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Effects of optical brighteners included in biopesticide formulations on the growth of crops

Dave Goulson [a,*], Lara C. Derwent [a], Dora I. Penagos [b], Trevor Williams [b]

[a] *Biodiversity and Ecology Division, School of Biological Sciences, University of Southampton, Biomedical Sciences Building, Bassett Crescent East, Southampton SO16 7PX, UK*

[b] *ECOSUR, AP 36, Tapachula 30700, Chiapas, Mexico*

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Abstract

Stilbene-derived optical brighteners are compounds that absorb ultraviolet (UV) radiation and emit visible blue wavelengths. When mixed with baculoviruses they can enhance the infectivity, rendering the viruses more efficient as control agents of insect pests. Formulations of baculoviruses with optical brighteners are being tested for control of crop and forest lepidopterous insects in North America. A possible consequence of field applications of optical brighteners on the growth of crops was examined. Application of the optical brightener Tinopal CBS increased reflectance of leaf surfaces, particularly in the region 420–470 nm. In glasshouse trials, foliar applications of 5% solutions reduced growth of maize by about 25%, while 1% solutions reduced growth of barley by about 30–40%. Foliar applications appeared to have no effect on growth of three dicotyledonous crops. Possible explanations for these differences between crops are discussed. Field trials are needed to ascertain whether the increased efficacy of biopesticides gained by including optical brighteners in formulations is sufficient to offset the reductions in growth that can be expected in some crops.

Keywords: Tinopal CBS; Reflectance; Growth rate; Yield

1. Introduction

Baculoviruses are widely used biocontrol agents of insect pests. They have advantages over conventional synthetic insecticides because they exhibit an extremely high degree of specificity and pose little or no threat to non-target organisms (Miller, 1997). A number of baculoviruses are currently manufactured on a commercial scale for field application, e.g. *Heliothis*/*Helicoverpa* nucleopolyhedrovirus (NPV) in the USA (Gemstar, Thermo Trilogy, Columbia, MD), *Anticarsia gemmatalis* Hübner NPV in Brazil (Moscardi, 1999), *Spodoptera exempta* (Walker) and *S. littoralis* (Boisduval) NPV in Egypt and Kenya (McKinley et al., 1989).

Infection of the host occurs when the insect consumes foliage contaminated by the baculovirus. The most common baculovirus formulations are water-dispersible liquids or powders that are applied to crops as aqueous sprays using conventional spray equipment (Hunter-Fujita et al., 1998). One limitation of baculoviruses as biopesticides is that they are rapidly deactivated by exposure to ultraviolet (UV) light (Ignoffo et al., 1989). The incorporation of UV-protectant sunscreens into baculovirus formulations is therefore a subject of considerable

* Corresponding author. Tel.: +44-1703-594212; fax: +44-1703-594269.
E-mail address: dg3@soton.ac.uk (D. Goulson).

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interest (Shapiro et al., 1983; Williams and Cisneros, 2001).

Stilbene optical brighteners substantially enhance the infectivity of baculoviruses (Hamm and Shapiro, 1992; Dougherty et al., 1996). It appears that they change the pH of the insect midgut and also block the sloughing of primary infected midgut cells leading to a higher probability of establishment of infection in larvae simultaneously fed virus and optical brightener compared with virus alone (Sheppard et al., 1994; Washburn et al., 1998). Several optical brighteners are known to interfere with chitin fibrillogenesis, and increase the permeability of this structure which normally acts as a defensive barrier to microbial invasion (Wang and Granados, 2000). The inclusion of optical brighteners in formulations may substantially increase the viability of baculoviruses used for biocontrol as higher pest mortality can be achieved with lower applications of virus (Hamm et al., 1994; Thorpe et al., 1999).

Although optical brighteners are widely used for domestic and industrial applications they have not been previously sprayed in the environment. If they were used in bioinsecticide-based control systems, they could be applied to crops over large areas. Little is known of the potential consequences of such actions. One possible effect may be on the growth of the crops. Coating the leaves with a compound that absorbs UV may suppress photosynthesis and reduce crop yield.

A pilot study was carried out to address this possibility by examining the effects of an optical brightener on the reflectance of light by plant leaf surfaces, and on the growth rate of crops treated with field application rates. Tinopal CBS was selected for this study as it is currently being evaluated as an enhancer for a baculovirus bioinsecticide of the principal pest of maize, *Spodoptera frugiperda* (J.E. Smith) in Mexico (Martínez et al., 2000).

2. Methods

The growth rate of the following five crops was studied: (a) maize, *Zea mays* L. (var. Challenger F1 hybrid); (b) broad bean, *Vicia faba* L. (var. the Sutton); (c) tomato, *Solanum lycopersicum* L. (var. Alicante); (d) barley, *Hordeum vulgare* L. (var. Alexis); (e) cabbage, *Brassica oleracea* L. (var. Greyhound).

Seeds were sown in Levingtons no. 2 compost (Levington Horticulture, Ipswich, UK) in individual 5 cm pots, and maintained in an unheated glasshouse. Plants were watered every week. No fertilizers were applied. Seedlings were randomly assigned to treatments 16 (maize, barley, cabbage and broad bean) or 29 days after sowing (tomato). Positions of the plants from each treatment were randomised. The height of each plant above soil level and the number of leaves was recorded. All crops were then sprayed to run-off with 5 or 1% Tinopal CBS solution or distilled water plus wetting agent as described above. Thereafter each plant was measured and re-treated every week for a further 4 weeks. Plants were then harvested, and the aboveground portion of each plant dried for 24 h in an oven at 80 °C before weighing. The number of replicates per treatment was 20 for maize, barley and cabbage, 18 for tomatoes and 17 for broad beans.

The height and number of leaves were analysed for each plant species by repeated measures ANOVA according to treatment in SPSS 8.0 (SPSS, Chicago, IL). Dry weights were compared across treatments using one-way ANOVA.

Forty four days after sowing, lots of 15 *B. oleracea* plants were sprayed to run-off with 1 or 5% (w/v) Tinopal CBS solution (Ciba Speciality Chemicals Holding, Basle, Switzerland) in distilled water (plus 2 drops/l Farman Blue wetting agent) using a hand sprayer, or with water and wetting agent only. After drying, one leaf was removed from each plant. The reflectance spectrum of 2 cm diameter leaf discs were measured using an analytical field spectrometer (FieldSpec Pro, Analytical Spectral Devices, Boulder, CO, USA), which is sensitive to wavelengths between 350 and 800 nm. Reflectance values were calibrated by reference to a Spectralon^{TM} reflectance panel. Percentage reflectance was compared between treatments using separate analysis of variance for each 1 nm wavelength band, to determine at which wavelengths Tinopal had a significant effect on reflectance.

3. Results

Applying Tinopal CBS to leaves increased reflectance at all wavelengths (Fig. 1). The difference between treatments was significant ($p < 0.001$) at every wavelength. *Post-hoc* tests (Tukey HSD) revealed

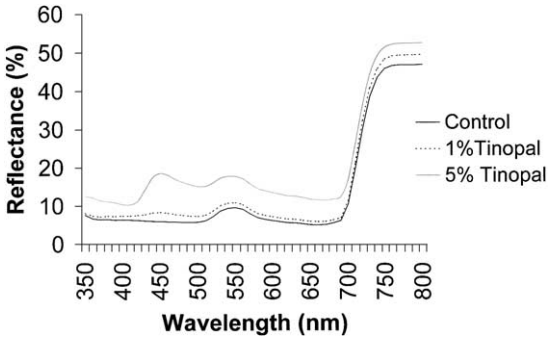

Fig. 1. Effects of applications of Tinopal CBS on the reflectance spectrum of cabbage leaves (mean of 15 replicates).

that the differences between controls and plants treated with 5% Tinopal CBS, and between plants treated with 1 and 5% Tinopal were significant at all wavelengths ($p < 0.05$). However, differences between controls and plants treated with 1% Tinopal CBS were only significant within the ranges 418–435 and 725–800 nm (Tukey HSD, $p < 0.05$).

The biggest difference between control and treated leaves was between 400 and 700 nm, within which range the reflectance of leaves treated with 5% Tinopal CBS was approximately doubled, compared to controls. The reflectance of plants treated with 1% Tinopal CBS was intermediate between plants treated with 5% and controls at all wavelengths. Reflectance curves were similar in shape for all treatments, except for the region 420–470 nm, where there was a marked hump

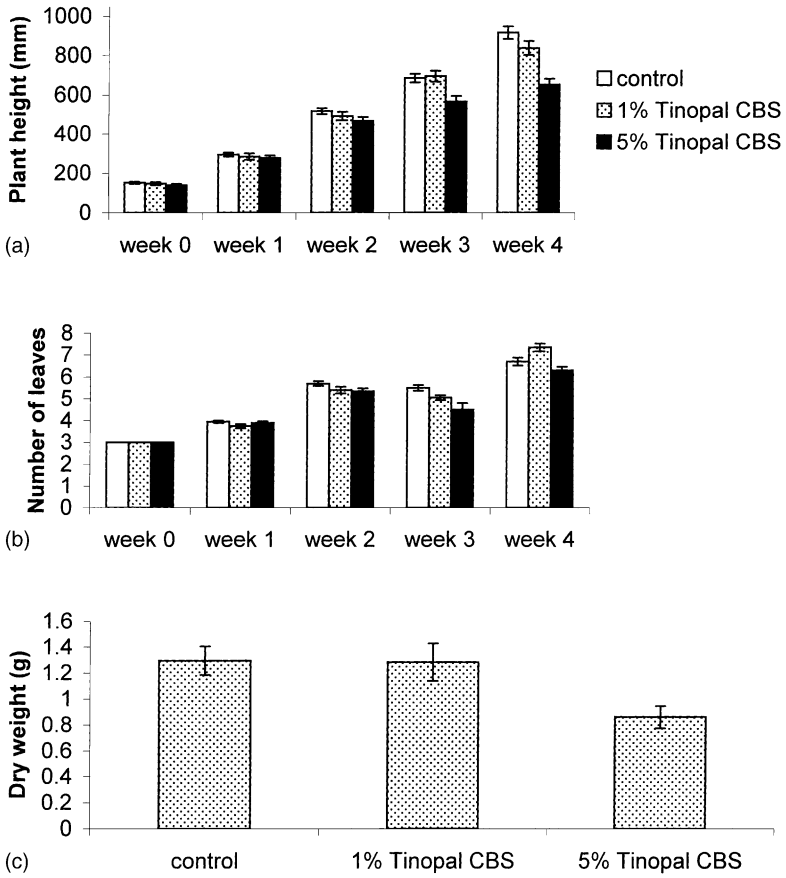

Fig. 2. Assessment of maize plants sprayed with 0, 1 or 5% Tinopal CBS over a 4-week period ($n = 20$ per treatment): (a) mean height (±S.E.); (b) mean number of leaves per plant (±S.E.); (c) dry weight of aboveground foliage.

in the reflectance of plants treated with Tinopal CBS which was not evident in controls.

No significant difference in growth rates was detected between treatments in any of the three dicotyledonous crop species, cabbage, tomatoes and broad beans, either in terms of number of leaves, height of plants, or final dry weight ($p > 0.15$ for all analyses).

Maize control plants were significantly taller than plants treated with Tinopal CBS ($F_{2,57} = 7.04$, $p = 0.002$) (Fig. 2a). The difference between controls and plants treated with 1% Tinopal CBS was not significant (Tukey HSD, $p = 0.68$), but both controls and plants treated with the 1% solution were taller than plants treated with 5% Tinopal CBS (Tukey HSD, $p = 0.002$ and 0.021, for controls versus 5% and 1% versus 5%, respectively). Treatment also affected the number of leaves per plant ($F_{2,57} = 4.07$, $p = 0.022$). Control plants and those treated with 1% Tinopal CBS tended to have more leaves than those treated with 5% Tinopal CBS (Fig. 2b) (Tukey HSD, $p = 0.90$, 0.027 and 0.077, for controls versus 1%, controls versus 5% and 1% versus 5%, respectively). Finally, treatment affected the final aboveground dry weight of plants ($F_{2,57} = 4.59$, $p = 0.014$) (Fig. 2c). Again, differences between controls and plants treated with 1% Tinopal were small, and *post-hoc* tests revealed that the difference in final weight was not significant (Tukey HSD, $p = 0.997$). Both controls and plants treated with 1% Tinopal CBS were significantly heavier than plants treated with 5% (Tukey HSD, $p = 0.027$ and 0.033, for controls versus 5% and 1% versus 5%, respectively). Overall, plants treated with 5% Tinopal CBS experienced an approximately

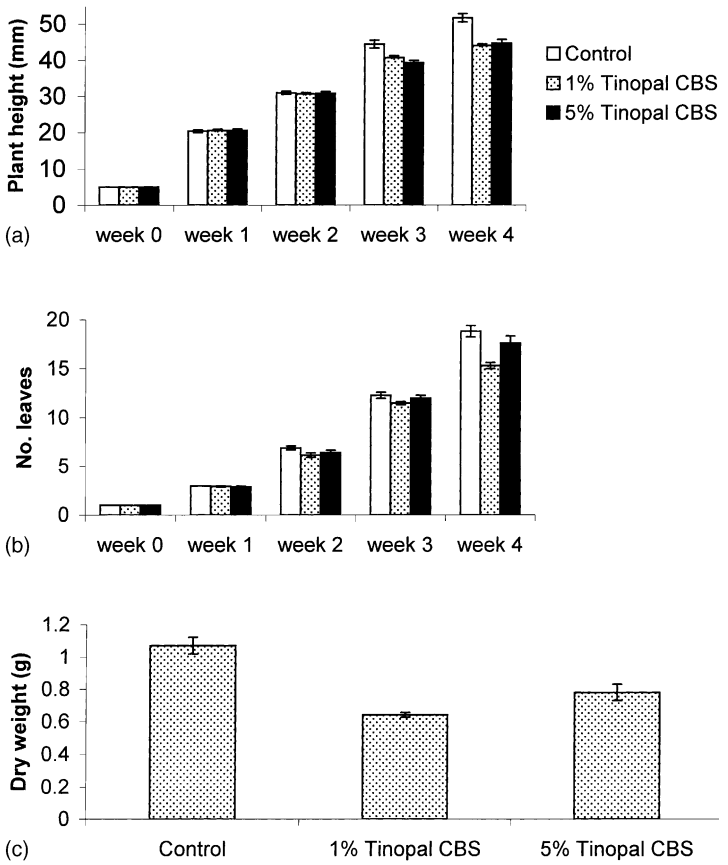

Fig. 3. Assessment of barley plants sprayed with 0, 1 or 5% Tinopal CBS over a 4-week period ($n = 20$ per treatment): (a) mean height (±S.E.); (b) mean number of leaves per plant (±S.E.); (c) dry weight of aboveground foliage.

25% reduction in height and aboveground dry weight, compared to controls.

Barley control plants were taller than those treated with Tinopal CBS ($F_{2,57} = 10.7$, $p < 0.001$) (Fig. 3a). *Post-hoc* tests revealed that controls were significantly taller than plants treated with either 1 or 5% Tinopal CBS (Tukey HSD, $p < 0.001$, 0.001 and $p = 0.909$, for controls versus 1%, controls versus 5% and 1% versus 5%, respectively). The number of leaves per plant also differed according to treatment, control plants having more leaves ($F_{2,57} = 7.82$, $p = 0.001$) (Fig. 3b). However, only the difference between controls and plants treated with 1% Tinopal CBS was significant (Tukey HSD, $p < 0.001$, $p = 0.256$ and 0.059, for controls versus 1%, controls versus 5% and 1% versus 5%, respectively). Control plants reached a greater final aboveground mass than those treated with Tinopal CBS ($F_{2,57} = 23.9$, $p < 0.001$) (Fig. 3c), the difference between controls and plants treated with both 1 and 5% Tinopal CBS being significant (Tukey HSD, $p < 0.001$ for both). There was no significant difference between plants treated with 1% versus 5% (Tukey HSD, $p = 0.072$). Overall, plants treated with Tinopal were about 15% shorter and 30–40% lighter than controls.

4. Discussion

Application of 5% Tinopal CBS markedly increased reflectance of cabbage leaves across the spectrum from 350 to 800 nm. It seems reasonable to assume that similar effects would be observed on other plant species. Increased reflectance will reduce the amount of light available for photosynthesis. Tinopal CBS increased reflectance disproportionately within the range 420–470 nm, which is one of the main absorption peaks for photosynthesis.

Application of Tinopal CBS reduced growth of both monocotyledonous crops tested. On barley, even applications of 1% Tinopal CBS significantly reduced height, number of leaves and final aboveground weight compared to control. On maize, only applications of 5% Tinopal CBS produced a significant effect. In contrast, Tinopal CBS had no detectable effects on the growth of the three dicotyledonous crops tested.

Growth trials on more plant species are needed to determine if applications of Tinopal CBS affect monocotyledonous plants more than dicotyledonous plants, but the present results suggest that this is the case. Differences between plant species may reflect variation in plant growth patterns and architecture, or in the physical properties of the leaf surface (which may influence how much Tinopal CBS clings to the leaf). Variation in leaf surface chemistry may also result in differential effects on plant species. Stilbene brighteners are deactivated by divalent ions (Ca^{++}, Mg^{++}, etc.) or high pH. Thus on the alkaline surface of cotton leaves, Tinopal LPW (M2R) almost completely disappeared within 3–7 days of application (Vail et al., 1999). Leaf surface chemistry was not assessed, but it is possible that the differences observed between crops were due in part to differences in persistence of Tinopal CBS on leaves.

Some baculoviral pesticides are applied on a very large scale, e.g. against gypsy moth over large forest areas in North America and more than 1 million ha of soya bean is treated with NPV in Brazil (Moscardi, 1999). Optical brighteners included in some formulations could disrupt natural communities through reduced growth rates of some wild plants species. When applied to oak in Maryland, USA, Blankophor BBH (another optical brightener) was still present at the same concentration 28 days post-application and between 28 and 40% of the brightener was recovered from treated foliage 6 months later (Webb et al., 1994). The cumulative effects of reduced photosynthesis through an entire season could have a profound effect on tree growth and reproduction.

The environmental benefits of baculovirus pesticides compared to conventional pesticides are considerable. Optical brighteners offer a means of rendering baculovirus pesticides more efficient and therefore more attractive as control agents against a range of insect pests. However, they also have undesirable side effects. Recently, Goulson et al. (2000) demonstrated that 0.1% solutions of Tinopal CBS on flowers can disrupt the behaviour of pollinators, reducing their ability to recognise flowers and locate nectaries. For crops where insect pollination is necessary to obtain an adequate yield, this effect could be of considerable importance.

Any negative effect on plant growth is clearly an undesirable property in a pesticide. The current study demonstrated significant effects on growth rates of two major crops, with reductions in aboveground

dry mass of up to 40%. However, the experimental setup probably represented a worst-case scenario. The optical brightener was applied at weekly intervals, something that is unlikely to occur with biopesticide formulations, except when very frequent applications are required either for control of pests such as *Spodoptera exigua* in Thailand (Kolodny-Hirsch et al., 1997), or when the cosmetic appearance of the product determines its value, such as damage to apple and pear by larvae of the codling moth, *Cydia pomonella* L. (Huber and Dickler, 1977).

Reductions in crop growth could be offset by improvements in crop protection obtained by including optical brighteners in biopesticide formulations. Clearly the net effect on crop yield and any undesirable side effects need to be properly evaluated in field-scale trials.

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