Plant Phenolics as Radiation Protectants for the Beet Armyworm (Lepidoptera: Noctuidae) Nucleopolyhedrovirus

Martin Shapiro,3 Said El Salamouny,4 B. Merle Shepard,2,3 and D. Michael Jackson5,6

ABSTRACT Thirteen phenolics were tested as ultraviolet (UV) protectants for the nucleopolyhedrovirus (SeMNPV) of the beet armyworm, *Spodoptera exigua* (Hübner). After 30-minute exposure to UVB radiation (in the 280–320 nm range), eleven SeMNPV/phenolic combinations provided good to excellent UV protection when used at a concentration of 0.050 M. At a concentration of 0.0050 M caffeic acid, chlorogenic acid, esculin, gallic acid, and tannic acid still provided good UV protection. As the concentration was reduced to 0.0005 M, only caffeic acid, chlorogenic acid, and gallic acid provided some UV protection.

KEY WORDS Plant phenolics, *Spodoptera exigua*, Insecta, ultraviolet radiation, protectants

One of the major limitations to the widespread use of insect pathogens, especially baculoviruses, is their sensitivity to sunlight (David & Gardner 1966, Jaques 1968, Sajap et al. 2007), particularly to the UVB portion (280–320 nm) of the solar spectrum (David 1969, Griego et al. 1985, Shapiro & Domek 2002). Under field conditions, the activity of the nucleopolyhedrovirus (HzSNPV) from the cotton bollworm, *Helicoverpa zea* (Boddie), was greatly reduced within the first 24 h after application (Bullock 1967, Young & Yearian 1974). Jones et al. (1993) demonstrated that there was greater than 90% inactivation of the nucleopolyhedrovirus (SpliNPV) from the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval), within four hours and greater than 99% inactivation within eight hours under natural conditions. In the case of the nucleopolyhedrovirus (SpltNPV) from the tobacco cutworm, *Spodoptera litura* (F.), “complete inactivation occurred after 12 h of direct sunlight” (Sajap et al. 2007).
For more than a decade, we have been investigating the use of plant materials as insect control agents (Shapiro et al. 1994, Abudulai et al. 2001), virus enhancers (Shapiro et al. 2007a,b), and sunlight protectants (Shapiro et al. 2008, El Salamouny et al. 2009). We showed that an aqueous green tea, *Camellia sinensis* L., was an excellent ultraviolet (UV) radiation protectant for the nucleopolyhedrovirus (SeMNPV) from the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), under both laboratory and field conditions (Shapiro et al. 2008). Subsequently, we demonstrated that black tea (El Salamouny et al. 2009) and selected medicinal herbs and spices (Shapiro et al. unpublished data) provided excellent UV protection for SeMNPV under laboratory conditions. In our initial study on plants as UV protectants, we selected green tea because it is an excellent source of antioxidants (Prior & Cao 1999, Katiyar 2003, Chen et al. 2009) as well as a rich source of UVB-absorbing polyphenols (Bradford & Penny 1948, Coxon et al. 1972, Caffin et al. 2004). Our studies on green tea and black tea (Shapiro et al. 2008, El Salamouny et al. 2009) utilized aqueous extracts. However, we did not determine whether UV protection was due to a single compound or to an interaction of chemicals. Our present study was undertaken to determine whether individual phenolic chemicals occurring in tea (Kuhr & Engelhardt 1991, Pan et al. 2003, Caffin et al. 2004) and other plants (Kim et al. 2000, Soong et al. 2006, Zhang et al. 2008) could also act as UV protectants for SeMNPV.

**Materials and Methods**

**Insects, virus inoculum.** The colonized strain of the beet armyworm, established and maintained by USDA-ARS, Tifton, GA, was used. Larvae were reared on the Multiple Species Diet (Southland Products, Inc., Lake Village, AR). The nucleopolyhedrovirus (SeMNPV) for beet armyworm larvae, registered as Spod-X®, was obtained from Certis USA (Columbia, MD).

**Phenolics.** Thirteen phenolics (caffeic acid, caffeine, chalcone, chlorogenic acid, ellagic acid, esculin, ferulic acid, gallic acid, morin, quercetin, rutin, tannic acid, and theophylline) were obtained from Sigma-Aldrich Chemicals (St. Louis, MO) as technical powders. Each phenolic was weighed and added to distilled water to obtain a standard concentration of 0.050 M. Subsequently, 10- and 100-fold dilutions were made from each phenolic solution.

**Radiation source.** Radiation was provided by two 15-watt UVB tubes (382 mm long, Fotodyne, Inc., New Berlin, WI), which were mounted in parallel within a Pelco UV-2 cryo chamber (Ted Pella, Inc., Redding, CA) and were 203.2 mm above the test dishes (refer to Shapiro & Domek 2002 for radiation emission profiles).

**Exposure of SeMNPV to UVB irradiation.** Preliminary bioassays were conducted to determine the virus concentration that caused 90–95% mortality prior to exposure to UVB radiation. As a result of these assays, SeMNPV was diluted in distilled water (controls) or in a phenolic solution to obtain a final virus concentration of $1 \times 10^6$ viral occlusion bodies (OBs) per ml ($=74.7$ OB/mm$^2$ of diet surface). Phenolic concentrations of 0.0500 M, 0.0050 M, and 0.0005 M were tested. Four milliliters of virus suspension was pipetted into a 60 × 15 mm glass Petri dish (Fisher Scientific, Pittsburg, PA) that was exposed to UVB radiation for 30 min (there also was an untreated control of virus suspension in distilled water). After...
the exposure period, the volume was determined and distilled water was added to each dish to replace water lost by evaporation. Lids were then placed on all dishes, and dishes were stored at 4°C until they were used for bioassays.

**Bioassay procedure.** When dishes were removed from the refrigerator, 0.1 ml of virus suspension (SeMNPV + distilled water or SeMNPV + phenolic solution) was applied to the surface of each 30-ml cup containing Multiple Species Diet. In addition, non-irradiated SeMNPV (74.7 OB/mm²) was applied to a companion set of cups. Second instars (5-day-old larvae) were placed individually into each cup and larvae were reared at 27°C. Tests were repeated six times with 10 larvae per treatment, 10 untreated larvae, and 10 phenolic-treated controls per replicate. Mortality was assessed initially at day 5 and every 2–4 days thereafter until day 14.

**Statistical methods.** Data were first converted to percent original virus activity remaining (OAR) by dividing the mortality in a phenolic treatment by the mortality for the non-irradiated control and multiplying this figure by 100. Converted data were then analyzed by Analysis of Variance (ANOVA) using SAS PROC GLM (SAS 1999). After ANOVA, treatment means for each concentration of phenolic were separated according to Fisher’s Protected Least Significant Difference (LSD) Test. For each phenolic compound, the effect of the three rates was analyzed by regression analysis using SAS PROC REG (SAS 1999).

**Results**

Irradiation of the aqueous suspension of SeMNPV for 30 min reduced virus-caused mortality from 98% (non-irradiated control) to 3% (irradiated control). When tested at the highest phenolic concentration (0.0500 M), five phenolics (caffeine, chlorogenic acid, esculin, morin, and quercetin) provided excellent UV protection (greater than 90% OAR) that was not statistically different from the non-irradiated control (Table 1). Another group of six phenolics (cafeic acid, ferulic acid, gallic acid, rutin, tannic acid, and theophylline) provided good (78–84% OAR) UV protection at the highest concentration. On the other hand, two phenolics (chalcone and ellagic acid) gave significantly less protection (31% and 62% OAR, respectively) (Table 1).

At a concentration of 0.0050 M, five phenolics (caffeic acid, chlorogenic acid, esculin, gallic acid, and tannic acid) still provided good to excellent UV protection (OAR greater than 76%) (Table 1). However, OARs for caffeine and quercetin were reduced by 40–50 (Table 1). Rutin, theophylline, and feulic acid lost 80–90% of their UV protective activity as the concentration was reduced ten-fold. Chalcone, ellagic acid, and morin provided no UV protection at the 0.0050 M concentration (Table 1).

When the phenolic concentration was reduced to 0.0005 M, only caffeic acid (≈27% OAR), chlorogenic acid (≈21% OAR), and gallic acid (≈29% OAR) provided UV protection greater than water alone (Table 1). Regression analysis revealed that there was a significant positive concentration effect for each of the phenolics tested.

**Discussion**

For more than 30 years there has been an effort to minimize the detrimental effects of UV radiation on baculoviruses by testing UV absorbers (Jaques 1971,
Table 1. Average original virus activity remaining (OAR) for beet armyworm nucleopolyhedrovirus (SeNPV) mixed with various phenolics, exposed to ultraviolet light (UVB), and tested against second-instar beet armyworms.

| Phenolic treatment | Original virus activity remaining (OAR) | phenolic concentration of
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0500 M</td>
<td>0.0050 M</td>
</tr>
<tr>
<td>Distilled water (no UVB)</td>
<td>100 a b A c</td>
<td>100 a b A c</td>
</tr>
<tr>
<td>Quercetin</td>
<td>100 a A</td>
<td>57 e B</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>96 a A</td>
<td>88 bc B</td>
</tr>
<tr>
<td>Esculin</td>
<td>94 a A</td>
<td>94 ab A</td>
</tr>
<tr>
<td>Caffeine</td>
<td>94 a A</td>
<td>41 f B</td>
</tr>
<tr>
<td>Morin</td>
<td>94 a A</td>
<td>0 h B</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>84 b A</td>
<td>86 bcd A</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>84 b A</td>
<td>82 cd A</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>84 b A</td>
<td>76 d A</td>
</tr>
<tr>
<td>Rutin</td>
<td>84 b A</td>
<td>17 g B</td>
</tr>
<tr>
<td>Theophylline</td>
<td>80 b A</td>
<td>17 g B</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>78 b A</td>
<td>8 gh B</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>62 c A</td>
<td>0 h B</td>
</tr>
<tr>
<td>Chalcone</td>
<td>31 d A</td>
<td>0 h B</td>
</tr>
<tr>
<td>Distilled water (30 min UVB)</td>
<td>3 e A</td>
<td>4 h A</td>
</tr>
</tbody>
</table>

aSeMNVP used at a final concentration of 74.7 OBs/mm² of diet surface. Six replicates; 10 larvae per treatment per replicate; 10 untreated control per replicate; 10 phenolic-treated larvae per replicate. Before bioassaying, the virus was exposed to UVB in distilled water (standard) or in phenolic solution for 30 min.

bMeans within a column followed by the same lowercase letter were not significantly different (SAS GLM Procedure, Fisher Least Significant Difference Test at P < 0.05).

cMeans within a row followed by the same uppercase letter were not significantly different (SAS GLM Procedure, Regression Analysis at P < 0.05).
flavonoids, lignins, salicylates, stilbenes, and tannins) also are synthesized via the phenylpropanoid pathway in response to various stresses (Dixon et al. 2002, Ferrer et al. 2008, Kawasaki et al. 2006), including UVB radiation (Möhle & Wellman 1982, Libkind et al. 2004, Brandt et al. 2008), and are effective UV protectants (Stapleton & Walbot 1994, Bieza & Lois 2001, Close & McArthur 2002). Twenty-four phenolics were identified from green tea (Caffin et al. 2004), and UV absorbance profiles indicated that each of these had maximal absorbance in the UVB portion of the solar spectrum (Bradfield & Penny 1948, Roberts & Williams 1958, Coxon et al. 1972, Caffin et al. 2004).

In a previous study, we determined that a 1% (wt:wt) green tea extract provided excellent (greater than 90% OAR) protection following UVB irradiation (30 min) in laboratory tests. Under field conditions, 1% and 5% green tea extracts were ineffective as UV protectants for SeMNPV, but at a 10% concentration, some UV protection was provided. UV protection further increased in a concentration-dependent manner as extract concentrations increased to 20% and 30% (Shapiro et al. 2008). Subsequently we found that black tea and lignin also provided excellent UVB protection for SeMNPV following irradiation for as long as 300 min (El Salamouny et al. 2009). In addition, we tested 64 medicinal herbs and spices and found that 27 of them provided excellent UV protection for SeMNPV (UVA/UVB irradiation, 30 min). When these 27 extracts were subjected to a more severe UV treatment (UVB, 30 min), 15 provided excellent UV protection. Subsequently, other samples from the 15 extracts were irradiated for 300 min (UVB) and three (kudzu, peppermint, skullcap) provided almost complete UV protection for SeMNPV (Shapiro et al. unpublished data).

Plants are a rich source of antioxidants (Miller et al. 2000, Szeto et al. 2002, Halvorsen et al. 2006), which have been shown to play a role in disease prevention (Duthrie et al. 2000, Blomhoff 2005, Serafini 2006). Theaflavins in black tea and catechins in green tea are excellent antioxidants (Valcic et al. 1999, Leung et al. 2001, Lee & Lee 2002). Extraction of these compounds increases as brewing time at 90°C is increased (Robinson et al. 1997, Prior & Cao 1999, Langley-Evans 2000). In our experiments, however, we observed no increase in UV protection as the brewing time was increased from 5 to 60 min. From this, we inferred that UV absorption by tea phenolics (Caffin et al. 2004) played a more important role in UV protection than did antioxidants in green tea (Shapiro et al. 2008). Based upon our results that demonstrated UV protection for SeMNPV from a wide variety of plant compounds and because phenolics act as UVB absorbers (Escandar & Sala 1991, Steerenberg et al. 1997, Taniguchi et al. 1997), we set up a test to determine whether plant phenolics could protect SeMNPV from UVB radiation. It has also been reported that several phenolics are induced by UVB radiation (Kalbin et al. 2005, Swinny & Ryan 2005, Izaguirre et al. 2007) and some of them possess antioxidant activity (Bors & Michel 1999, Kreft et al. 2002, Jiang et al. 2008).

For our study, we used these criteria to assess UV protection: (1) the virus concentration (OBs/ml) should cause approximately 90–95% larval mortality prior to irradiation; (2) the UV exposure should decrease virus-caused mortality to less than 10%; (3) UV protection should be measured in terms of original activity remaining (％OAR) post-irradiation (Ignoffo & Batzer 1971); and (4) our criterion for excellent UV protection should be above 90% OAR (Shapiro et al. 1983). Using these criteria, $1 \times 10^6$ OBs/ml were used as the LC$_{95}$ of SeMNPV. In
our laboratory tests, virus-caused mortality was reduced about 94% following 30 min of UVB irradiation of SeMNPV in distilled water (Table 1). At a concentration of 0.050 M, five of the phenolics (caffeine, chlorogenic acid, esculin, morin, and quercetin) provided greater than 90% UV protection for SeMNPV, five (caffeic acid, gallic acid, rutin, tannic acid, and theophylline) provided 80–90% protection, two provided 60–80% protection (ellagic acid and ferulic acid), and one (chalcone) provided only about 30% protection (Table 1). As the phenolic concentration was reduced 10-fold to 0.0050 M, two phenolics (esculin, gallic acid) retained approximately 90% of their effectiveness.

On the other hand, around 60% of the phenolics (caffeine, chalcone, ellagic acid, ferulic acid, morin, quercetin, and rutin) lost greater than 50% of their effectiveness as UV protectants. Surprisingly, caffeic acid, chlorogenic acid, and gallic acid still provided some UV protection (over 20% OAR) at a concentration of 0.0005M (Table 1). Although caffeic acid, chlorogenic acid, and gallic acids are UV absorbers (Hulme 1953, Katwa et al. 1981, Slawińska et al. 2007, Sun et al. 2007), as well as antioxidants (Soderquist et al. 1990, Olthof et al. 2001, De Leonardis et al. 2008), we do not know why they were more effective UV protectants than the other 10 phenolics tested. Although we have demonstrated that individual phenolics can provide UV protection for SeMNPV, we do not know whether additive or synergistic effects could be obtained with combinations of phenolics. Based upon our results, however, we feel that phenolics should be further investigated as adjuvants for insect pathogenic viruses in pest management of agriculturally important insects.

Acknowledgements

We thank Paul Goforth, Mark Schaffer, and Chad Smith for their excellent technical assistance. Technical Contribution No. 5538 of the Clemson University Experiment Station.

References Cited


Shapiro, M., J. L. Robertson & R. E. Webb. 1994. Effects of neem seed extract upon the gypsy moth (Lepidoptera: Lymantriidae) and its nuclear polyhedrosis virus. J. Econ. Entomol. 87: 356–360.


Soong, Y-Y. & P. J. Barlow. 2006. Quantification of gallic acid and ellagic acid from longan (Dimocarpus indica Lour.) seed and mango (Mangifera indica L.) and their effects on antioxidant activity. Food Chem. 97: 524–530.


