Evaluation of Production Method and Formulation for Optimizing In-vitro Produced Gypchek

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Abstract

Gypchek* (USDA Forest Service, Washington, DC), a product with the *Lymantria dispar* multi-enveloped nucleopolyhedrovirus (LdMNPV) as the active ingredient, is a general use biopesticide for use against the gypsy moth. Successful field trials with Gypchek incorporated with the commercially-produced Carrier 038 (Valent BioSciences Corp., Libertyville, IL) and environmental concerns over the effects of non-specific insecticides applied to forest ecosystems have stimulated interest in the use of Gypchek. Gypchek, as currently produced in vivo, has some limitations. It is expensive to produce, contains extraneous material, and lacks potency at affordable dosages. A strain of LdMNPV (strain 203) has been developed at the USDA Forest Service's Forest Science Laboratory at Delaware, OH, that is as potent as the strain LDP 226 currently used in Gypchek, kills a bit faster, and is stable in cell culture. Its use would result in an inherently clean product with the potential for industrial-scale production at reduced cost. Reduced cost would permit a higher dosage application that should result in improved efficacy. Gypchek produced in-vivo has a half-life of about 12 hours in direct sunlight. An in-vitro produced commodity should be more susceptible to degradation by sunlight since it would lack the insect melanins, etc., present in the partially purified in-vivo product. Valent BioSciences Corp. has addressed the sunlight degradation problem along with improving mixing characteristics in their new product, Carrier 038A. Production techniques may affect potency possibly explaining some variable results seen in earlier studies with strain 203 produced in-vitro.

We thus designed a test with the following goals: (1) We sought to evaluate in vivo produced LDP226 against in-vitro produced strain 203. (2) Strain 203 was evaluated as produced by three different methods to determine if method of production affects performance. (3) We sought to evaluate the effectiveness of Carrier 038A for protecting the virus products from sunlight.

We designed a study that compared in-vivo produced strain LDP226 against strain 203 produced by three different methods. All strains were tested at two doses: 1 x 1012 polyhedral inclusion bodies (PIBs) per ha and 1 x 1011 PIBs per ha. All of the above combinations were applied with and without Carrier 038A, and all the above were evaluated as 1-hour, 1-day, and 2-day residues. There was a Carrier 038A control and a distilled water control. We report the results of 18 treatments arranged in six randomized blocks.

We used a "bugs-in-bags" approach (developed at the University of Massachusetts by Joe Elkinton and students) at a research site at Cedar Swamp, DE, where we have access to large swathes of low, accessible oak

foliage. Two branch tips were pre-selected at each point for each treatment combination and residue date. A cloth bag was placed over each branch tip with leaves bearing treatment residues, and the larvae were added. Larvae were allowed to feed on the leaves for 7 days, were removed from the bags, placed individually in 30-ml diet cups half filled with gypsy moth diet, and held until death or pupation. All cadavers were necropsied.

Analysis of variance revealed that time (residue period) effects, dose effects, and carrier (with or without) effects, but not method of production effects were significant at P = 0.05. We concluded that: (1) In-vitro produced 203 was equivalent in potency to in-vivo produced LDP226. (2) Any affect of production method on potency of strain 203 was minor. (3) Product potency (either strain produced by any method) was greatly enhanced when applied with Carrier 038A. (4) Carrier 038A provided excellent protection from sunlight for at least 2 days.