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INACTIVATION OF BACULOVIRUS BY ISOFLAVONOIDS ON CHICKPEA (*Cicer arietinum*) LEAF SURFACES REDUCES THE EFFICACY OF NUCLEOPOLYHEDROVIRUS AGAINST *Helicoverpa armigera*.

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Chickpea isoflavonoids inhibit *Helicoverpa armigera* NPV

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ABSTRACT-Biological pesticides based on nucleopolyhedroviruses (NPVs) can provide an effective and environmentally benign alternative to synthetic chemicals. On some crops, however, the efficacy and persistence of NPVs is known to be reduced by plant specific factors. The present study investigated the efficacy of *Helicoverpa armigera* NPV (*Hear*NPV) for control of *H. armigera* larvae and showed that chickpea reduced the infectivity of virus occlusion bodies (OBs) exposed to the leaf surface of chickpea for at least one hr. The degree of inactivation was greater on chickpea than on previously reported on cotton and the mode of action is different to that of cotton. The effect was observed for larvae that consumed OBs on chickpea leaves but also occurred when OBs were removed after exposure to plants and inoculated on to artificial diet, indicating that inhibition was leaf surface related and permanent. Despite their profuse exudation from trichomes on chickpea leaves and low pH, organic acids – primarily oxalic and malic acid – caused no inhibition. When *Hear*NPV was incubated with biochanin A and sissotrin, however, two minor constituents of chickpea leaf extracts, the OB activity was reduced significantly. These two isoflavonoids increased in concentration by up to 3 times within one hr of spraying the virus suspension onto the plants and also when spraying only carrier, indicating induction was in response to spraying and not a specific response to the *Hear*NPV. Although inactivation by the isoflavonoids did not account completely for the level of effect recorded on whole plants this work constitutes evidence for a novel mechanism of NPV inactivation in legumes. Expanding the use of biological pesticides on legume crops will be dependent upon the development of suitable formulations for OBs to overcome plant secondary chemical effects.

Key Words- Baculovirus, Biopesticide, Nucleopolyhedrovirus, *Helicoverpa armigera*, Chickpea, Induced resistance, Plant leaf chemistry, Isoflavonoid.

INTRODUCTION

Helicoverpa armigera (Hubn.) is a major crop pest in Asia, Africa and Australasia attacking a wide range of important crops including cotton, maize, tomato, peppers, chilies, and legumes such as chickpea and pigeonpea (Gowda, 2005; King, 1994). Its status as arguably the world's most important agricultural pest can be attributed to its wide geographical and host range coupled with its ability to develop high levels of resistance to chemical insecticides (Armes et al., 1992b; Kranthi et al., 2002). The baculovirus biopesticide *Helicoverpa armigera* nucleopolyhedrovirus (*Hear*NPV) is an ecologically benign alternative to chemical insecticides that is effective and can overcome problems of chemical insecticide resistance (Moscardi, 1999; Grzywacz et al., 2005). *Hear*NPV is now commercially produced in Australia, Thailand, India and China for control of *H. armigera* (Buerger et al., 2007, Sun and Peng, 2007, Singhal, 2004). However, the utility of baculoviruses for insect pest management is compromised by the fact that some host plants adversely influence the severity of viral disease in insects and so reduce pest control efficacy (Felton and Duffey, 1990; Duffey et al., 1995; Hoover et al., 1998a; Cory and Hoover, 2006). It has for some time been recognized that *Heliothis zea* NPV, a closely related baculovirus, performed poorly on some crops such as cotton (Young and Yearian, 1974; Forschler et al., 1992,), a phenomena linked to the direct action of glandular secretions in reducing the persistence of occlusion bodies (OBs) the infective stage of the virus (Young and Yearian, 1977; Ellerman and Entwistle, 1985). OBs are a protective crystalline protein matrix in which virions are embedded during transmission and in hostile environments (Hunter-Fuijita et al., 1998). The maintenance of OB integrity is crucial to viral persistence outside the host and for initiating infections in new host insects. Host plant effects on biological pesticides are not restricted to baculoviruses, as plants such as cotton have been shown to reduce the efficacy of other biopesticides, especially *Bacillus thuringiensis* (Kushner and Harvey, 1962; Johnson, 1982; Ali et al., 2004). Inhibition of NPV infections on cotton has also been attributed to high peroxidase activity and subsequent free radical generation which was associated with an increase in the sloughing off of midgut cells that are the point of entry for the NPV virions, thereby reducing virus-induced mortality (Hoover et al., 1998a; Hoover et al., 1998b; Hoover et al., 2000). While the use of *Hear*NPV has been shown to be effective on chickpea (Jayaraj et al., 1987; Rabindra et al., 1992; Cherry et al., 2000) field trials have indicated OB persistence and activity to be much lower on chickpea leaf surfaces than on other crops such as tomato (Rabindra et al., 1994), suggestive of some degree of adverse interaction on chickpea. Chickpea produces copious glandular secretions rich in organic acids and the leaf surface can subsequently have a very low pH (<3) (Rembold and Weigner, 1990; Stevenson and Aslam, 2006). This could make it a challenging host plant for biopesticide use because earlier work on *Lymantria dispar* NPV has shown that larvae can be less susceptible to OBs when inoculated on highly acidic (pH 3.8-4.6) oak foliage rather than other less acidic aspen foliage (Keating and Yendol, 1987) an effect associated with low pH and high levels of organic acids (Keating et al., 1989).

The present study was undertaken to investigate the efficacy of *Hear*NPV on chickpea in comparison with tomato, a known favorable host (Forschler et al., 1992; Farrar et al., 2000), and cotton, a host plant known to impair OB infectivity, to better understand what plant factors affect virus efficacy with a view to developing better recommendations for the efficacy of NPV-based insecticides on legume crops and to assist in the development of a suitable formulation for OBs for use on crops such as chickpea.

MATERIALS AND METHODS

Virus. The virus strain (NRI#0210) was provided by Professor R.J. Rabindra of Tamil Nadu Agricultural University, India, and stored at -80°C. This strain is typical in activity of strains of *Hear*NPV used in biopesticides products in India having a mean LC_{50} of 2.78 x 10^3 OB ml^{-1} for neonate larvae similar to that reported by others including Somasekar et al. (1993) and had been used previously in field trials on chickpea in India (Cherry et al., 2000). It was multiplied up in third instars of *H. armigera* then harvested and purified using a standard NPV purification protocol (Hunter-Fuijita et al., 1998). The virus was enumerated using a standard Neubauer haemocytometer and phase contrast microscope at X400 magnification (Wigley, 1980). The identity of the source and progeny of the virus was checked using a standard DNA restriction analysis protocol for NPVs with EcoR1 (Hunter-Fuijita et al., 1998).

Insects. The insects for the bioassays were derived from a culture of *H. armigera* provided by the NERC Centre for Ecology and Hydrology at Oxford which had been maintained there for a number of years. The insects were reared at 26 ± 2 °C with a relative humidity of 50 ± 5% and a 14:10 hr light:dark regime. Larvae were reared in groups in 250 ml plastic pots on an artificial wheatgerm casein diet until the second instar and then individually in 30 ml plastic pots on wheatgerm diet using a method previously described (Armes et al., 1992a).

Plants. The plants used in the study were cotton (*Gossypium hirsutum*,) variety Ankur 651 (Ankur Seeds Ltd. Nagpur, India), chickpea (*Cicer arietinum*) variety ICC 11322 provided by ICRISAT, Hyderabad, India and tomato (*Lycopersicon esculentum*) 'Moneymaker' variety. All were grown in plastic pots on John Innes no. 2 potting compost at 28 ± 2^{o}C in a glasshouse with a 14:10 hr light:dark cycle and a relative humidity of 60%. Plants were used at 5 weeks old. The surface area of leaves was measured using a Quantimet 520-image analyser (Leica Microsystems Cambridge Ltd., UK). Thus, the concentration of different compounds in a sample could be equated to an area of leaf surface to ensure that insects were presented with naturally occurring concentrations during feeding bioassays. These data together with the chemical analysis were used to calculate chemical concentration of leaf extracts in terms of unit area so that surface contamination bioassays could be calibrated to match concentrations found on leaf surfaces.

*Viral Bioassays***.** To assess OB activity both leaf dip and surface contamination neonate larval bioassays were used under standard larval rearing conditions, 26^{o}C with a 14/10 hour light dark cycle. In the leaf dip assays a standard methodology was used (Evans and Shapiro, 1997). The *Hear*NPV stock suspensions were prepared as fivefold dilution series in 50 ml of 0.02 % Triton X-100 immediately prior to use in bioassays. The leaves were cut from the plant at the stem and dipped in the *Hear*NPV dilutions. Control leaves were dipped in 0.02 % Triton X-100 only. After dipping, the stem of the treated leaves was mounted in molten agar in 250 ml round plastic containers, either one cotton leaf, two tomato leaves and six compound chickpea leaves were used per container ; fifty neonate larvae less than 18 hours old were used for each treatment with 25 being placed in each container. Larvae were allowed to feed on the leaves for 24 h, after which they were transferred to 25 ml individual pots and reared individually on clean artificial diet, the mortality was recorded after 5 and 7 days. To ascertain OB activity separately from leaf surfaces OB treatments the mass surface contamination bioassay was employed (McKinley, 1985; Jones, 2000). Again fivefold series dilutions of OBs in distilled water were prepared and then dispensed as 75 µl aliquots onto the surface of artificial diet in 30ml plastic pots, spread evenly by tilting and left to dry. Two larvae were added to each pot, reared for 7 days under standard conditions and mortality counted on days 5 & 7. Fifty larvae were used for each treatment replicate. All assays were replicated 5-7 times with each assay including a control and a stock solution positive control and the results were subjected to probit analysis (Finney, 1971) in SPSS. Comparisons of LC_{50} were performed on log transformed data, to equalize variances, using ANOVA procedure in SIGMASTAT software and treatment means were compared using LSD test. In some bioassays where means differed by several orders of magnitude transforming the data did not normalize variances so the non-parametric Kruskal-Wallis test with Tukey multiple comparison procedure was adopted.

Effect of exposure of Hear*NPV to cotton, tomato and chickpea leaf surfaces*. To study plant surface chemistry and its effect on *Hear*NPV, OBs suspended in distilled water were applied to the leaf surfaces on whole plants at a concentration of $3x10^7$ OB ml^{-1} in 0.02% triton using a hydraulic hand sprayer and applied at a rate sufficient to evenly wet the leaves. The plants used in experiments were after application of OB maintained in the laboratory at 26°C under the 14/10 hour light dark cycle and the virus was then left on the leaves for 1 or 24 hr after which OBs were recovered using a standard washing technique in water containing 0.1% sodium dodecycl sulphate for one hr (Jones 1988). The samples and the OBs concentrated by centrifugation at 2500g at 5^{o}C for 30 min (Hunter Fuijita et al., 1998a). The supernatant was discarded, and the OBs were re-suspended in distilled water then stored at -20^{o}C prior to counting and bioassay. This procedure was found to have no significant effect on the LC_{50} of virus and recovery of OBs from leaf surfaces was ascertained to be >95%; similar to that reported by other workers using this technique (McKinley, 1985; Jones, 1988).

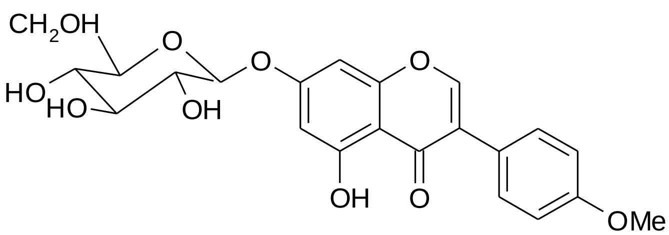
Analysis of organic acids in methanol extract of chickpea leaf surface by GC-MS. The surfaces of 50 leaves were extracted in methanol 300ml and analyzed by GC-MS. Purification of organic acid fraction was carried out according to Stumpf and Burris (1979). The residue was resuspended in pyridine (50μl) (Sigma-Aldrich) with a glutaric acid internal standard (1mg ml^{-1}) (Sigma-Aldrich). Ten min before injection 25 μl of *N*, *O*-bis (tri-methylsilyl)-acetamide (Supelco) was added; the vial shaken and left to stand at room temperature for 5 min before injection. GC-MS was carried out on a Hewlett Packard HP6890 GC linked to an Ion detector (HP 5973 Mass Selective Detector) operated in Electron Ionisation (EI) mode. A fused silica capillary column (30 m x 0.25 mm i.d., coating 0.25um) coated with non-polar HP-5MS (5% Phenyl Methyl Siloxane, Agilent 1909 IS-433) was used with a split/splitless injector and helium as a carrier gas (0.5kg cm^{-2}). The oven temperature was held at 60°C for 2 min and then raised to 250°C at 6°C per min. Compounds were identified by comparing EI-MS and GC retention indices with synthetic standards under the same operating conditions. A set of organic acid standards as reported to occur on chickpea leaf surfaces (Rembold and Weigner, 1990) was prepared in sterile distilled water, derivatised and analysed as described above.

Effect of organic acids present on the chickpea leaf surface on the infectivity of OBs against H. armigera *neonates***.** Organic acids (Sigma Aldrich, USA) were mixed together, at the concentration present on leaf surface as determined above, in 10 ml of sterile distilled water. A sample of *Hear*NPV (1 x 10^{10}OB) was added to the organic acid solution and then left in a rotator at 30 rpm for one hr. OBs were then recovered by centrifuging at 2500g for 30 minutes then re-suspended in 5 ml of distilled water and counted. Serially diluted suspensions of OBs in distilled water were bioassayed alongside a control OB suspension not exposed to the organic acids.

HPLC analysis of chickpea leaf extracts after spraying with OB *suspension.* To determine the effect of *Hear*NPV OBs on the chickpea leaf chemistry, a suspension of 3 x 10^7 OB ml^{-1} in 0.02% Triton was sprayed onto to the leaf surfaces of whole plants using a hydraulic hand sprayer sufficient to evenly wet the leaves. Control plants were sprayed with 0.02% Triton. The leaves were excised within 5 min or after 1, 4 or 24 h after spraying and surface extracted in methanol for 40 sec, and the extracts filtered (Whatman No. 1), and evaporated to dryness under reduced pressure. The dried extracts were redissolved in 1 ml of 100% HPLC grade methanol for analysis. Aliquots (10 ul) were injected onto a reverse-phase column (Spherisorb 5ODS analytical column, 4.6 mm i.d. x 250 mm) and eluted at 1 ml/min using the gradient 90% A: 10% B at t = 0 min to 50% A: 50% B at t =20 mins to 20% A: 80% B at t = 25 mins to 100% B at t = 30 mins and 90% A: 10% B at t = 37 mins (A is 2% acetic acid and B is 2% acetic acid in acetonitrile).

Isolation of leaf surface compounds and their effect on the activity of Hear*NPV OBs against* H. armigera *larvae.* Compounds **1** and **2** were isolated by repetitive HPLC as described above and fractions were collected manually at approximately 22 and 29 min.

The combined fractions were evaporated under reduced pressure and weighed. LC-MS was carried out on a Thermo-Finnigan LC/MS/MS system consisting of a ‘Surveyor’ autosampling LC system interfaced to a LCQ Classic quadrupole ion trap mass spectrometer. Chromatographic separation was performed on a 150 mm × 4.6 mm i.d. (5 μm particle size) Phenomenex Luna C18 column using a linear mobile phase gradient of 1 ml min^{-1} flow rate with water (A): MeOH (B): 5% Acetic Acid in MeOH (C). Initial conditions were 80% A, 0% B and 20% C changing to 0% A, 80% B and 20% C at t = 20 min and maintained at these conditions to t = 25 min. Injection volume was 10 μl and data analysis was performed using Xcalibur 1.2 software. The ion trap MS was fitted with an Atmospheric Pressure Chemical Ionisation (APCI) source operated under standard conditions; i.e. vaporiser temperature 450 ºC, needle current 5 mA, heated capillary temperature 150 ºC, sheath and auxiliary nitrogen gas pressure 80 and 20 psi, and the source voltages tuned for the optimal transmission of protonated rutin. The ion trap was set to monitor ions from *m/z* 125-1200 with collision energy of 45 %. Authentic samples of genistein, daidzein, pratensein, biochanin A and formononetin (Aldrich-Sigma) were co-chromatographed with methanol leaf extracts of chickpea leaf surface that had been sprayed with *Hear*NPV (suspended in 0.02% Triton X-100) and indicated that **2** was biochanin A. Compound **1** had a similar UV spectrum to **2** but eluted earlier (22 min) indicating a more polar nature and suggesting a glycoside. An aliquot of **1** that had been isolated from the leaf extracts as described above was analysed by LC-MS and recorded a molecular ion signal in positive mode $[M + H]^+$ at *m/e* = 447 indicating the molecular weight of 446 and a molecular formula $C_{22}H_{22}O_{10}$. Comparison of the mass spectrum with the library confirmed the structured to be biochanin A 7-*O*-glucoside (sissotrin) with good match in the lower range (*m/e* =100-300) of the spectrum. For example, the signal observed at $[M + H]^+$ *m/e* = 285 indicated loss of a glucose moiety $[M - 162 + H]^+$ and corresponded to biochanin A with a base peak at *m/e* = 270 correlating to the loss of glucose and a methyl from the methoxy at C-4' and a further fragment at *m/e* = 253 correlating to $[M - 162 - OCH_3]^+$ with the loss of the methoxy group. Subsequent co-chromatography using an authentic standard of sissotrin from natural products collection at Royal Botanic Gardens, Kew, confirmed this identification.



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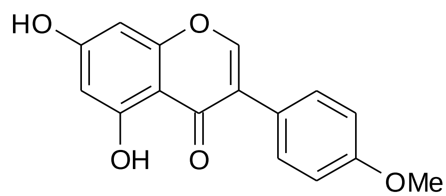

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Compounds **1** and **2** were used subsequently in bioassays to evaluate their effects on *Hear*NPV.

The surface area of the leaves was measured as described above. A 200 µl aliquot of sissotrin (25 µg ml^{-1}) in methanol containing the equivalent sissotrin from 1250 mm^2 of chickpea leaf surface and equal to the surface area of artificial diet in a 30ml container was placed onto the diet surface and allowed to evaporate. The control diets were treated with 200 µl methanol. *Hear*NPV concentrations on a five-fold dilution scale were prepared in distilled water. A control dose containing only distilled water was also prepared. An aliquot of each virus concentration was dispensed in a volume of 75µl onto the surface of the diet and allowed to dry after which 10 neonate larvae were released into each of the 5 pots. Larvae were allowed to feed for 24 hr and then were transferred to clean artificial diet pots at a rate of two per pot and reared under standard conditions and mortality recorded after 7 days. The experiment was replicated three times

Effect of biochanin A on the efficacy of Hear*NPV against* H. armigera *larvae.* Biochanin A (Sigma Aldrich, USA) was diluted to 500, 250, 100 and 10 ppm in distilled water and was also tested against *Hear*NPV. A 200 µl aliquot of biochanin A at 500, 250, 100 or 10 ppm was spread over the surface of artificial diet. Control pots were treated with same amount of biochanin A. Bioassays were carried out as described above for sissotrin with 50 larvae $treatment^{-1}$ and the experiment was again replicated three times.

RESULTS

Effect of cotton, tomato and chickpea plants on Hear*NPV against* H. armigera *larvae using a leaf dip bioassay method*. The leaf dip bioassay showed that exposure of *Hear*NPV on chickpea leaf could impair *Hear*NPV activity. The LC_{50} values (Fig 1) for the different plants were significantly different ($F = 14.6$, $df = 2,20$, $P = <0.001$) and the LC_{50} for *Hear*NPV on chickpea was of 3.96×10^4 OB ml-1 was significantly higher than that on tomato (2.65×10^3 OB ml^{-1}) and cotton (9.36×10^3 OB ml^{-1}). The result on tomato was not different from the mean LC_{50} of this virus strain obtained on artificial diet which was 2.78×10^3 OB ml^{-1}. The bioassays of *Hear*NPV OBs exposed to tomato, cotton and chickpea leaf surfaces also showed highly significant differences after 1 hr ($H = 10.851$, $df = 3$, $P = 0.017$) and 24 hr ($H=11.033$, $df = 3$, $P = 0.012$) (Fig 2); OBs on chickpea were markedly less infectious than OBs on tomato or cotton which did not differ significantly from the LC_{50} of unexposed control OBs. Thus, exposure of OBs to the surface of chickpea for 1 and 24 hr resulted in inactivation even after OBs were removed from the leaf surface. The LC_{50} values of *Hear*NPV OBs exposed to chickpea for 1 and 24 hr did not differ significantly, indicating that the observed inactivation reaches its maximum effect within one hr and exposure beyond that does not further affect OB infectivity.

Analysis of organic acids in methanol extract of chickpea leaf surface by GC-MS. The leaf surfaces of chickpea extracted with 100% methanol contained oxalic, malonic, malic, citramalic and citric acid (Fig 3). The compounds with retention times 13.47-13.48 and 16.01 min were silane impurities while those at 24.80-24.81 min were sugars. Glucose-6-phosphate, oxalacetate, succinic and fumaric acids were not found in any of the solvent extracts despite having been identified earlier by Rembold et al. (1980).

Effect of organic acids present on the chickpea leaf surface on the efficacy of Hear*NPV against* H. armigera *neonates.* The mean LC_{50} values of *Hear*NPV exposed to organic acids and for untreated *Hear*NPV using a surface contamination bioassay system to neonates of *H. armigera* were 8.05 x 10^2 OB ml^{-1} and 6.16 x 10^2 OB ml^{-1} respectively and were not significantly different ($t = 0.484$, $P = 0.762$).

HPLC analysis of chickpea leaf surfaces after spraying with NPV. Chickpea plants were sprayed with *Hear*NPV in a 0.02% Triton X-100 suspension (to optimize spreading) and surface extracted in methanol within 5 min and after 1, 4 and 24 hr. After 1 hr there was a more than four-fold increase in the concentration of **1** to 22 µg cm^{-2} compared with unsprayed leaf surfaces (5 µg cm^{-2}) in which the presence of **1** is constitutive. After 2, 4 and 24 hr the concentration of **1** was similar to pre spray quantities and remained there up to 24 hr. Analysis of control plants that were sprayed with 0.02%

Triton only also showed higher levels of **1** after 1hr indicating that the process of spraying in the absence of virus was itself sufficient to induce the production of this compound and was not induced by the presence of the *Hear*NPV.

Effect of sissotrin on the efficacy of Hear*NPV against* H. armigera *larvae*. The mean LC_{50} after exposure of *Hear*NPV to sissotrin for 1 hr at a concentration equivalent to that found on the leaf surface after spraying was 1.23 x 10^4 OB ml^{-1} and was significantly higher than untreated *Hear*NPV at 2.30 x 10^3 OB ml^{-1} ($F = 44.24$, df = 1,4, $p = 0.003$). However, this increase in LC_{50} for sissotrin treated *Hear*NPV are small compared to the LC_{50} values when *Hear*NPV OBs were exposed to chickpea plant surface for 1 hr suggesting that sissotrin does reduce the efficacy of *Hear*NPV but does not account for all the inhibition observed when *Hear*NPV was applied to the leaf.

The mean LC_{50}s of *Hear*NPV after exposure to different concentrations of biochanin A are shown in Fig.4. There was a significant difference ($F = 4.16$, df = 4, 10, $p = 0.031$) between the treatments and it was shown using least significant difference tests that mean LC_{50} values for *Hear*NPV exposed to biochanin A were not significantly different from each other but were significantly greater than the untreated sample, indicating that biochanin A even at concentrations as low as 10 ppm. As with sissotrin, however, the effect of biochanin A does not explain fully the 5-fold increase in LC_{50} seen in *Hear*NPV after exposure on chickpea plants suggesting that other factors must be involved.

DISCUSSION

This study showed that the efficacy of *Hear*NPV OBs was inhibited considerably more on chickpea than on cotton and that the effect was caused, at least in part, by surface isoflavonoids and not by organic acids. This was surprising since chickpea leaf surfaces have pH of <3 due the presence of organic acids (Rembold and Weigner, 1990), and there is a well known association between low pH with NPV inactivation (Ignoffo and Garcia, 1966). This study has also demonstrated that the inactivation of OBs on leaves is caused by their direct interaction with surface chemicals since OBs that had been exposed to the leaf surface were still inactive once removed and thus differs from the mechanism of peroxidase inactivation reported previously for cotton (Hoover et al., 1998a; 1998b,). The present work does not support an earlier proposition that the reduced efficacy of *Hear*NPV on chickpea could be related to a slower feeding rate of *H. armigera* on chickpea, thus reducing the rate of OB ingestion (Rabindra et al., 1992). Sissotrin accumulated on the leaf surface at least for a short period of time after plants that were sprayed with the OB suspension in 0.02% Triton or even with the 0.02% Triton control. This indicates that the process of spraying was sufficient to induce the production of these compounds and was not induced by the presence of the *Hear*NPV. Thus the induction of these compounds is not a specific response to the application of *Hear*NPV but a response to either wetting or the presence of surfactant. The increased secretion of biologically active antimicrobial compounds by chickpea in response to wetting would be biologically explicable as chickpea is subject to the damaging fungal diseases such as Botrytis grey mould during periods of heavy dew or precipitation (Pande et al., 2005).

Plant chemicals have previously been shown to inhibit OB dissolution by binding irreversibly to OB structural proteins (Schultz and Keating, 1991), a mechanism that is enhanced at least for orthodihydroxy moieties in the presence of peroxidases and polyphenoloxidases, particularly in damaged plant tissues (Felton and Duffey, 1990). The present data do not shed light on the mechanism by which isoflavonoids impair NPV infectivity. Further work to understand this would be useful since the inactivation mechanism reported here may impact on other biological pesticides such as Bt or entomopathogenic fungi, given that chickpea isoflavonoids are toxic to numerous organisms including viruses, bacteria, fungi and insects (Aslam et al., 2009; Getti et al., 2007; Ito et al., 2003; Simmonds and Stevenson, 2001; Stevenson and Haware, 1999; Stevenson et al., 1997). The identification of a new group of compounds affecting OBs, however, adds to the existing literature on this topic and the importance of the finding is highlighted by the LC_{50}s of OBs exposed on leaf surfaces being 3-5 orders of magnitude greater than that reported in cotton in both the present and earlier studies (Young and Yearian, 1974; Forschler et al., 1992). It is not known if this mechanism is present or as profound in all chickpea varieties. However, selective breeding for disease resistance (Pande et al., 2005) may have resulted in varieties with more biologically active compounds and may explain the high OB inactivation reported here.

This study showed that *Hear*NPV OBs were inactivated when consumed on cotton leaf material, but showed no sign of inactivation when bioassayed on diets after exposure on and then removal from cotton; a result that concurs with those of Hoover et al. (1998a; 1998b). However, there was no evidence of the OB inactivation by ionic cotton gland secretions reported previously (Ellerman and Entwistle, 1985) on Ankur 651, the cotton variety tested here. This may again be explained by varietal differences in the chemistry of Ankur 651 and the Deltapine varieties studied earlier. Some Indian cotton are reportedly more detrimental to OB infectivity than chickpea (Rabindra et al., 1994).

While sissotrin and biochanin A have a significant inactivating action, the magnitude of inactivation by these compounds did not fully account for the effects observed on leaf surface assays. Therefore, other chemicals are likely to contribute to this inactivation and further work will be required to identify these.

In considering the results reported here it may be surprising that *Hear*NPV is effective as a biopesticide on chickpea (Jayaraj et al., 1987; Rabindra et al., 1989; Cherry et al., 2000; Ahmed and Chandel, 2004). However, on some crops 90% of *H. armigera* larvae killed by *Hear*NPV sprayed onto plants acquire the infection within one hr of application (D Murray, pers. comm.). The interaction of *Hear*NPV with chickpea may also be influenced by the variety of chickpea. Cowgill and Bhagwat (1996) for example reported a field trial in which *Hear*NPV was more effective at killing *H. armigera* when applied to the *H. armigera* susceptible genotype (ICCC 37) of chickpea than on a *H. armigera* resistant genotype (ICC 506). This may have been due to differences in their chemistry since the production of isoflavonoids in chickpeas is known to vary between cultivars at least in association with resistance to plant pathogens such as Botrytis and Fusarium (Stevenson et al., 1997).

Additives, including milk powder, casein, molasses and Robin blue dye are reported to improve *Hear*NPV performance on chickpea (Rabindra et al., 1989) and although it has been assumed that they improved UV stability (Rabindra and Jayaraj, 1988) given the present findings, it is possible that some additives may also contribute to improving OB efficacy by inhibiting chemical inactivation of OBs or by encouraging feeding and rapid viral acquisition before the OB inactivation processes have taken effect.

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