Fruit and Vegetable Extracts as Radiation Protectants for the Beet Armyworm Nucleopolyhedrovirus[1]

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ABSTRACT Extracts from 37 fruits and vegetables were tested as ultraviolet (UV) protectants for the nucleopolyhedrovirus (SeMNPV) of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Only one extract (black currant) provided almost complete protection following UVB/UVA irradiation for 30 minutes under laboratory conditions. As a group, fruit and vegetable extracts were significantly less effective than published values for herb and spice extracts. Based on analyses of antioxidant capacity and total phenolic content of selected plants, it was determined that (1) herbs and spices contained much higher levels of antioxidants and phenolics than fruits and vegetables, (2) neither high levels of antioxidants nor high levels of phenolics alone could account for UV protection, and (3) selection of extracts with high levels of both antioxidants and total phenolics resulted in increased UV protection.

KEY WORDS *Spodoptera exigua*, Insecta, ultraviolet radiation, protectants, SeMNPV

Because of their specificity and safety, insect viruses are attractive alternatives to broad-spectrum insecticides and ideal components of integrated pest management (IPM) systems (Lacey et al. 2001). Viruses have been used successfully in cropping systems in Brazil (Moscardi 1999, Oliveira et al. 2006), China (Gelernter 2007, Sun & Peng 2007), and India (Skovmand 2007). On the other hand, they have not been used widely in North America, despite efforts expended in the private and public sectors (Black et al. 1997, Ravensberg 2011). The main reasons for the limited use of insect viruses in pest control are: (1) slowness of kill, (2) susceptibility to solar radiation, (3) costs of production, and (4) reliance on chemical pesticides (Lacey et al. 2001, Rosell et al. 2008).

UV absorption, antioxidant activities, and radical scavenging are critical for UV protection in plants (Close & McArthur 2002, Agati et al. 2007, Grace 2011), and much of this activity has been attributed to plant phenolics (Cai et al. 2004, Huang et al. 2005, Surveswaran et al. 2007). For the past several years, we have

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studied the use of plant extracts as virus enhancers (materials that increase infectivity) (Shapiro et al. 2007a,b) and sunlight protectants (Shapiro et al. 2008, 2012, El Salamouny et al. 2009a,b). Aqueous extracts of green tea and black tea, *Camellia sinensis* (L.) (Theaceae); cocoa, *Theobroma cacao* L. (Malvaceae); and coffee, *Coffea arabica* L. (Rubiaceae), provided excellent UV protection for the nucleopolyhedrovirus (SeMNPV) from the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), under both laboratory and field conditions (Shapiro et al. 2008, El Salamouny et al. 2009a,b). Subsequently, we screened extracts from 67 herbs and spices as UV protectants for SeMNPV under different UV regimens. Initially, all SeMNPV/aqueous extracts (1%) were exposed to one UVB and one UVA (UVB/UVA) tube for 30 minutes, and those combinations that retained at least 90% of original activity post radiation were selected for the next UV regimen (Shapiro & Domek 2002). While unprotected SeMNPV lost approximately 90% of its activity (i.e., virus-caused mortality was reduced from 98% to 9%), 25 of 67 extracts were successful in protecting SeMNPV, and the most effective ones were then exposed to two UVB tubes for 30 minutes (Shapiro & Domek 2002). Fifteen of these SeMNPV/extract combinations provided at least 90% UV protection, so they were subsequently exposed to two UVB tubes for 300 minutes (El Salamouny et al. 2009a). In this final exposure scheme, extracts of kudzu, *Pueraria lobata* (Willd.) Ohwi (Fabaceae); peppermint, *Mentha* × *piperita* L. (Lamiaceae); skullcap, *Scutellaria lateriflora* L. (Lamiaceae); and thyme, *Thymus vulgaris* (Lamiaceae) still provided at least 90% UV protection after radiation (Shapiro et al. 2009a). An extract of kudzu was chosen for subsequent field testing. Under field conditions, a 5% kudzu formulation provided significant protection for SeMNPV, but a kudzu (5%)/cottonseed oil (0.5%) formulation provided significantly greater sunlight protection for at least seven days (Shapiro et al. 2012). In the present study, we expanded our search for UV protectants to include extracts from 37 fruits and vegetables (hereafter called fruit/vegetable extracts). We also compared the relative activities of the fruit/vegetable extracts with the published activities of 67 herb/spice extracts (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b).

Materials and Methods

Insects and virus inoculum. A colony of the beet armyworm was established and maintained at the Coastal Research and Education Center, Clemson University, Charleston, SC using the methods of Shapiro et al. (2008, 2009a,b). Larvae were reared on Multiple Species Diet (Southland Products, Inc., Lake Village, AR). The nucleopolyhedrovirus SeMNPV, registered as Spod-X® LC, was obtained from Certis USA (Columbia, MD). This product contains a minimum of two billion occlusion bodies (OB)/ml and it is formulated with inert ingredients to 0.64% OBs (Certis USA 2015).

Plant extracts. Thirty-seven types of fruits and vegetables were obtained from local grocery stores. The extracts were made from apple, *Malus domesticus* Borkh (Rosaceae); apricot, *Prunus armenica* L. (Rosaceae); avocado, *Persea americana* Mill. (Lauraceae); banana, *Musa accuminata* Colla (Muscaceae); bell pepper (yellow, orange, and red), *Capsicum annum* (L.) (Solanaceae); blackberry, *Rubus allegheniensis* Porter (Rosaceae); blueberry, *Vaccinum angustifolium* Aiton (Ericaceae); cantaloupe, *Cucumis melo* Naudin (Curcubitaceae); carrot,

Daucus carota L. (Apiaceae); celery, *Apium graveolens* L. (Apiaceae); cherry, *Prunus avium* L. (Rosaceae); chickpea, *Cicer arietinum* L. (Fabaceae); sweet corn (maize), *Zea mays* L. (Poaceae); cranberry, *Vaccinum macrocarpon* Aiton (Ericaceae); cucumber, *Cucumis sativus* L. (Curcubitaceae); currant (black), *Ribes nigrum* L. (Grossulariacaceae); dates, *Phoenix dactylifera* L. (Arecaceae); grape (red and white), *Vitis vinifera* L. (Vitaceae); honeydew melon, *Cucumis melo* L. (Curcubitaceae); Kiwano melon, *Cucumis metuliferus* E. Mey (Curcubitaceae); kiwi, *Actinidia deliciosa* Liang & Ferguson (Actinidaceae); mango, *Mangifera indica* L. (Anacardiaceae); onion, *Allium cepa* L. (Alliaceae); orange, *Citrus sinensis* (L.) (Rutaceae); papaya, *Carica papaya* L. (Cariceae); passion fruit, *Passiflora edulis* Sims (Passifloraceae); peach, *Prunus* (L.) (Rosaceae); pear, *Pyrus communis* L. (Rosaceae); plum, *Prunus domestica* L. (Rosaceae); pomegranate, *Punica granatum* L. (Lythraceae); raspberry, *Rubus idaeus* L. (Rosaceae); strawberry, *Fragaria* × *ananassa* Duchesne (Rosaceae); tomato, *Solanum lycopersicum* L. (Solanaceae); and watermelon, *Citrullus lanatus* (Thunb.) (Cucurbitaceae). Unprocessed fruits and vegetables were gently washed before extracts were made from them. Extracts were made by blending ten grams of a fruit or vegetable in 90 ml distilled water at room temperature and then filtering it through coarse cheesecloth. The filtrates were refrigerated (4°C) until used within two weeks.

Exposure of SeMNPV to UVB/UVA. Fruit/vegetable extracts were removed from refrigeration, diluted in distilled water, and combined with SeMNPV to obtain final concentrations of 10^6 OB/ml and 0.9% extract (wt:vol). Four milliliters of each virus-extract suspension was pipetted into a separate 60 x 15 mm Petri dish (Fisher Scientific, Pittsburg, PA) and exposed to UVB/UVA radiation for 30 minutes. Radiation was provided by one UVA tube and one UVB tube (15 watt, 382 mm, Fotodyne, Inc., New Berlin, WI) (UVB/UVA), that were mounted in parallel within a Pelco UV-2 Cryo Chamber (Ted Pella, Inc., Redding, CA) approximately 20 cm above the test dishes (see Shapiro & Domek 2002 for output and radiation emission profiles). After the exposure period, additional distilled water was added to each dish to replace the water lost by evaporation. This was done by weighing each dish before and after irradiation and adding back the necessary weight of distilled water.

Bioassays. Bioassays were conducted using 30-ml plastic cups (Sweetheart Cup Co., Chicago, IL) containing Multiple Species Diet. For each treatment, 0.1 ml of irradiated SeMNPV-extract suspension was pipetted onto the surface of the diet, which gave a final concentration of about 72 OBs/mm^2. Then, one second instar (5-day-old) beet armyworm was placed individually into each cup and reared for 14 days at 27°C. For each replication, ten cups were setup for each treatment, and the experiment had eight replications. Thus, there was a total of 80 larvae tested for each treatment, including controls. Water-control treatments consisted of irradiated SeMNPV with distilled water and non-irradiated SeMNPV with distilled water. Mortality was assessed initially at day 5, and every 3-4 days thereafter until day 14, when the test was terminated.

Statistical methods. Mean percent mortality and standard error of the mean were determined for each treatment based on survivability of 80 larvae after 14 days (eight replications of ten larvae each). Using the water controls as standards, mortality data were converted to the percentage of original activity remaining (% OAR) after UV exposure based on the value of

virus-caused mortality post-irradiation and pre-irradiation (Ignoffo et al. 1977, Shapiro 1989).

Percent OAR values for 37 fruit/vegetable extracts (present study) and 67 herb/spice extracts (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b) were correlated with published values for Oxygen Radical Antioxidant Capacity (ORAC) (Haytowicz & Bhagwat 2010) and Total Phenolic (TP) content (Haytowicz & Bhagwat 2010). In addition, using data from the present study, Shekar et al. (2012), and our published UV studies (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b), we correlated (1) antioxidant activity and % OAR, (2) UV absorption at 290 nm and % OAR, and (3) UV absorption at 320 nm and % OAR. Correlation coefficients (r^2) were determined using an online correlation coefficient calculator (Anonymous 2015). Means of % OAR, ORAC, and TP for fruit/vegetable extracts and herb/spice extracts were compared using a paired-t test (Steel & Torrie 1960).

Results and Discussion

Ultraviolet irradiation (UVB/UVA) for 30 minutes reduced SeMNPV-caused mortality from 95.3% for untreated NPV (non-irradiated water control) to 11.2% for UV-treated NPV (irradiated water control), which was a reduction in activity of 87.8% (Table 1). NPV-caused mortalities ranged from 13.8% (14.5% OAR) (SeMNPV with pomegranate extract) to 88.8% (93.2% OAR) (SeMNPV with black currant extract). Twenty-two of the 37 fruit/vegetable extracts (59.5%) provided less than 50% UV protection, and 14 fruit/vegetable extracts (37.8%) provided 50-76% UV protection (50-76% OAR) (Tables 1 & 2). However, only black currant extract provided UV protection over 90% (Table 1).

The mean % OAR for the 37 fruit/vegetable extracts in this study was 45.5±7.8% (±SEM), which compared to an average of 77.1±5.6% (±SEM) for 67 herb/spice extracts from published studies (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b). Only one of the 37 fruit/vegetable extracts (black currant) compared with 37% of the 67 herb/spice extracts provided at least 90% protection of SeMNPV (Table 2). While 59% of the fruit/vegetable extracts provided less than 50% UV protection, only 11% provided at least 70% UV protection. In contrast, only 18% of the herb/spice extracts provided less than 50% UV protection, while 72% of the herb/spice extracts provided at least 70% UV protection (Table 2). The average % OAR for the ten fruit/vegetable extracts with the highest values (68.3% OAR) was not significantly different ($P = 0.05$) from the average % OAR for the ten herb/spice extracts with the lowest values (64.9% OAR).

Because of the large difference between the overall UV protection provided by herb/spice extracts compared to fruit/vegetable extracts (Table 2), we searched the literature for possible factors that could lead to this disparity. Shekar et al. (2012) evaluated antioxidant activities and UV absorbance spectra of several herbal sunscreens. Using their data, we determined that antioxidant activity and UV absorption at 290 nm were highly correlated ($r^2 = 0.82$), while the correlation was lower ($r^2 = 0.56$) at 320 nm. From our published UV studies (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b) that included some of the same herbs and spices as Shekar et al. (2012), we found a significant correlation coefficient ($r^2 = 0.92$) between antioxidant activity and % OAR.

Table 1. UV protection provided to beet armyworm nucleopolyhedrovirus (SeMNPV) by extracts from 37 unprocessed fruits and vegetables after exposure to UVB/UVA for 30 minutes.

Treatment[a]	Percent mortality (± SEM)	% OAR[b]
Water control (non-irradiated)	95.3 ± 1.5	100.0
Currant (black)	88.8 ± 4.0	93.2
Banana	72.5 ± 4.9	76.1
Dates	67.5 ± 4.5	70.8
Raspberry	67.5 ± 4.5	70.8
Bell pepper (orange)	62.5 ± 3.7	65.6
Celery	60.0 ± 4.6	63.0
Cranberry	60.0 ± 3.8	63.0
Blackberry	58.9 ± 3.3	61.8
Blueberry	57.8 ± 3.9	60.7
Papaya	56.3 ± 3.2	59.1
Mango	55.0 ± 4.6	57.7
Peach	52.5 ± 3.7	55.1
Cucumber	50.0 ± 2.7	52.5
Apple	50.0 ± 3.8	52.3
Apricot	50.0 ± 2.7	52.3
Kiwano melon	47.5 ± 3.7	49.8
Grape (red)	47.5 ± 4.5	49.8
Chickpea	45.0 ± 3.3	47.2
Watermelon	45.0 ± 4.2	47.2
Bell pepper (yellow)	41.3 ± 3.0	43.3
Passion fruit	40.0 ± 4.6	42.0
Carrot	40.0 ± 2.7	42.0
Corn (maize)	38.8 ± 4.8	40.7
Cantaloupe	33.8 ± 3.8	35.5
Orange	31.3 ± 3.0	32.8
Grape (white)	31.3 ± 3.0	32.8
Bell pepper (red)	28.8 ± 3.5	30.2
Onion	28.8 ± 3.5	30.2
Cherry	27.5 ± 8.0	28.9
Honeydew melon	27.5 ± 3.1	28.9
Kiwi	25.0 ± 2.7	26.2
Strawberry	25.0 ± 3.3	26.2
Tomato	24.4 ± 1.9	25.6
Avocado	23.8 ± 3.8	25.0
Pear	21.3 ± 3.0	22.4
Plum	20.0 ± 2.7	21.0
Pomegranate	13.8 ± 1.8	14.5
Water control (irradiated)	11.2 ± 1.7	11.8

[a]SeMNPV plus 1% extract applied to diet surface at 0.1 ml for a final concentration of 72.0 OBs/mm^2 of diet surface; eight replicates with 10 larvae per treatment per replicate. Before bioassays, SeMNPV and plant extract (0.9% wt:vol) or distilled water (irradiated water control) were exposed to UV irradiation for 30 min.

[b]For % Original Activity Remaining (OAR), treatments were compared to the non-irradiated water control treatment, which was defined as 100% OAR.

Table 2. Percent of 37 fruit/vegetable extracts and 67 herb/spice extracts used as UV protectants for the beet armyworm nucleopolyhedrovirus (SeMNPV) in each percent original activity remaining (OAR) class.

	Percent of treatments in each % OAR[a] class					
Treatment[b]	<10.0%	10.0-29.9%	30.0-49.9%	50.0-69.9%	70.0-89.9%	90.0-99.9%
Fruits & Vegetables (n=37)[c]	0.0	24.3	35.1	29.7	8.1	2.7
Herbs & Spices (n=67)[d]	1.5	1.5	14.9	10.4	34.3	37.3

[a]Original Activity Remaining (OAR); all virus treatments were compared to a non-irradiated control (100.0% OAR).
[b]SeMNPV in 0.9% aqueous plant extract (wt:vol) was exposed to UVB/UVA irradiation for 30 min.
[c]Values from present study; mean %OAR was 45.5% for these 37 fruit/vegetable extracts.
[d]Values from published studies (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b); mean %OAR was 77.1% for these 67 herb/spice extracts.

However, there was little association between UV protection (% OAR) and UVB absorption at 290 nm ($r^2 = 0.05$) or 320 nm ($r^2 = 0.15$).

Based on studies of the association of antioxidant, free radical scavenging activities, and UV absorption (Chen & Ahn 1998, Shekar et al. 2012, Yu et al. 2012), we hoped to utilize these parameters and their relationships as predictors of UV protection for NPVs. Because comparative UV absorption data were not available for many of the plant extracts, we relied upon the association between (1) antioxidant capacity or activity with total phenolics (TP) (Velioglu et al. 1998, Cheung et al. 2003), (2) antioxidants with UV absorbance (Levizou & Manetas 2002, Yu et al. 2012), and (3) plant phenolics and UV absorption (Cerovic et al. 2002, Levizou & Manetas 2002), and correlated these values with % OARs from the present and published studies (Shapiro et al. 2002, 2007a,b, 2008, 2009a,b, 2012, El Salamouny et al. 2009a,b). For these comparisons, we relied upon published data on total phenolic (TP) content and Oxygen Radical Absorbance Capacity (ORAC) (Haytowicz & Bhagwat 2010). However, the USDA database (Haytowicz & Bhagwat 2010) included only 33 of the 37 fruits and vegetables we used in the present study and 26 of the 67 herbs and spices we used in previous studies (Shapiro et al. 2002, 2008, 2009a, 2012, El Salamouny et al. 2009a,b). The average % OAR from the present study for the shared 33 fruit and vegetables was 46.8%, while the average % OAR for the all 37 fruit/vegetable extracts was 45.5% and these values were not significantly different ($P > 0.05$). In addition, the average % OAR for the shared 26 herb/spice extracts was 83.6%, while the average % OAR for all 67 herb/spice extracts from our published studies was 77.1%, and these values also were not significantly different ($P > 0.05$). Thus, the similarities in % OARs indicate that the plants reported by Haytowicz & Bhagwat (2010) are representative of the fruit/vegetables and herb/spices that we evaluated.

In Haytowicz & Bhagwat (2010), the average ORAC values for the shared 33 fruits and vegetables (2,307 μmol Trolox Equivalents [TE]/100g) were significantly lower ($P \leq 0.01$) than the average ORAC values of the shared 26 herbs and spices (47,825 μmol TE). However, correlations between the published ORAC values and UV protection (% OAR) from the present study were weak for both fruits/vegetable extracts ($r^2 = 0.36$) and herbs/spice extracts ($r^2 = 0.23$). There also was a weak correlation ($r^2 = 0.12$) between antioxidant values from Halvorsen et al. (2002) (FRAP assay, reduction of Fe^{3+} to Fe^{2+}) and % OAR from our study. Therefore, we conclude that antioxidants (as measured by ORAC) are associated with, but not solely responsible for UV protection, as high ORAC values do not necessarily result in high UV protection.

In the present study, average total phenolic (TP) values (from Haytowicz & Bhagwat 2010) for the shared 33 fruits and vegetables (246 mg gallic acid equivalents [GAE]/100 g) were significantly lower ($P \leq 0.05$) than the average TP values (2,307 GAE) of the shared 26 herbs and spices. However, correlations between TP and % OAR for fruit/vegetable extracts ($r^2 = 0.45$) and for herb/spice extracts ($r^2 = 0.25$) were weak. It appears that neither high antioxidant nor high phenolic levels are solely responsible for UV protection in these plant extracts.

The goal of the present study was to assess the effectiveness of 37 aqueous fruit and vegetable extracts as UV protectants for the beet armyworm baculovirus SeMNPV, as part of screening program on non-chemical control of insect vegetable pests. However, only black currant extract provided adequate protection (at least 90% OAR) from UVB/UVA for 30 minutes. Herb/spice extracts tested

previously had higher average % OAR and contain higher levels of antioxidants and phenolics than the fruit/vegetable extracts evaluated in the current study. Careful selection of fruits/vegetable extracts or herbs/spice extracts with high levels of both antioxidants and phenolics could result in increased UV protection, and help insure that future field studies include the best UV protectants.

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