Ultraviolet protection of a granulovirus product using iron oxide

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(Received 28 June 2004; Accepted 9 February 2005)

Abstract
Ultraviolet (UV) protection of a granulovirus (GV) product using iron oxide was investigated under laboratory conditions with fluorescent lamps. The UV protective activity was evaluated by comparing the pathogenicity of a GV product to the neonates of the oriental tea tortrix, Homona magnanima, in the presence or absence of iron oxide under UV irradiation. The infection rates of GV suggested that the addition of iron oxide at 1–4 mg/ml could reduce the inactivation of GV by UV irradiation to 1/6–1/18 as compared to that without it, indicating that iron oxide could be a useful UV protectant for microbial insecticide GV products.

Key words: Homona magnanima; granulovirus; GV; UV-protectant; UV-protection; iron oxide

INTRODUCTION
The oriental tea tortrix, Homona magnanima, is one of the major leaf-rolling pests that injure tea plants in Japan. H. magnanima granulovirus (HomaGV) was isolated from the diseased larvae of H. magnanima in 1975 (Sato et al., 1980). Prior to the identification of HomaGV, another granulovirus (GV), Adoxophyes orana GV (AdorGV), was described as a pathogen of the summer fruit tortrix, A. orana fasciata, in 1963 (Aizawa and Nakazato, 1963). AdorGV was reisolated from A. orana fasciata larvae in 1967, and its effectiveness as a possible biological agent had been demonstrated against A. orana fasciata in apple fields (Shiga et al., 1973; Yamada and Oho, 1973). The pathogenicity of AdorGV to the smaller tea tortrix, Adoxophyes honmai, was also recognized to be the same that of A. orana fasciata (Yamada and Oho, 1976).

All the GVs of Baculoviridae have been isolated only from lepidopterous insects worldwide thus far, and their host ranges are rather narrow. HomaGV showed virulence only against H. magnanima (Sato et al., 1980), and AdorGV was limited to certain insects that were closely related species in Genus Adoxophyes, like A. orana fasciata and A. honmai (Yamada and Oho, 1976). Based on a large number of field tests with HomaGV and AdorGV for controlling H. magnanima and A. honmai, respectively, it was concluded that the successful control of pests in tea fields using such GVs requires at least both an adequate concentration and a proper application time in early generations of the target pest species (Kodomari, 1987; Kodomari and Ohba, 1989, 1990; Nonaka et al., 1994).

Generally, viral pesticides are more advantageous than chemical-based insecticides for several reasons: safety of non-target living organisms, longer persistence beyond generations, and so on. On the other hand, viral pesticides exhibit several unfavorable properties, one of them being quick inactivation by natural sunlight, especially the UV portion of the spectrum. UV-B (280–310 nm) was considered to be mostly responsible for the inactivation of insect pathogens (viruses, bacteria, fungi and protozoa) under natural conditions (David, 1969; Morris, 1971; Ignoffo et al., 1977). The accumulated energies in the UV-B region (in kJ/m²) days after the application of a granulovirus product were found to show a negative correlation with the residual GV activity on tea foliage under natural conditions (Asano et al., 2002a, b, 2003). Therefore, if some effective and economical materials were available as UV protectants, the use of microbial insecticides, especially the virus products could be more efficiently used in an integrated pest management (IPM) program.

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DOI: 10.1303,uez2005.359
Jaques (1971) evaluated 29 materials and their combinations as UV protectants for the cabbage looper, *Trichoplusia ni*, nucleopolyhedrovirus (NPV), and showed that all the materials exhibited some UV protective ability. Kunimi (1986) reported that the addition of white carbon to *Spilosoma imparilis* NPV increased the viral residual activity on mulberry leaves, indicating the existence of some prevention from inactivation by UV radiation. Shapiro and Robertson (1990) tested 79 dyes as UV protectants for *Lymantria dispar* NPV and six of them were effective. Shapiro (1992) tested 23 brighteners as UV protectants for *L. dispar* NPV and the most effective ones belonged to the stilbene group such as Tinopal LPW. Ignoffo and Garcia (1994) reported that three antioxidants could provide some level of UV protection for *Heliothis zea* NPV.

The author attempted to screen some materials as UV protectants for a GV product, as a mixture of HomaGV and AdorGV, under laboratory irradiation with fluorescent lamps. In the present study, it was found that the addition of iron oxide (black) to a GV product could confer a UV protectant effect for HomaGV. Recently, several kinds of iron oxides are used as components of cosmetic foundations, powders, colors and sunscreens worldwide. The present study suggests that some iron oxides might also be possibly utilized as UV protectants for microbial insecticide GV products.

**MATERIALS AND METHODS**

**Insects.** A laboratory colony of the oriental tea tortrix, *H. magnanima* (originally collected from tea fields in Shizuoka Prefecture, Japan), were maintained on an artificial diet, Insecta-LFS (Nihon Nosanko Co.), at 25°C and 70% R.H. for 5 y. Neonates were used in the bioassays to examine the pathogenicity of HomaGV.

**Test materials.** A GV product named “Hamaki-Tentek” (Arysta LifeScience Co.) was used in this experiment. The product was formulated in aqueous form and was composed of 2 GV species, HomaGV and AdorGV, at $1 \times 10^{11}$ granular inclusion bodies (GIBs)/ml for each, based on the manufacturer's standard. The material used for the UV protection test was an iron oxide (black) of reagent grade (Kanto Kagaku Co.).

**UV source and exposure conditions.** Fluorescent lamps (Toshiba Lightech Co., FL20S·E, peak 315 nm: ranges 270–370 nm) were used as the UV source and placed on top of a radiation box made of stainless steel (35×100×35 cm in height). The distance between the position of the lamp and the bottom of the box where the test foliage was exposed was adjusted to 20 cm. UV irradiation was performed on both the upper and lower sides of the foliage for 10 min each.

**Bioassay.** The GV product was diluted at desired concentrations with distilled water, fresh tea leaves were dipped into the test solution for a few seconds, and then dried on a paper towel at room temperature. Next, some leaves were exposed to fluorescent light while others were not. Tea leaves with and without UV irradiation were cut in half and placed in a styrol petri dish 9 cm in diameter and 3 cm in height (Takano Rika Glass Co.). Ten neonates of *H. magnanima* were transferred to each dish with a brush and kept at 25°C and 70% R.H. Tea leaves as controls were treated in the same way except for the use of distilled water instead of the test solution. For each bioassay, 5 dishes per treatment (a total of 50 larvae) were used and the experiment was replicated three times on different days. Twenty-four hours later, an artificial diet (Insecta-LFS) was introduced into the dish. On day 7, the tea leaves were removed from the dish, leaving the artificial diet. The diet was added when the larvae required it for development until pupation. Three weeks later, when pupation began, the control and infected larvae were scored based on the appearance of milky-white body color, which indicated a typical pathogenic symptom caused by HomaGV.

**Statistical analysis.** The percentages of GV infection were calculated based on the number of larvae infected with GV to the total number of observed larvae (infected, healthy and others). In order to compare the degrees of infection among treatments under UV irradiation, the percentages of infection ($p$) were transformed to arcsine of square root ($\sin^{-1}\sqrt{p}$) and analyzed by one-way classification analysis of variance (ANOVA). Means were then separated by the Bonferroni multiple comparison method, using the statistical program (11.0J) of SPSS Co. Infection rates under no UV irradiation were analyzed using two-way ANOVA using the concentration of GV product and the presence/absence of iron oxide as factors. The relationships
between concentrations of products (ppm in logarithm) and GV infection (in probit) were analyzed by probit analysis to estimate the EC₅₀ (concentration giving 50% infection) values (Finney, 1972).

RESULTS

Pathogenicity of GV product against neonates of H. magnanima

The pathogenicity of a GV product against the neonates of H. magnanima was evaluated at concentrations of 1, 10 and 100 ppm based on the weight of formulation µg/ml (Table 1). The average infection rates among treatments in Table 1 differed significantly by one-way ANOVA ($F_{3,8}=79.152, p<0.05$). Using probit analysis, the resultant regression line was obtained as $Y=1.001X+4.174$ (df=1, $r^2=0.429$), where $X$ and $Y$ mean probit and concentration of GV product in logarithm, respectively. The EC₅₀ and its 95% confidential limits were estimated as 6.691 ppm and 4.503–9.839 ppm, respectively.

UV protection of GV product using iron oxide

The UV protective activity of iron oxide for the GV product was evaluated by comparing the percentages of GV infection to H. magnanima neonates with and without the presence of iron oxide under exposure to UV irradiation (Table 2). The average infection rates among treatments in Table 2 differed significantly by one-way ANOVA ($F_{3,8}=126.013, p<0.05$). The infection rate at 1,000 ppm of GV alone under no UV irradiation was 100%, and decreased to 51% with UV irradiation. On the other hand, the addition of 2 mg/ml of iron oxide led to a recovery to 80% under UV irradiation, indicating that iron oxide was able to protect GV from UV irradiation. The development time (i.e., from inoculation to pupation) of uninfected larvae treated with GV alone was not different from that with the mixture of GV-iron oxide (data not shown). This suggests that 2 mg/ml of iron oxide mixed with 1,000 ppm of GV product exhibits no inhibitory effect on the larval development of H. magnanima.

Effective doses of iron oxide for UV protection of GV product

In order to determine whether the UV protective activity of iron oxide displays dose dependency, iron oxide was added to GV product at three dose levels, and the pathogenicity to H. magnanima neonates was examined and compared with that of GV alone (Table 3). The average infection rates among the treatments listed in Table 3 differed significantly using one-way ANOVA ($F_{5,12}=63.367$,

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>No. of larvae observed</th>
<th>% GV infection mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>92</td>
<td>87.0±1.2 a</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>60.3±7.4 a</td>
</tr>
<tr>
<td>1</td>
<td>108</td>
<td>19.0±6.4 b</td>
</tr>
<tr>
<td>0</td>
<td>93</td>
<td>1.0±1.0 c</td>
</tr>
</tbody>
</table>

The concentration of the GV product was based on formulation weight (µg/ml).

The total number of larvae observed in three replicated tests.

There was no significant difference (at $p=0.05$) between same letters as analyzed by the Bonferroni method.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>UV irradiation</th>
<th>No. of larvae observed</th>
<th>% GV infection mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV product (ppm)</td>
<td>Iron oxide (mg/ml)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1,000</td>
<td>2</td>
<td>-</td>
<td>115</td>
</tr>
<tr>
<td>1,000</td>
<td>0</td>
<td>-</td>
<td>115</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>-</td>
<td>112</td>
</tr>
</tbody>
</table>

The concentration of the GV product was based on formulation weight (µg/ml).

Irradiation was done by exposing treated leaves to UV lamps for 10 min.

The total number of larvae observed in three replicated tests.

There was no significant difference (at $p=0.05$) between same letters as analyzed by the Bonferroni method.
Table 3. Effective doses of iron oxide for UV protection of the GV product against the neonates of *H. magnanima*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>UV irradiationa</th>
<th>No. of larvae observedc</th>
<th>% GV infection mean±SEd</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV product (ppm)a Iron oxide (mg/ml)</td>
<td>+</td>
<td>112</td>
<td>85.3±7.0ab</td>
</tr>
<tr>
<td>1,000</td>
<td>4</td>
<td>109</td>
<td>72.0±4.4bc</td>
</tr>
<tr>
<td>1,000</td>
<td>2</td>
<td>98</td>
<td>71.3±7.8bc</td>
</tr>
<tr>
<td>1,000</td>
<td>1</td>
<td>97</td>
<td>42.7±5.7cd</td>
</tr>
<tr>
<td>1,000</td>
<td>0</td>
<td>114</td>
<td>100±0.0a</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>93</td>
<td>1.0±1.0e</td>
</tr>
</tbody>
</table>

a The concentration of the GV product was based on formulation weight (μg/ml).  
b Irradiation was done by exposing treated leaves to UV lamps for 10 min.  
c The total number of larvae observed in three replicated tests.  
d There was no significant difference (at *p*=0.05) between same letters as analyzed by the Bonferroni method.

Table 4. Influence of iron oxide on the pathogenicity of a GV product against the neonates of *H. magnanima* under no UV irradiation

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>No. of larvae observedc</th>
<th>% GV infection mean±SEd</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV product (ppm)a Iron oxide (mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>138</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>129</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>112</td>
</tr>
</tbody>
</table>

a The concentration of the GV product was based on formulation weight (μg/ml).  
b The total number of larvae observed in three replicated tests.  
c There was no significant difference (at *p*=0.05) between same letters as analyzed by the Bonferroni method.

*p*<0.05). The infection rates at 1,000 ppm of the GV product alone without and with UV irradiation were 100 and 43%, respectively. On the other hand, the addition of iron oxide at 1, 2 and 4 mg/ml led to the infection rates recovering to 71, 72 and 85%, respectively. Although the infection rates with the addition of iron oxide were not statistically significant, except for 4 mg/ml, compared with that of GV alone under UV irradiation, this suggests that higher concentrations of iron oxide might have greater UV-protective activity.

**Synergistic activity between iron oxide and GV product**

To determine whether the GV product and iron oxide displayed a synergistic activity, the infection rates for *H. magnanima* neonates were compared between GV alone and the mixture of GV-iron oxide under no UV irradiation. Iron oxide at 2 mg/ml was mixed with 1,000 ppm of GV product and the infection of *H. magnanima* neonates was examined (Table 4). The average infection rates among treatments in Table 4 differed significantly by two-way ANOVA (*F*4,10=131.583, *p*<0.05). When the same concentration of GV product was used, there was no difference in the infection rates between the presence and absence of iron oxide. The infection rate of tested *H. magnanima* larvae was significantly affected by the concentration of GV product (*F*1.8=181.5, *p*<0.05) but not by the presence/absence of iron oxide (*F*1.8=0.009, *p*>0.05). Additionally, interaction between the concentration of GV product and presence/absence of iron oxide was not statistically significant (*F*1.8=0.146, *p*>0.05). Results show that iron oxide neither affected infection by product, nor had any interactive effect (synergism or antagonism) on the relationship between the GV product and infection.
rate under no UV irradiation. Additionally, this suggests that iron oxide might have no detrimental effect on the larval development of *H. magnanima*, since no distinct differences were observed in the larval period for treatments of GV alone and mixtures of GV-iron oxide (data not shown).

**DISCUSSION**

It has been reported that several substances show a protective effect on the inactivation of insect viruses by UV irradiation under laboratory conditions (Jaques, 1971; Kunimi, 1986; Shapiro and Robertson, 1990; Shapiro, 1992; Ignoffo and Garcia, 1994). However, few substances could be utilized to improve the residual activity of virus products in practical pest control. One of the reasons for not using them is, there might be a difference in effectiveness as an UV protectant due to the difference in laboratory and field conditions. The cost performance of UV protectants must also be considered for practical use. Since the fluorescent lamps (peak 315 nm: range 270–370 nm) used as the UV source in the present study were kept of a distance at 20 cm above the exposed tea foliage, the intensity was estimated to be 66 mW/cm², which was sufficient to inactivate HomaGV on tea foliage following exposure for 5–10 min (Asano et al., 2003). The present study demonstrated that iron oxide displays a UV protective ability for HomaGV.

In order to estimate the degrees of UV protection for a GV product resulting from the addition of iron oxide at 1–4 mg/ml, the percentages of GV infection in *H. magnanima* at various mixtures of GV-iron oxide with UV irradiation were compared with that of GV alone under no UV irradiation. The concentrations that showed rates similar to the infection rates observed with the application of GV alone or a mixture of GV-iron oxide under UV irradiation were estimated using a concentration-infection regression equation (*Y* = 1.001*X* + 4.174) derived from the results of GV alone under no UV irradiation (Table 1). The comparison of concentrations with and without UV irradiation was used as the index for UV protection. For example, the infection rate at 1,000 ppm of GV product alone under UV irradiation (Table 2) was 42.7%, corresponding to that of 4.4 ppm of GV product alone under no UV irradiation. This suggests that the activity of the GV product decreased to 4.4/1,000 (or 1/230) after UV irradiation. In a similar way, the infection rates using mixtures of GV (1,000 ppm) and iron oxide at 4, 2 and 1 mg/ml with UV irradiation were 85.3, 72.0 and 71.3%, corresponding to those of 74.8, 25.6 and 24.4 ppm of the GV product alone without UV irradiation, respectively. Therefore the reduction in GV activity as the results of UV irradiation was 74.8/1,000 (1/13), 25.6/1,000 (1/39) and 24.4/1,000 (1/41), respectively. This suggests that the addition of iron oxide at 4, 2 and 1 mg/ml decreases the GV reduction activity of UV irradiation to 13/230 (1/18), 39/230 (1/6) and 41/230 (1/6), respectively, as compared to the case without iron oxide.

As iron oxide did not exert any synergistic or antagonistic activity with the GV product under no UV irradiation, this suggests that iron oxide alone has no effect on the biological activity of GV or the volume of GV adhering to the treated tea leaves. Additionally, this also suggests that iron oxide alone did not cause any inhibitory effects on the larval development of *H. magnanima*, since there was no distinct difference observed in pupation time when treated with GV alone or a mixture of GV-iron oxide.

The UV protective activity of iron oxide was also observed against the neonates of *A. orana fasciata* in preliminary tests with the same GV product, as well as *H. magnanima* (Asano unpublished). However, before the practical use of iron oxide as an UV protectant for GV products, several problems related to future tests should be clarified, such as the practical effectiveness and economical concentrations under field conditions, the influence on the quality of the tea plant, safety for humans, the mode of action for UV protection, and so on.

**ACKNOWLEDGEMENTS**

The author thanks Drs. M. Matsui and H. Noguchi (National Institute of Agro-Environmental Sciences) for their support in the present study.

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