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Beet Armyworm (Lepidoptera: Noctuidae) Nucleopolyhedrovirus: Screening of Extracts[1]

Martin Shapiro,[2] Said El Salamouny,[3] and B. Merle Shepard[2,4]

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ABSTRACT Sixty-seven plant-derived extracts were tested as ultraviolet (UV) protectants for the nucleopolyhedrovirus (SeMNPV) of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). In the initial laboratory screening experiment, 25 of the 67 extracts provided protection following UVA/UVB irradiation for 30 min. Fifteen of these 25 extracts provided good UV protection when they were subjected to a more severe UV treatment of UVB/UVB irradiation for 30 min. Four of these 15 extracts (kudzu, peppermint, skullcap, and thyme) provided excellent UV protection for SeMNPVwhen they were irradiated with an even more stringent UV regime of UVB/UVB for 300 min. These findings indicate that these plant extracts may be useful UV protectants for the SeNPV and they should be investigated further to obtain more efficacious formulations for the control of agriculturally important insect pests.

KEY WORDS Plant extracts, *Spodoptera exigua*, ultraviolet radiation protectants, nucleopolyhedrovirus

More than 40 y ago it was determined that insect pathogenic viruses were very susceptible to solar radiation (Bullock 1967, David 1969, Jaques 1971) and that efficacy of these microbials was greatly reduced within the first 24–48 h post-spraying (Bullock 1967, Ignoffo & Batzer 1971, Young & Yearian 1974). Moreover, studies in several laboratories demonstrated that the UVB portion (=280–320 nm) of the solar spectrum was primarily responsible for virus inactivation (David 1969, Bullock et al. 1970, Griego et al. 1985). Inactivation, however, also occurred after exposure to UVA (=320–400 nm) (David 1969, Morris 1983, Shapiro & Robertson 1990), but at a slower rate (Bullock et al. 1970, Morris 1971). In addition, Griego & Spence (1978) reported that "the inactivation of *B. thuringiensis* spores by sunlight is due in part to wavelengths of the visible spectrum (near 400 nm) that spores readily absorb, as shown by its absorption profile." These studies were important in determining the mechanisms responsible for solar inactivation (Ignoffo & Garcia 1978, Witt 1984, El Salamouny et al. 2009a,b) and for testing synthetic (Ignoffo & Batzer 1971,

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[2]Clemson University, Coastal Research and Education Center, 2700 Savannah Highway, Charleston, South Carolina 29414, USA.

[3]Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, 11451 Riyadh, P.O. Box 2460, Kingdom of Saudi Arabia.

[4]Corresponding author (mshprd@clemson.edu).

Shapiro & Robertson 1990, Asano 2005) and natural products (Batzer & Ignoffo 1978, Leland & Behle 2005, Shapiro et al. 2008) as UV protectants.

Although studies on UV inactivation and UV protection have provided much information, researchers employed different UV irradiation systems with different peak absorbances and energy levels, different UV irradiation exposure times, different heights from the UV source, different pathogens, different initial pathogen-caused mortality levels, and different criteria for "success." For example, the following UV irradiation systems have been used: (1) UVC (peak at 254 nm) (Bull 1978, Liu et al. 1993, Filho et al. 2001); (2) UVC (peak at 254 nm/UVA peak at 366 nm) (Ignoffo & Batzer 1971, Witt & Stairs 1975); (3) UVB (peak 312–315 nm) (Brassel & Benz 1979, Rangel et al. 2004, Shapiro et al. 2008); (4) UVB (peak 312 nm)/UVA (peak 366 nm) (Cohen et al. 2001, Shapiro & Domek 2002, El Salamouny et al. 2009a); (5) UVA (peak 366 nm) (Shapiro & Domek 2002); (6) UVA/visble light (Griego & Spence 1978, Shapiro & Domek 2002); (7) visible light (400–800 nm) (Shapiro & Domek 2002); (8) Xenon solar simulator 295–1100 nm (Fargues et al. 1996, McGuire et al. 2000, Farrar et al. 2003); and (9) natural sunlight (Broome et al. 1974, Nickle & Shapiro 1994, Arivudainambi et al. 2000).

Although we have used different UV irradiation systems to test several chemicals (Shapiro et al. 1983, Shapiro 1989, Shapiro & Robertson 1990) and plant extracts (Shapiro et al. 2008, El Salamouny et al. 2009a, b), our criteria have remained constant in terms of initial virus-caused mortality prior to UV irradiation of the virus/water standard, post-irradiation larval mortality in virus/water standard, and in the definition of "success" of UV protectants. These criteria are: (1) the pre-irradiation virus-caused mortality should be 90–95% larval mortality; (2) the post-irradiation virus-caused mortality should be reduced to 10% or less in the virus/water standard; (3) UV protection for each UV treatment should be compared to initial pre-irradiation virus-caused mortality in virus/water standard; and (4) a "successful" UV protectant should retain at least 90% of its activity after UV irradiation (Shapiro & Robertson 1990, Shapiro et al 2008, El Salamouny et al. 2009a,b). While these criteria may be subjective, they have been successful in identifying UV protectants in laboratory tests for subsequent testing under natural conditions (Nickle & Shapiro 1994, Farrar et al. 2003, Shapiro et al. 2008). Recently, we showed that green tea (Shapiro et al. 2008), black tea (El Salamouny et al. 2009a), and cocoa & coffee (El Salamouny et al. 2009b) were excellent UV protectants for the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) NPV (SeMNPV). Our present study was undertaken to screen 67 plant-derived extracts as photoprotectants for SeMNPV in order to obtain the most effective UV protectants for field testing.

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 nuclepolyhedrovirus (SeMNPV) for *S. exigua*, registered as Spod-X®, was obtained from Certis USA, Columbia, MD.

Plant extracts. Sixty-seven medicinal herbs and spices were obtained from several sources, including Foodhild USA, Inc., Landover, MD; McCormick & Co.,

Hunt Valley, MD; Natrol, Charsworth, CA; Natures' Herbs, American Fork, UT; Sigma-Aldrich Chemicals, St. Louis, MO; Solaray, Park City, UT; Soofer Co. Inc., Los Angeles, CA; Swanson Vitamins, Fargo, ND; St. John's herb garden Inc., Bowie, MD., A.L. Verna Co. Inc., Philadelphia, PA; and Vitamin Shoppe, Bergen, NJ (Table 1). One gram of powder from each plant product was blended in 99 grams of distilled water and then filtered through coarse cheesecloth. The filtrates were stored under refrigeration (4°C) until used.

Radiation source. In the initial test, radiation was provided by a UVA tube (15 watt, 382 mm, Fotodyne, Inc., New Berlin, WI) and a UVB tube (15 watt, 382 mm, Fotodyne, Inc.), which were mounted in parallel within a Pelco UV-2 cryo chamber (Ted Pella, Inc., Redding, CA) 203 mm above the test dishes. This treatment was designated as UVA/UVB. In subsequent tests, radiation was provided by 2 UVB tubes (UVB/UVB). Radiation emission profiles for these UV sources are described by Shapiro & Domek (2002).

Exposure of SeMNPV to UVA/UVB irradiation, preliminary test. A preliminary bioassay was conducted to determine the SeMNPV concentration that caused 90–95% larval mortality. The result was 1×10^6 viral inclusion bodies (OBs) per ml diluted in distilled water (standard). Subsequently, four milliliters of virus suspension were pipetted into a 60 × 15 mm glass petri dish (Fisher Scientific, Pittsburg, PA) and exposed to UVA/UVB radiation for 5, 10, 15, 30, or 60 min to obtain the time necessary to reduce virus activity (=virus-caused larval mortality) from 90–95% to less than 10%. After each exposure period, the volume in each dish 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 used. When dishes were removed from the refrigerator, 0.1 ml of virus suspension (e.g., SeMNPV/water) was applied to each 30-ml cup (W.L. Enterprises Inc., Newark, NJ) containing Multiple Species diet (10 cups per treatment). The result of the subsequent bioassay with second instar *S. exigua* larvae indicated that SEMNPV should be exposed to UVA/UVB radiation for 30 min to reduce virus activity below 10%.

Primary screen: UVA/UVB for 30 min. Sixty-seven plant extracts (0.9% final concentration) were tested as UV protectants for the SeMNPV that was exposed to UVA/UVB for 30 min. In addition, non-irradiated SeMNPV/H_2O, irradiated SeMNPV/H_2O, plant-extract only, and untreated larvae were tested as controls. Second stage *S. exigua* larvae were placed individually into each container and reared for 14 d at 27°C under ambient laboratory conditions. Tests were repeated five times with 10 larvae per replication. Because it was impossible to irradiate all SeMNPV/H_2O and SeMNPV/plant extract treatments at the same time, treatments were tested in batches. Thus, extra control treatments were setup so that 400 larvae were tested each in the SeMNPV/H_2O (0 UV) and 400 SeMNPV/H_20 (30 min UV) treatments; whereas 50 larvae were tested in other 0 UV treatments (H_20 only, plant extract only) and in the 30 min UV SeMNPV/plant extract treatments. Mortality was initially assessed at five days post-treatment and every 2–4 d thereafter until day 14, when the experiment was terminated.

Secondary screen: UVB/UVB for 30 min. Only those plant extracts that provided greater than 90% protection of SeMNPV from UVA/UVB in the primary screen were selected for testing in the secondary screen. In this test, fresh SeMNPV/H_2O and SeMPNV/plant extract samples were exposed to two UVB

Table 1. Plant extracts tested as ultraviolet (UV) protectants for the beet armyworm nucleopolyhedrovirus (SeMNPV).

Plant	Scientific name	Family
Angelica	*Angelica archangelica* (L.)	Umbelliferae
Anise	*Pimpinella anisum* (L.)	Umbelliferae
Arrowroot	*Maranta membranaceus* (L.)	Marantacae
Astralagus	*Astralagus membranaceus* (Fisch.)	Leguminosae
Basil, sweet	*Ocimum basilium* (L.)	Labiatae
Basil, holy	*Ocimum sanctum* (L.)	Labiate
Caraway	*Carum carvi* (L.)	Umbelliferae
Catnip	*Nepeta cataria* L.	Labiatae
Cayenne	*Capsicum minimum* (Roxb.)	Solanaceae
Celery	*Apium graveolens* (L.)	Umbelliferae
Chicory	*Cichorium intybus* (L.)	Compositae
Cilantro	*Coriandrum sativum* L.	Umbelliferae
Cinnamon	*Cinnamum zeylanicum* (Nees.)	Lauraceae
Cloves	*Eugenia caryophyllate* (Thumb.)	Myrtaceae
Cranberry	*Vaccinum oxycoccus* L.	Ericeae
Cumin	*Cuminum cyminum* (L.)	Umbelliferae
Curcumin	Main ingredient of turmeric	
Curry	Mixture (cilantro, cumin, turmeric)	
Dandelion	*Leontodon taraxacum* L.	Compositae
Dill	*Peucedanum gravolenss* (Benth.)	Compositae
Eucalyptus	*Eucalyptus globulus* (Labille)	Myrtaceae
Eugenol	Chief constituent of oil of cloves	
Fenugreek	*Foenus-graecum* (L.)	Leguminaceae
Fennel	*Foeniculum vulgare* (Gaert.)	Umbelliferae
Feverfew	*Tanacetum parthenium* (L.)	Compositae
Garlic	*Allium sativum* (L.)	Liliaceae
Ginger	Zingiber officinale Roscoe	Zingiberaceae
Ginseng, Siberian	*Eleutherococcus senticosus* Maxim	Araliaceae
Ginseng, Panax	*Panax quinquefolium* (L.)	Araliaceae
Goldenseal	*Hydrastis canadensis* (L.)	Ranunculaceae
Hawthorn	*Crataegus oxyacantha* (L.)	Rosaceae
Horse chestnut	*Aesculus hypocastanum* L.	Sapindaceae
Juniper	*Juniperus communis* (L.)	Coniferae
Kudzu	*Pueraria lobata* (Wild.)	Fabaceae
Lavender	*Lavandula angustifolia* Stoechas	Labiatae
Licorice	*Glyrriza glabra* (L.)	Leguminosae
Mace	*Myristica fragrans* (Houtt.)	Myristiceae
Marjoram	*Origanum majorana* (L.)	Labiatae
Magnolia	*Magnolia grandiflora* (L.)	Magnoliacae
Marshmallow	*Althaea officinalis* (L.)	Malvaceae
Mustard	*Brassica alba* (Boiss.)	Cruciferae
Noni	*Morinda cirtifolia* L.	Rubiaceae
Nutmeg	*Myristica fragrans* (Houtt.)	Myristicaceae
Onion	*Allium capa* (L.)	Liliaceae
Oregano	*Origanum vulgare* (L.)	Labiatae
Paprika	*Capsicum annum* (L.)	Solanaceae
Parsley	*Petroselinum crispum* (Mill.)	Apiaceae

Table 1. Continued.

Plant	Scientific name	Family
Paw Paw	*Asimina triloba* L.	Annonaceae
Pepper	*Piper nigrum* (L.)	Piperaceae
Peppermint	*Mentha piperita* (L.)	Labiatae
Popcorn tree	*Triadica sebifera* (L.)	Euphorbiaceae
Rosemary	*Rosmarinus officinalis* (L.)	Labiatae
Radish,wild	*Raphanus raphanistrum* (L.)	Cruciferae
Sage	*Salvia officinalis* (L.)	Labiatae
Sarasparilla	*Smilax glabra* Roxb.	Liliaceae
Saw palmetto	*Serenoa repens* Hook	Arecaceae
Skullcap	*Scutellaria lateriflora* L.	Labiatae
Slippery elm	*Ulmus rubra* Muhl.	Urticaceae
Sour grape	*Ribes uva-crispa* (L.)	Ribesiaceae
Spearmint	*Mentha viridis* (L.)	Labiatae
St. John's wort	*Hypericum perforatum* L.	Hypericaceae
Sumac	*Rhus coriaria* L.	Anacardiaceae
Tansy	*Tanacetum vulgare* (L.)	Compositae
Tarragon	*Artemisia dracunculus* (L.)	Compositae
Thyme	*Thymus vulgaris* (L.)	Labiatae
Turmeric	*Curcuma longa* (L.)	Zingiberaceae
Valerian	*Valeriana officinalis* L.	Valerianaceae

tubes (UVB/UVB) for 30 min. The protocol for the secondary screen was the same as that for the primary screen and tests were repeated five times. Three hundred larvae were used in both the SeMNPV/H_20 (0 UV) and the SeMNPV/H_20 (30 min UV) treatments; 50 larvae were used in the other no UV treatments (H_2O only, plant extract only) and in the 30 min UV SeMNPV/plant extract treatments.

Tertiary screen: UVB/UVB for 300 min. Only those plant extracts that provided greater than 90% protection of SeMNPV from UVB/UVB in the secondary screen were selected for testing in the tertiary screen. Fresh SeMNPV/H_2O and SeMNPV/plant extract samples were exposed to UVB/UVB for 300 min. The test was repeated five times. Three hundred larvae were used in both the SeMNPV/H_2O (0 UV) and SeMNPV/H_2O (300 min UV) treatments; 50 larvae were used in the other no UV treatments (H_2O only, plant extract only) and in the 300 min UV SeMNPV/plant extract treatments.

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 (Ignoffo & Batzer 1971). Converted data were then analyzed by Analysis of Variance (ANOVA) using SAS PROC GLM (SAS 2000). After ANOVA, treatment means were separated according to Fisher's protected Least Significant Difference (LSD) Test.

Results

Primary screen: UVA/UVB, 30 min. UVA/UVB irradiation of an aqueous suspension of SeMNPV for 30 min reduced virus-caused mortality from 98.0% (0

Table 2. Primary screen: SeMNPV/plant extracts exposed to UVA/UVB irradiation for 30 min.

Treatment[a]	UV exposure (min)[b]	Avg. % mortality ± SE	OAR (%)[c]
SeMNPV/H_2O	0	98.0 ± 1.5 a	100.0
SeMNPV/H_2O	30	9.3 ± 2.7 q	9.5
Angelica	30	82.0 ± 3.7 fghi	83.7
Anise	30	94.0 ± 2.5 abcd	95.9
Arrowroot	30	2.0 ± 2.0 q	2.0
Astralagus	30	92.0 ± 2.0 abcde	93.9
Basil, sweet	30	80.0 ± 5.5 ghi	81.6
Basil, holy	30	82.0 ± 3.7 fghi	83.7
Caraway	30	82.0 ± 4.9 fghi	83.7
Catnip	30	84.0 ± 4.0 bcdef	85.7
Cayenne	30	50.0 ± 3.2 lm	51.0
Celery	30	64.0 ± 5.1 k	65.3
Chicory	30	22.0 ± 3.7 p	22.4
Cilantro	30	96.0 ± 2.5 abc	98.0
Cinnamon	30	96.0 ± 2.5 abc	98.0
Cloves	30	96.0 ± 4.0 abc	98.0
Cranberry	30	82.0 ± 3.7 fghi	83.7
Cumin	30	86.0 ± 5.1 cdefg	87.8
Curcumin	30	82.0 ± 3.7 fghi	83.7
Curry	30	80.0 ± 7.1 fghi	81.6
Dandelion	30	46.0 ± 3.1 mn	46.9
Dill	30	96.0 ± 4.0 abc	98.0
Eucalyptus	30	88.0 ± 3.7 bcdefg	89.8
Eugenol	30	80.0 ± 5.5 ghi	81.6
Fenugreek	30	92.0 ± 3.7 abcde	93.9
Fennel	30	78.0 ± 5.8 ghi	79.6
Feverfew	30	96.0 ± 2.5 abc	98.0
Garlic	30	64.0 ± 5.1 k	65.3
Ginger	30	72.0 ± 3.7 hij	73.5
Ginseng, Siberian	30	48.0 ± 3.7 m	49.0
Ginseng, Panax	30	36.0 ± 2.5 no	36.7
Goldenseal	30	42.0 ± 5.5 mno	42.9
Hawthorn	30	80.0 ± 7.1 fghi	81.6
Horse chestnut	30	76.0 ± 2.5 hij	77.6
Juniper	30	42.0 ± 5.8 mno	42.9
Kudzu	30	100.0 ± 0.0 a	100.0
Lavender	30	78.0 ± 5.5 ghi	79.6
Licorice	30	100.0 ± 0.0 a	100.0
Mace	30	70.0 ± 5.5 ijk	71.4
Magnolia	30	98.0 ± 2.0 ab	100.0
Marjoram	30	96.0 ± 2.5 abc	98.0
Marshmallow	30	48.0 ± 3.7 m	49.0
Mustard	30	62.0 ± 3.7 k	63.3
Noni	30	40.0 ± 3.2 mno	40.8
Nutmeg	30	52.0 ± 3.7 k	53.1
Onion	30	72.0 ± 3.7 hij	73.5
Oregano	30	96.0 ± 2.5 abc	98.0

Table 2. Continued.

Treatment[a]	UV exposure (min)[b]	Avg. % mortality ± SE	OAR (%)[c]
Paprika	30	100.0 ± 0.0 a	100.0
Parsley	30	66.0 ± 5.1 jk	67.3
Paw Paw	30	84.0 ± 4.0 bcdef	85.7
Pepper	30	84.0 ± 2.5 defgh	85.7
Peppermint	30	96.0 ± 2.5 abc	98.0
Popcorn tree	30	90.0 ± 5.5 abcdef	91.8
Rosemary	30	94.0 ± 3.7 abcd	91.8
Radish, wild	30	70.0 ± 3.2 ijk	71.4
Sage	30	92.0 ± 3.7 abcde	93.9
Sarasparilla	30	76.0 ± 5.1 hij	77.6
Saw palmetto	30	60.0 ± 4.5 kl	61.2
Skullcap	30	96.0 ± 2.5 abc	98.0
Slippery elm	30	32.0 ± 3.7 op	32.7
Sour grape	30	40.0 ± 3.2 mno	32.7
Spearmint	30	90.0 ± 3.2 abcdef	91.8
St. John's wort	30	92.0 ± 3.7 abcde	93.9
Sumac	30	96.0 ± 2.5 abc	98.0
Tansy	30	84.0 ± 5.1 defgh	85.7
Tarragon	30	96.0 ± 2.5 abc	98.0
Thyme	30	98.0 ± 2.0 ab	100.0
Turmeric	30	36.0 ± 4.0 no	36.7
Valerian	30	96.0 ± 2.5 abc	98.0

[a]SeMNPV was used at a final concentration of 72.0 OBs/mm^2 of diet surface; aqueous plant extracts were used at a final concentration of 0.9%. Five replicates, 10 larvae per treatment per replicate; total: 50 larvae per SeMNPV/plant extract (UVA/UVB, 30 UV); 50 untreated control larvae; 50 plant extract-treated larvae. In the SeMNPV/H_2O (0 UV) treatment: 10 larvae per test; total = 400; in the SeMNPV/H_2O (30 UV) treatment: 10 larvae per test; total = 400 larvae.

[b]SeMNPV was exposed to UVA/UVB irradiation in deionized water (standard) or in aqueous plant extract for 30 min.

[c]For % Original Activity Remaining (% OAR), all virus treatments were compared to SeMNPV/H_2O (0 UV) where % OAR = 100.0%.

UV standard) to 9.3% (=9.5% OAR). The percent original activities of the 67 SeMNPV/plant extract combinations ranged from 2.0% for SeMNPV/arrowroot to greater than 99% for SeMNPV/kudzu, SeMNPV/licorice, SeMNPV/magnolia, SeMNPV/paprika, and SeMNPV/thyme (Table 2). Twelve extracts provided 0 to 50% protection, while 23 extracts provided 70–90% protection. Twenty-five extracts provided at least 90% protection (Table 2), and they were selected for the secondary screen.

Secondary screen: UVB/UVB, 30 min. UVB/UVB irradiation of an aqueous suspension of SeMNPV for 30 min reduced virus-caused mortality from 96.8% (0 UV standard) to 3.7% (=3.8% OAR). The percent original activities of the 25 extracts tested ranged from 6.2% for SeMNPV/sumac to greater than 99% for SeMNPV/kudzu and SeMNPV/skullcap (Table 3). Fifteen extracts provided at least 90% protection (Table 3), and they were selected for the tertiary screen.

Tertiary: UVB/UVB, 300 min. UVB/UVB irradiation of an aqueous suspension of SeMNPV for 300 min reduced virus-caused mortality from 96.7%

Table 3. Secondary screen: SeMNPV/plant extracts exposed to UVB/ UVB irradiation for 30 min.

Treatment[a]	UV exposure (min)[b]	Avg. % mortality ± SE	OAR (%)[c]
SeMNPV/H_2O	0	96.8 ± 2.5 ab	100.0
SeMNPV/H_2O	30	3.7 ± 2.0 h	3.8
Anise	30	74.0 ± 5.1 c	76.4
Astralagus	30	54.0 ± 4.0 f	55.8
Cilantro	30	78.0 ± 4.9 abcd	80.6
Cinnamon	30	82.0 ± 3.7 de	84.7
Cloves	30	94.0 ± 2.5 a	97.1
Dill	30	94.0 ± 2.5 a	97.1
Fenugreek	30	90.0 ± 2.0 abcd	93.0
Feverfew	30	92.0 ± 3.7 abc	95.0
Kudzu	30	98.0 ± 2.0 a	100.0
Licorice	30	92.0 ± 2.0 abc	95.0
Magnolia	30	96.0 ± 2.5 a	99.2
Marjoram	30	94.0 ± 2.5 ab	97.1
Oregano	30	92.0 ± 2.0 abc	95.0
Paprika	30	86.0 ± 2.5 bcd	88.8
Peppermint	30	94.0 ± 2.5 a	97.1
Popcorn tree	30	84.0 ± 4.0 cd	86.8
Rosemary	30	92.0 ± 3.7 abc	95.0
Sage	30	82.0 ± 3.7 de	84.7
Skullcap	30	98.0 ± 2.0 a	100.0
Spearmint	30	90.0 ± 3.2 abcd	93.0
St. John's wort	30	20.0 ± 3.2 g	20.7
Sumac	30	6.0 ± 3.2 h	6.2
Tarragon	30	90.0 ± 4.5 abcd	93.0
Thyme	30	90.0 ± 3.2 abcd	93.0
Valerian	30	86.0 ± 2.5 bcd	88.8

[a]SeMNPV was used at a final concentration of 72.0 OBs/mm^2 of diet surface; aqueous plant extracts were used at a final concentration of 0.9%. Five replicates, 10 larvae per treatment per replicate; total: 50 larvae per SeMNPV/Plant extract (UVB/UVB, 30 min); 50 untreated control larvae; 50 plant extract-treated larvae. In the SeMNPV/H_2O (0 UV) treatment: 10 larvae per test, total = 300; in the SeMNPV/ H_2O (30 UV) treatment: 10 larvae per test, total = 300 larvae.

[b]SeMNPV was exposed to UVB/UVB irradiation in deionized water (standard) or in aqueous plant extract for 30 min.

[c]For % Original Activity Remaining (% OAR), all virus treatments were compared to SeMNPV/H_2O (0 UV) where % OAR = 100.0%.

(0 UV standard) to 0.7% (=0.7% OAR). The percent original activities of the 15 SeMNPV/plant extract combinations ranged from 8.3% for SeMNPV/dill to greater than 99% for SeMNPV/kudzu, SeMNPV/peppermint, and SeMNPV/ skullcap (Table 4).

Discussion

Laboratory *in vitro* or *in vivo* screening of chemicals, natural products, or microorganisms for bioactivity against insect parasitoids and predators (Helyer

Table 4. Tertiary screen: SeMNPV/plant extracts exposed to UVB/UVB irradiation for 300 min.[a]

Treatment[a]	UV exposure (min)[b]	Avg. % mortality ± SE	OAR (%)[c]
SeMNPV/H_2O	0	96.7 ± 2.5 a	100.0
SeMNPV/H_2O	300	0.7 ± 0.3 b	0.7
Cloves	300	82.0 ± 3.7 bcd	84.8
Dill	300	8.0 ± 2.0 g	8.3
Fenugreek	300	14.0 ± 2.5 fg	14.5
Feverfew	300	16.0 ± 2.5 f	16.5
Kudzu	300	97.0 ± 2.0 a	100.0
Licorice	300	36.0 ± 2.5 e	37.2
Magnolia	300	34.0 ± 2.5 e	35.2
Marjoram	300	84.0 ± 2.5 bc	86.9
Oregano	300	80.0 ± 3.2 cd	82.7
Peppermint	300	96.0 ± 2.5 a	99.3
Rosemary	300	76.0 ± 4.0 d	78.6
Skullcap	300	96.0 ± 2.5 a	99.3
Spearmint	300	84.0 ± 2.5 bc	86.9
Tarragon	300	86.0 ± 4.0 bc	88.9
Thyme	300	88.0 ± 2.0 b	91.0

[a]SeMNPV was used at a final concentration of 72.0 OBs/mm^2 of diet surface; aqueous plant extracts were used at a final concentration of 0.9%. Five replicates; 10 untreated larvae per replicate; 10 plant extract-treated larvae per replicate; 10 larvae per replicate; 10 larvae per SeMNPV/plant extract (UVB/UVB, 300 min) treatment per replicate. In the SeMNPV/H_2O (0 UV) treatment: 10 larvae per test, total = 300 larvae.; in the SeMNPV/H_2O (300 min) treatment: 10 larvae per test, total = 300 larvae.

[b]SeMNPV was exposed to UVB/UVB irradiation in deionized water (standard) or in aqueous plant extract for 300 min.

[c]For % Original Activity remaining (% OAR), all virus treatments were compared to SeMNPV/H_2O (0 UV), where % OAR = 100.0%.

1991, van de Veire et al. 1996), as microbial control agents (USEPA 1996), as insecticides (Somasundaram et al. 1990, Alexenizer & Dorn 2007), as pharmaceuticals for human usage (Fabricant & Farnsworh 2001, Sawangjaroen et al. 2005), or for UV radiation protection (Cockell & Knowland 1999, Solovchenko & Merziyak 2008) is an efficient and cost-effective means of discovering bioactive compounds or natural products before testing them in the field. For example, "most large pharmaceutical manufacturers and some small biotechnology firms have the ability to screen 1000 or more substances per week using high throughput *in vitro* assays. In addition to synthetic compounds from their own programs, some companies screen plant, microbial, and marine organisms" (Fabricant & Farnsworth 2001).

In the search for effective UV protectants for the cabbage looper NPV, Jaques (1971) tested 29 materials or combinations in the laboratory, using a UVC (peak emission at 254 nm) system. Dyes such as brilliant yellow, buffalo black, methylene blue, and safranin provided good UV protection in both laboratory and subsequent field tests. He later tested 22 materials and mixtures in the laboratory against *Trichoplusia ni* (Lepidoptera: Noctuidae) and found that six of these (charcoal, yeast extract, brewer's yeast, egg albumen, skim milk powder,

and molasses) "greatly extended activity of deposits of *T. ni* NPV." Based on these results, Jaques (1972) selected material and mixtures for field testing and showed that charcoal, skim milk powder, and a charcoal plus egg albumen mixture provided excellent protection for the *T. ni* NPV.

Because the overall goal of our research is to maximize the use of insect pathogenic viruses as microbial control agents, we have investigated different materials (e.g., chemicals, plant extracts) as enhancers (Dougherty et al. 1996, Shapiro et al. 2007a,b) and UV protectants (Shapiro et al. 1983, Shapiro et al. 2008, El Salamouny et al. 2009b) for insect viruses. In our initial UV study (Shapiro et al. 1983), we tested different sunscreens and reported their relative effectiveness as UV protectants for the gypsy moth (*Lymantria dispar* [L.]) (Lepidoptera: Lymantriidae) NPV (LdMNPV), using a UV irradiation system with peak emissions at 313 mm (UVB) and 366 nm (UVA). We found that the most effective sunscreen was benziline sulfonic acid (=89% OAR at 5% concentration and 100% OAR at 10% concentration) (Shapiro et al. 1983). In a later study (Shapiro & Robertson 1990), we utilized the same UV irradiation system to evaluate 79 dyes and utilized an LC_{90} for the virus concentration and determined the OAR post-irradiation as a criterion for "success." Dyes were then ranked as <10%, 11–30%, 31–50%, 51–70%, and >70% OAR, based upon their abilities to protect *L. dispar* NPV from UV radiation inactivation. Based upon these categories, we (Shapiro & Robertson 1990) found that acridine yellow, alkali blue, brilliant yellow, congo red, lissamine green, and mercurochrome provided excellent UV protection (>70% OAR), but these were not tested in the field. Moreover, we determined that both effective dyes (>70% OAR) and ineffective dyes (<10% OAR) had similar UVB absorbances, however the effective dyes also were good UVA absorbers (Shapiro & Robertson 1990).

In general, this criterion (>70% OAR, Shapiro et al. 1983) has been useful in selecting the most promising UV protectants for subsequent field testing (Shapiro et al. 2008). However, in the present study, we used a more stringent criterion for "success" (>90% OAR). We assumed that a UVA/UVB exposure of 30 min would be sufficient to eliminate many candidates from further consideration, since a 30 min UV exposure reduced virus-caused mortality from around 95% to less than 10% (=9.5% OAR) (Table 2). Although the 30 min exposure to a combination of UVA and UBV tubes resulted in about a 90% reduction in activity of the SeMNPV/H_2O sample, the treatment was not severe enough to eliminate enough plant-derived extracts from further consideration. If we had used the same criterion for "success" that we used previously (70% OAR), approximately 72% of the plant-derived extracts would have met this criterion. Moreover, 37% of the plant-derived extracts also met our more-stringent criterion for success (>90% OAR). These results were consistent with those obtained in our study of black tea and lignin (El Salamouny et al. 2009a). In that study, a 60 min UV exposure time resulted in about a 98% loss in activity (El Salamouny et al. 2009a).

Instead of increasing the exposure time of the UVA/UVB treatment or using extracts at lower concentrations (Shapiro et al. 2008), we decided to utilize two UVB tubes as the more-stringent UV irradiation treatment for the secondary screening. The UVB/UVB setup emits a significantly greater amount of UVB energy than the UVA/UVB setup (Shapiro & Domek 2002, El Salamouny et al. 2009b). Whereas a 30 min exposure of SEMNPV to UVA/UVB resulted in a loss in virus activity to 9.5% OAR, a 30 min UVB/UVB exposure resulted in a loss of

activity to 3.8% OAR (Table 3). Although UV-caused inactivation of SeMNPV/H_2O for the UVB/UVB 30 min exposure was 60% greater than for the UVA/UVB 30 min exposure, only 10 of the 25 samples provided less than 90% UV protection and could be eliminated for further consideration (Table 3). Therefore, the 15 SeMNPV/plant extract combinations that provided at least 90% UV protection were subjected to the most severe treatment (UVB/UVB exposure, 300 min) (Shapiro et al. 2008, El Salamouny et al. 2009a). Although El Salamouny et al. (2009a) found that viral DNA was totally destroyed after a UVA/UVB exposure of 240 min, we used 300 min UVB/UVB exposure time as a more severe treatment to maximize the probability of obtaining the most efficacious UV protectants. The 300 min UVB/UVB exposure resulted in a loss of virus activity in the unprotected SeMNPV/H_20 sample to 0.7% OAR (Table 4). Despite the greater than 99% reduction in activity of the unprotected virus (SeMNPV/H_20), six of the 15 plant extracts provided greater than 70% UV protection and four provided at least 90% UV protection (Table 4).

As a result of this study, we were compelled to examine the criteria used for "success" in order to establish a standardized protocol for future tests. Our criterion for a "successful" UV protectants (at least 90% OAR post irradiation) evolved from earlier laboratory studies (Shapiro et al. 1983, Shapiro & Robertson 1990) and was designed to select only the most efficacious candidates for further laboratory and field tests. These criteria are critical to the success of any program involved in maximizing the effectiveness of microbial control agents. LC_{50}s are often used as a critical measurement of biological activity of insecticides, insect pathogens, and natural products (Koziol & Witkowski 1981, Fuxa & Richter 1990, Palacios et al. 2009), as well as in measurements of insect resistance (Tabashnik et al. 1994, Osorio et al. 2008, Djihinto et al. 2009). In our studies of UV inactivation and UV protectants, we used an $LC_{90–95}$ as a pre-irradiation standard in both laboratory (Shapiro & Domek 2002, Shapiro et al. 2008, El Salamouny et al. 2009a) and field tests (Shapiro et al. 2002, Farrar et al. 2003, Shapiro et al. 2008), where the relative effectiveness of candidate UV protectants was compared to the mortality level of the pre-irradiated NPV sample (OAR = 100.0%) (Ignoffo & Batzer 1971, Griego et al. 1985, Farrar et al. 2003).

Our results agree with published reports where inactivation of SeMNPV is dependent upon both the UV irradiation system used and the time of exposure (the total UVB energy emitted) (Nickle & Shapiro 1994, Smits et al. 1997, Shapiro & Domek 2002). While the $LC_{90–95}$s differ in different virus-host systems (Shapiro & Dougherty 1994, Shapiro et al. 1994, Shapiro & Hamm 1999), the use of this end point permits us to compare both UV inactivation and UV protection. In addition, the $LC_{90–95}$ values used in inactivation and UV protection studies are important, as the inactivation slopes (% OARs post virus application) are dependent upon initial virus-caused mortality. For example, in both laboratory and field tests involving NPVs from *L. dispar* (Shapiro & Domek 2002), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Shapiro et al. 2002, Farrar et al. 2003), and *S. exigua* (Shapiro et al. 2002), it was clearly shown that inactivation was inversely related to virus concentration. While pre-irradiation virus caused mortality is of critical importance, we also determined the post-irradiation mortality level of the SeMNPV/H_2O sample. Our end point of less than 10% OAR was chosen as a measure of the "success" of the UV irradiation system and was dependent upon (1) the initial virus mortality level used, (2) the

UV irradiation system used, and (3) the UV exposure time (Nickle & Shapiro 1994, Shapiro & Domek 2002, Farrar et al. 2003). This endpoint was also used to compare the relative effectiveness of chemicals and plant-derived extracts as UV protectants. Moreover, we also used the pre- and post-UV irradiation data in field tests to determine rates of inactivation as a consequence of virus concentration (=pre-irradiation $LC_{90–95}$), exposure time, and effectiveness (or not) of candidate UV protectants (Shapiro et al. 2002, Farrar et al. 2003, Shapiro et al. 2008).

In summary, we feel that we have optimized the laboratory screening system (e.g., LC_{95}, <10% OAR, UVB/UVB, 300 min) to discover efficacious UV protectants for further laboratory and field tests. The immediate goal of this study was to assess the effectiveness of 67 plant-derived extracts as UV protectants for the beet armyworm NPV (SeMNPV) as part of our program on non-chemical control of insect vegetable pests. In this regard, our goal was achieved as we identified four plant extracts (kudzu, peppermint, skullcap, and thyme) that provided a high level of protection and will be tested in future field experimants. Moreover, it is hoped that this research may lead to further interest in the use of plants and plant products as adjuvants for insect pathogenic viruses in pest management of agriculturally important insects.

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