edinburgh, U.K. Shoot cultures were maintained in test tubes containing 10 ml potato medium (PM) as described in Newell et al. (1991). The cultures were grown at 22 °C with a 16 h photoperiod. Shoots were subcultured monthly by excising ca. 1 cm of the shoot tip and transferring to fresh PM. When the shoots were about 12 cm in length, the internode sections were used in transformation experiments.

#### Plasmid construction

The genes used in this work encode the proteins bean chitinase (BCH; Broglie et al., 1986), snowdrop lectin (GNA; van Damme, 1991) and wheat α-amylase inhibitor (WAI; W. D. O. Hamilton & Merryweather, unpublished; Kashlan & Richardson, 1981). The coding region for each gene was isolated as follows: the BCH coding region was isolated from *Phaseolus vulgaris* genomic DNA by PCR using primers complimentary to the ends of the coding sequence and including a *BamH* site at each end; the GNA gene was obtained as clone LECGNA2 (van Damme, 1991) and PCR was used to flank the coding sequence with *BamH* and *Kpn* restriction sites; the WAI coding region was isolated from a cDNA library of devel-
oping wheat embryos and PCR used to flank the coding region with KpnI and SstI restriction sites. These coding region fragments were cloned individually between the cauliflower mosaic virus (CaMV) 35S promoter sequence and the nopaline synthase (NOS) transcriptional terminator sequence to produce expression cassettes. These expression cassettes were then combined in pairs (WAI/GNA and BCH/GNA) in an inverted repeat orientation, a short 150 bp spacer region of CaMV 35S DNA being placed between the two cassettes to facilitate the cloning. Finally, these pairs of expression cassettes were cloned into the T-DNA of the binary vector pAPT5 (A. Merryweather, unpubl.), adjacent to the right-border sequence. The T-DNA of this pRK290-type binary vector (Ditta et al., 1980) also carries aphlI and uidA genes encoding neomycin phosphotransferase II (NPTII) and β-glucuronidase (GUS), respectively. The resultant binary transformation vectors, pPWG6 (encoding WAI and GNA) and pPBG6 (encoding BCH and GNA), were transferred into Agrobacterium tumefaciens LBA4404 (Ooms et al., 1982) by electroporation according to the protocol of Wenjun & Forde (1989).

Transformation
For transformation, Agrobacterium tumefaciens LBA4404 (pPWG6) and LBA4404 (pPBG6) were each inoculated into 50 ml of liquid YEP (10 g l\(^{-1}\) bactopeptone, 10 g l\(^{-1}\) yeast extract, 5 g l\(^{-1}\) sodium chloride) medium containing 1 mg l\(^{-1}\) tetracycline in a 250 ml flask. The flasks were shaken overnight at 30 °C. After approximately 16 h growth, the suspension was centrifuged at 1800 \(\times\) g for 20 min and the pellet resuspended in a volume of liquid inoculation medium sufficient to give an A\(_{600}\) O.D. reading for the suspension of 0.5. The inoculation medium consisted of Murashige & Skoog (MS) (1962) medium (ICN Biomedicals Ltd.) with 30 g l\(^{-1}\) sucrose.

Transformation of stem internode explants was performed according to the method of Newell et al., (1991) with the following exceptions. The stem sections were incubated in 10 ml of bacterial suspension in a petri dish, 100 sections per dish, for 30 min; following inoculation they were transferred to co-culture plates containing a feeder layer of 1.5 ml of a Nicotiana benthamiana cell suspension, maintained by weekly subculturing in a liquid medium of MS salts, B5 medium vitamins (Gamborg et al., 1968), 30 g l\(^{-1}\) sucrose, 0.2 mg l\(^{-1}\) 6-benzylaminopurine (BAP) and 1 mg l\(^{-1}\) 2,4-dichloroacetic acid (2,4-D), and covered by a sterile filter paper. Shoots which developed were initially screened for GUS activity with the indigogenic substrate, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-gluc), according to the method of Jefferson et al. (1987) with some modifications. The substrate solution contained 0.5 mg ml\(^{-1}\) X-gluc, cyclohexylammonium salt (Biosynth Ag.) in 0.1 M sodium phosphate buffer, 0.5 mM K\(_3\)Fe(CN)\(_6\), 0.5 mM K\(_4\)Fe(CN)\(_6\), 10 mM Na\(_2\)EDTA, and 0.06% Triton-X. Sections of stem were immersed in substrate and incubated at 37 °C overnight before being transferred to 70% ethanol. Once confirmed, transgenic plantlets were assayed for activity of the introduced genes of interest.

**Determination of expression levels**

**Total RNA.** Total RNA was isolated from leaves of in vitro grown plants following the method of Verwoerd et al. (1989). For the dot blot assay, 4 μg RNA was loaded onto a nylon membrane filter (Amersham Hybond N+) in a Bio-Rad dot blot apparatus. The filter was hybridized with probes consisting of the entire coding region of the WAI, BCH or GNA gene. The probe was radioactively labelled with 32P-dCTP following the method of Feinberg & Vogelstein (1983). The filter was hybridized and washed according to the conditions recommended by the membrane manufacturer and autoradiography was performed using X-Omat AR X ray film and X-Omat Regul (Kodak) intensifying screens at -70 °C.

**Protein quantification.** The presence of BCH and GNA protein in plants transformed with the pPBG6 and pPWG6 constructs was confirmed using an immunoassay which incorporated the enhanced chemiluminescence detection (ECL) system of Amersham (M. Longstaff, pers.comm.). For extraction of protein, a leaf from each in vitro-grown plant was ground in 100 μl phosphate buffered saline (PBS) and centrifuged in a microfuge for 5 min. The supernatant was transferred to a fresh tube and the total amount of protein present quantified against BSA using the Bio-Rad protein assay system. For a dot-blot assay of GNA, 2–5 μg total protein was loaded onto 0.2 mm nitrocellulose (Schleicher & Schuell, BA83) in a Bio-Rad dot-blot apparatus. Pure GNA was also added to 2 μg total protein from an untransformed control plant, such that GNA was present at levels of 1.5, 1.0, 0.75, 0.5 and 0.1% of total protein; this provided a set of standards with which to compare GNA expression in transgenic
plants. After removal from the dot-blot apparatus the filter was washed in freshly prepared 2% periodic acid for 10 min, blocked for 1 h in 5% low-fat milk powder + 1% Tween 20 in PBS, then incubated with polyclonal rabbit antibody raised against GNA and diluted in 5% low-fat milk powder + 0.1% Tween 20 in PBS for 2 h at room temperature. The filter was washed in 5% low-fat milk powder + 0.1% Tween 20 in PBS, then incubated with HRP-conjugated goat anti-rabbit IgG (Bio-Rad) as the secondary antibody for 1.5 h. The filter was washed in PBS + 0.1% Tween 20, followed by a wash in water, and the GNA protein was detected by ECL following the manufacturer’s instructions (Amer sham plc, UK). The filter was placed between two sheets of acetate and exposed to Hyperfilm (Kodak) for approximately 1 min. Dot-blot assays of BCH protein were carried out in the same way, except that a polyclonal mouse antibody against BCH was the primary antibody and HRP-conjugated goat antimouse IgG (Bio-Rad) was used as the secondary antibody.

Western analysis using SDS-PAGE was used to detect both GNA and BCH protein in transgenic plants transformed with pPBG6. Aliquots containing 5 μg total protein were loaded onto 15% acrylamide gels containing 4M urea. Following separation by electrophoresis, the proteins were transferred electrophoretically onto Schleicher and Schuell BA83 nitrocellulose filter. This filter was processed as for the dot-blot procedure, and protein was detected by ECL as above.

In order to select for high levels of expression of BCH from primary transformants of plants transformed with the double construct PBG6, plants were also assayed for the presence of the enzyme using chitin labelled with Remazol brilliant violet. Lyophilised leaf material was extracted at 1 mg ml⁻¹ 0.1 M sodium acetate buffer pH 5.1 at 4 °C and then centrifuged 10 min at 9000 x g. Aliquots of the supernatant (0.5, 10, 20 μl) were incubated with 300 μl substrate (1 mg ml⁻¹ acetate buffer) at 25 °C for 1 h. Assays were terminated by the addition of 120 μl HCl, stood on ice for 10 min, centrifuged and the O.D. of the supernatant read at 540 nm in microtitre plates; enzyme assays were carried out using non-transformed leaf material as a control.

Plant propagation
Plants for insect bioassays were propagated in tissue culture from apical shoot cuttings. When a good root system had developed, the plantlets were potted out into John Innes No. 3 compost, and a polythene bag was placed over each plant until they had reached a height of ca. 10 cm, when the top of the bag was opened to enable ventilation; after a further week the bag was removed. Plants were grown under a regime of 20 °C, L16:D8.

Insects
The Myzus persicae clone (MP1; Woodford et al., 1988) was initially obtained from the Scottish Crop Research Institute, Invergowrie, Dundee, U.K. The aphids were reared continuously on Chinese cabbage plants under environmentally controlled conditions of 18 °C with L16:D8. Prior to any trials, the aphids were removed from these plants and starved for 4 h before being placed onto test plants.

Fecundity trials
From a synchronised Myzus population reared on cabbage plants, sexually mature females were removed from the rearing plants, starved for 4 h and then placed in clip-cages (as described by Honeyborne, 1969) on either control (i.e. non transgenic, tissue cultured-derived) or transgenic potato plants. In all cases aphids were caged on the abaxial leaf surfaces. Aphids were caged throughout the canopy, but avoiding immature or senescing leaves. For each plant line, 10–12 plants were used and 3 clip-cages were set up per plant thus giving 30–36 replicates per plant line. After 24 h the adults (A₁) were all removed, together with all but 2 first-instar nymphs; where necessary, plants were re-infested as above, due to mortality on the transgenic lines. This day was labelled day 0. The remaining 2 nymphs per clip-cage were left undisturbed until adulthood (A₂), recording at the same time each day nymphal mortality and developmental stage. At the teneral adult stage one aphid per cage was left so that the daily reproduction of all the individually-caged second generation adult aphids could be recorded. At each daily count, newly deposited nymphs were removed with minimal disturbance to the adult, and mortality of the A₂ aphids was monitored. Aphid bioassays were carried out under controlled environmental conditions of 25 °C, 16 h light, 8 h dark regime. If leaves started to senesce and die, clip cages were moved to fresh leaves on the same plant. The trials were conducted over a period of 36 days, by which time no more nymphs were being produced.
Data analysis

Aphid survival. Survival rates (%) were compared at a given time by standard % comparison test, also referred to as ‘Fisher’s binomial exact test’ (hypergeometric law, treatment vs. control).

Fecundity and $r_m$. All aphids were included in the computation of the rate of increase, except those which had died by day 12, which were considered to have died from experimental damage and not from the treatment. Biological performance of aphids was evaluated using several different parameters, as shown in Table 1. Effects were tested through one-way ANOVA followed by Bonferroni-Dunn multiple comparison tests (using Statview 4 software; Abacus Inc., USA).
Figure 2. Chitinase activity in transgenic potato plants transformed with the BCH/GNA double construct, as determined by digestion of dye-labelled chitin substrate; activity is expressed relative to endogenous activity in non-transformed control plants. Bars represent means ± SE for determinations on different clones of primary transformants.

Table 1. Adult performance of *Myzus persicae* reared on transgenic plants expressing the snowdrop lectin GNA, either alone (PWG6#85) or in combination with bean chitinase (PBG6#47)

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<td>Control</td>
<td>35</td>
<td>0.382 (0.005)a</td>
<td>0.367 (0.005)a</td>
<td>74.3 (3.2)a</td>
<td>7.06 (0.04)a</td>
<td>9.56</td>
<td>1.81</td>
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<td>PBG6#47</td>
<td>26</td>
<td>0.309 (0.008)b</td>
<td>0.294 (0.010)b</td>
<td>51.5 (3.9)b</td>
<td>8.35 (0.14)b</td>
<td>11.31</td>
<td>2.24</td>
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<tr>
<td>PWG6#85</td>
<td>22</td>
<td>0.301 (0.008)b</td>
<td>0.294 (0.008)b</td>
<td>62.1 (3.8)b</td>
<td>8.86 (0.24)c</td>
<td>12.01</td>
<td>2.30</td>
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Plant lines and definition of prereproductive, generation and doubling times are detailed in text. SDjack is a jackknife estimator of standard deviation.
* Numbers in a column followed by different letters are significantly different at P<0.05.

Prereproductive time (d) is the mean number of days from aphid birth to reproduction. The mean generation time (T) is the mean length of aphid generation, calculated after the approximation of Wyatt & White (1977): T = d/0.738. The doubling time DT is the time required by the aphid population to double its size, and comes from the standard definition of r_m as: DT = Log_e(2)/r_m, as used by Deloach (1974).

Values for the intrinsic rate of increase (r_m) were calculated either on the basis of individual aphids, or on the population data, using the following equation:

\[ \sum e^{-r_m x} L_x m_x = 1 \]

where: x = age in days; L_x = age specific survival; m_x = age specific fecundity (offspring/female/day) (Birch, 1948). The r_m values were estimated by successive approximations of this equation, and expressed as female/female/day (Sauvion et al., 1996). These values were also estimated by the method of Wyatt and White (1977) using the following equation:

\[ r_m = 0.738 \log_e (M_d)/d \]

where M_d is the mean number of progeny produced in a time equal to the prereproductive time d. r_m values presented in Table 1 are the group values; standard errors were calculated using the jack-knife technique (Miller, 1974); ANOVAs and multiple comparisons
on \( r_m \) were conducted on individual \( r_m \) values. All statistical analyses are described elsewhere (Sauvion et al., 1996).

**Results**

*Screening of transgenic potato plants for transgene expression*

Transgenic plants which expressed the \( \beta \)-glucuronidase enzyme were assayed for RNA and/or protein result-

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**Figure 3.** Western blot of primary transformants transformed with either the double construct BCH/GNA (PBG) or WAI/GNA (PWG). (a) Tracks: A, line PBG6#22; B, line PBG6#47; C, line PWG6#9C; D, PWG6#85; E, non-transformed potato (control); F, GNA standard; (b) Tracks: A, line PBG6#22; B, line PBG6#47; C, non-transformed potato (control); D, BCH standard.
Figure 4. Quantification of clonal replicates of lines PBG6#47 and PWG6#85 used in aphid clip-cage bio-assays for GNA expression. Wells A1-F3; GNA standards (0.0%-2.0%) spiked into control non-transformed potato. Wells A5-H6 control, non-transformed tissue culture-derived potato plants: A7-D9, clonal replicates of line PBG6#47; E9-H11, clonal replicates of line PWG6#85. Standards and tissue samples were duplicated down the wells i.e. A1 B1 etc.

ing from expression of the introduced genes. Altogether 66 plants containing the pPWG6 construct and 47 plants containing the pPBG6 construct were screened for RNA levels of WAI, GNA and BCH. Expression ranged from below the limits of detection to high signal levels; those lines which showed elevated levels of RNA were selected for further study (data not presented).

Transgenic plants with detectable levels of RNA were characterised further with respect to protein expression by immunodot blot analysis using polyclonal antibodies raised against the respective protein. For GNA (irrespective of the double construct) and BCH, expression levels of the foreign protein in these primary transformants ranged from undetectable to approximately 1.5% of the total soluble protein (Figure 1); high levels of message corresponded with high levels of protein. However, although RNA could be detected in all of the lines containing the WAI/GNA construct, no WAI protein could be detected, despite the fact that nontransformed control potato tissue spiked with purified WAI could be readily detected down to a level of 0.02% of total soluble protein. Non-transformed control leaf tissue clearly showed that there was no cross-reactivity with any of the antibodies, thus it was possible to accurately quantify the expression levels.

Not only was BCH determined in the original transformants using antibodies raised against the protein purified from senescing bean leaves, but it was also determined enzymatically using labelled chitin.

Although there is a low level of endogenous chitinase present in potato leaves, those levels in the plants transformed with the BCH/GNA construct were approximately 8-fold higher (significant at P<0.001). Between those plants selected on the basis of high mRNA levels, there was little difference in expression levels of the foreign protein (Figure 2).

In order to check that the foreign proteins had been correctly processed in the potato plants, leaf tissue samples from the different lines were run on SDS-PAGE, blotted on to nitrocellulose, and, following incubation with the appropriate antibodies, were visualised using the ECL system. These blots clearly showed that both GNA and BCH from transgenic plant tissue were of the correct molecular weight (M, 12,000 and M, 30,000 respectively) when compared with purified standard proteins (Figure 3). In the case of WAI, again no protein could be detected.

Selection of transformants for aphid bio-assay

On the basis of expression levels of the transgene, one potato line transformed with the BCH/GNA construct, and one with the WAI/GNA construct, were selected for aphid bio-assays; these were designated PBG6#47 and PWG6#85, respectively. Plants from each line were clonally propagated to provide sufficient numbers in order to carry out statistically valid trials. Control plants were also propagated in tissue culture. Just prior to infestation, when the plants had been potted out into compost for 9 weeks, leaf samples
were taken from all plants, ensuring that samples were taken from comparable leaves. The actual expression levels were determined by immunodot blot analyses, calibrated by using leaf extracts from control potato plants spiked with different concentrations of the purified protein; the levels of expression were then quantified by densitometric scanning. Assay of leaf samples taken from plants of line PBG6#47 showed the levels of BCH expression to be similar in all plants, and was in the region of 2% of the total soluble protein. Expression of GNA in all plants from both transgenic lines was approximately 1.5% of total soluble protein (Figure 4). These proteins were absent in leaves from control plants.

Effects of transgene expression on aphid development and fecundity

In order to determine whether expression of GNA, either on its own or in the presence of BCH, had any effects upon aphid development and fecundity, trials using clip-cages were carried out on plants of the transgenic lines PWG6#85 and PBG6#47, and aphid performance was compared to that on control plants.

The results suggested that both transgenic lines affected the initial establishment of aphids, since survival of the initial inoculum of aphids on the transgenic plants was up to 20% lower over a two-day period than on the control plants. This difference was statistically significant (P<0.02) for line PBG6#47 (Figure 5), but was not significant for line PWG6#85. However, on plants where adults had died, establishment of aphids could be 'forced' by repeating the inoculation, to result in one surviving adult aphid per clip cage. This procedure enabled r_m studies to be carried out.

Examination of progeny produced by the initial inoculum of aphids showed that the survival of A_2 aphids was similar (and >90%) prior to production of nymphs (days 1–5 of the trial) on all three lines; survival on line PWG6#85 was slightly lower than control, but not significantly so, whereas survival on control and line PBG6#47 was identical (data not presented). The number of aphids in each treatment was made the same at day 5 when nymph production commenced (see methods section). Subsequently, no differences in survival of A_2 aphids between the lines were observed over the first four days after nymph production commenced (i.e. days 5–9 after the commencement of the trial), and differences remained small and not significant, with survival ≥75%, until approximately 20 days of development. After this time the survival of aphids on all three lines declined, but the decline was more rapid on both transgenic lines than on the control. No aphids on the transgenic plants survived beyond 30 days of development, whereas aphids on control plants survived to 33 days of development (Figure 6).

Although effects on survival of the A_2 aphids were not significant, both transgenic lines had a marked and significant effect on aphid fecundity. The number of nymphs produced per female per day peaked at 4.1 and 4.2, for lines PBG6#47 and PWG6#85 respectively, compared to a value of 5.4 on control plants (Figure 6). The intrinsic rates of increase (r_m) were also significantly lower on both of the transgenic lines in comparison to that on control plants (ANOVA, P<0.001); however the differences between the two transgenic lines were not significant (Table 1; Bonferroni-Dunn test; P=90%). Overall, total nymphal production was again significantly lower on either of the transgenic lines compared to the control (ANOVA, P<0.001). In this case the difference between the transgenic lines was almost significant (P=0.058), with fewer nymphs being produced on line PBG6#47. This decrease in nymphal production on the transgenic lines was accompanied by a shift in the peak in maximal nymphal production (Figure 6). On line PBG6#47, there was a delay of 1.6 days, representing a delay of 15%, and on line PWG6#85 the delay was 3.2 days, representing a delay of approximately 30%. These
Figure 6. Adult performance (survival and fecundity) of *Myzus persicae* on transgenic potato plants expressing the snowdrop lectin GNA, either alone (PWG6#85) or in combination with bean chitinase (PBG6#47). A. adult survival; B. cumulative fecundity of surviving adults; C. Model curves for net adult fecundity (model used: see Materials and methods).

Figure 6. Adult performance (survival and fecundity) of *Myzus persicae* on transgenic potato plants expressing the snowdrop lectin GNA, either alone (PWG6#85) or in combination with bean chitinase (PBG6#47). A. adult survival; B. cumulative fecundity of surviving adults; C. Model curves for net adult fecundity (model used: see Materials and methods).

Delays were also reflected by the 1.3 and 1.8 days of increase in the prereproductive time on the transgenic plants of lines PBG6#47 and PWG6#85 respectively (Table 1).

Discussion

Previous studies identified mannose-specific lectins, and in particular GNA, to be toxic towards homopteran insects such as rice brown planthopper (Powell et al., 1993), and aphids, including *M. persicae* (Rahbé et al., 1995; Sauvion et al., 1996), when tested in artificial diet. Based upon these results, the gene encoding GNA was selected for transfer to crops in order to determine whether *in planta* it would afford the levels of protection predicted by the artificial diet studies. Expression of the foreign gene in transgenic plants was driven by the constitutive CaMV 35S promoter, and expression levels in the transgenic potato lines used were found to be approximately 1.5% of the total soluble protein; these levels are in keeping with other reported values using the same promoter (Ellis et al., 1988). However, it should be noted that this estimated level of expression does not necessarily reflect the actual level of GNA present in the phloem sap. The GNA preprotein coding sequence used in the constructs is sufficient to direct the mature protein into the phloem sap (Shi et al., 1994); this finding is supported by the immunological detection of GNA in guts of glasshouse potato aphids (*Aulacorthum solani*) which have fed on the transgenic potato lines PWG6#85 and PBG6#47 (unpubl. data).

In the present study both line PWG6#85, where only expression of GNA protein was detectable, and line PBG6#47, which expresses GNA and BCH at approximately the same levels, showed a significant decrease in aphid fecundity compared to that on non-transformed control plants. Furthermore, the model fecundity curves (Figure 6) show that the presence of the lectin alters the dynamics of fecundity by shifting the reproductive peak by 3.2 days and 1.6 days for the two lines respectively. These results are reflected in the rm values which show that the rate of population increase on control plants is significantly higher than on the transgenic plants. If the estimated rm values were stable over a 30 day period, they would theoretically result in a population increase of $\times 9.5 \times 10^4$ on control plants and only $\times 8.5 \times 10^3-10.5 \times 10^3$ on the transgenic plants (after the equation: Population ratio $= \exp (r_m \times \text{days})$). Such a change in the population growth rate is very similar to that observed on
artificial diets containing ca. 400 μg ml⁻¹ of GNA, which leads to a population increase over 30 days that was approximately 6.5 times lower on lectin diet than on control diet (Sauvion et al., 1996). It is interesting to note that whilst the reduction in fecundity between artificial diet studies and transgenic plants were similar to one another, the shift in the reproductive peak caused by GNA was much greater on the transgenic plants than on diet, particularly in line PWG6#85. Data have been presented recently to show that the rate of growth of a population of *M. persicae* was slower on GNA transgenic tobacco plants than on control plants, with the doubling times being 4.8 ± 0.2 days and 4.4 ± 0.2 days respectively (Hilder et al., 1995); in the present study the deleterious effects of GNA on the aphid were much greater.

The effects of GNA on aphid survival are less pronounced than on fecundity, with two effects being observed. First, there is an ‘aversion effect’ when aphids are first exposed to GNA, with a lower survival over the initial inoculation period. Secondly, there is an effect of GNA on the longevity of aphids, where the decline in survival of an aging aphid population occurs more rapidly in the presence of GNA. Both these effects have also been observed in bioassays of *M. persicae* against GNA in artificial diet (Sauvion et al., 1996).

It is difficult to estimate what effect expression of BCH in the transgenic potato line PBG6#47 is having on *M. persicae*. In terms of intrinsic rate of increase (rₘ), it would appear that its presence is having little effect. However, overall nymphal production throughout the trial period was decreased on this line compared to line PWG6#85, although the decrease in level was just outside the P<0.05 level of significance. The presence of the chitinase does not appear to be having any significant effect on delaying the reproductive peak; on the contrary it reduces this parameter by one day when compared to the GNA-only expressing line, thus ‘restoring’ the rₘ value, which is very sensitive to the dynamics of reproduction. The results from the present study suggest that whilst the presence of BCH may not influence the rate of the population build-up, it may help reduce aphid damage through reduced adult survival (Figure 6A) and reduced late fecundity (Table 1 and Figure 6). The results obtained from the two different transgenic lines can be interpreted to suggest that the primary role of GNA is to delay the peak of reproduction, with a secondary effect of reducing total fecundity; in any event its presence strongly affects the rₘ and population build-up. BCH, on the other hand, appears to reduce total fecundity, but with little effect overall on population build-up.

The present study shows that in small-scale trials carried out under controlled environmental conditions, expression of GNA in transgenic potato plants has deleterious effects on the development of *M. persicae*, reducing fecundity and delaying the reproductive peak, and thus resulting in a significant reduction in the population build-up. The relevance of these results to crop protection in the field, or indeed in glasshouses, has yet to be tested. However, the results are encouraging, and suggest that this strategy could make a valuable contribution to crop protection, particularly when used as part of an integrated pest management system. Aphid infestation is not only a cause of crop losses in itself, but causes further losses (which can be considerably more serious) by virus transmission. Thus the consequences of expressing foreign proteins on the probing behaviour, and subsequent uptake and transmission of these viruses by the aphid, will have to be investigated. Preliminary studies are now underway to address these points.

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**References**


