FORMULATION DEVELOPMENT TO INCREASE NEMATODE EFFICACY IN SUPPRESSION OF THE LESSER PEACHTREE BORER, *Synanthedon pictipes*

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ABSTRACT

The overall goal is to develop a non-toxic formulation to protect beneficial insect-killing nematodes from ultraviolet radiation and/or desiccation to increase efficacy in suppression of the lesser peachtree borer and other insects that attack crops aboveground. Specifically, in this project we addressed the following objectives: 1) toxicity tests to determine if any potential formulations may harm the nematodes, and 2) field tests to determine if a gel formulation could be combined with nematodes in a single effective spray. The first step to developing a formulation is to determine whether or not the chemicals within the formulation are toxic to the nematodes. In laboratory experiments we conducted toxicity trials with five possible adjuvants/formulations; none were found to be toxic to the nematode *Steinernema carpocapsae* (Sc All strain), which is the nematode that is most effective in suppressing the lesser peachtree borer. Included were four powdered starches (Soybean Oil ratio 46.7% and SoyScreen ratio 49.2%, 32.7% and 24.4%) as well as Nemaprotect. Additional tests were conducted to test the efficacy of Barricade gel in protecting nematodes from UV/desiccation. Barricade was previously shown to greatly enhance nematode efficacy suppressing lesser peachtree borer; however, a major shortcoming was that the gel was applied at a high concentration separately from the nematodes, which makes the process less attractive to growers. Thus, we wanted to determine if Barricade could be effective in protecting the nematodes when applied at a lower concentration that can be sprayed with the nematodes. The assay involved nematodes mixed with Barricade at three different concentrations (0.5%, 1%, 2%). A control of water only and an aqueous solution of infective juvenile staged nematodes (IJs) were also a part of the assay. The assay was split into five time intervals, allowing the infectivity of the IJs to range from immediate infection to two hours. The addition of a sentinel insect, *Galleria mellonella* (as a model host) was exposed to the nematode applications for thirty minutes. The *G. mellonella* were checked after 48 hours to determine the survival of the nematodes. Results of the assay found infectivity in Barricade treatments to last even after a full hour in direct sunlight whereas nematodes without Barricade failed. In our study, Barricade was an effective agent to protect nematodes from hazardous environmental conditions and induce infectivity within the nematodes. The single spray approach of using Barricade should prove more attractive to farmers.

INTRODUCTION

Nematodes constitute a diverse phylum (Nematoda). Most nematodes are free-living and benign, whereas others are parasitic (some are harmful to plants, humans or livestock). In contrast, entomopathogenic nematodes in the genera *Steinernema* or *Heterorhabditis*, also known as beneficial nematodes, are natural pesticides and are not harmful to humans or other non-target organisms. The nematodes kill their insect hosts with the aid of symbiotic bacteria that is carried in the nematode gut. One issue that prevents entomopathogenic nematodes from being used on a wider scale is their susceptibility to environmental factors such as UV radiation and desiccation; thus many insect pests are found in environments that are adverse to nematode survival (e.g., insects that attack crops aboveground) cannot be targeted with beneficial nematodes. For example, in a study aimed at suppressing the lesser peachtree borer, *Synanthedon pictipes*, which attacks the trunk and limbs of peach trees, nematode applications made in an aqueous suspension failed due to exposure to UV radiation and desiccating conditions. If protected long enough, infective juvenile nematodes (IJs) will be able to attack targeted hosts aboveground and eventually suppress pest populations. Therefore, our overall goal is to develop a non-toxic formulation to protect the nematodes from UV radiation and/or desiccation and thereby increase efficacy in suppression of above ground pests, in particular, *S. pictipes*. Specifically, in this project our first objective was to conduct toxicity tests to determine if any potential formulations may harm the nematodes. Our second objective was to determine if a gel formulation (Barricade) could be combined with nematodes in a single effective spray. Barricade is formulated from a gel that is best known for being used in residential and commercial structures in preventing the spread of fire. The gel works by creating a barrier between the structure and the fire, minimizing further fire damage. Barricade is non-toxic to nematodes and environmentally friendly. The Barricade gel (applied at 5%) had previously been shown to be effective when sprayed separately after the nematodes – but farmers may not want to make two consecutive sprays for a single pest. Therefore the single spray approach using a lower concentration will increase the likelihood of grower adoption.

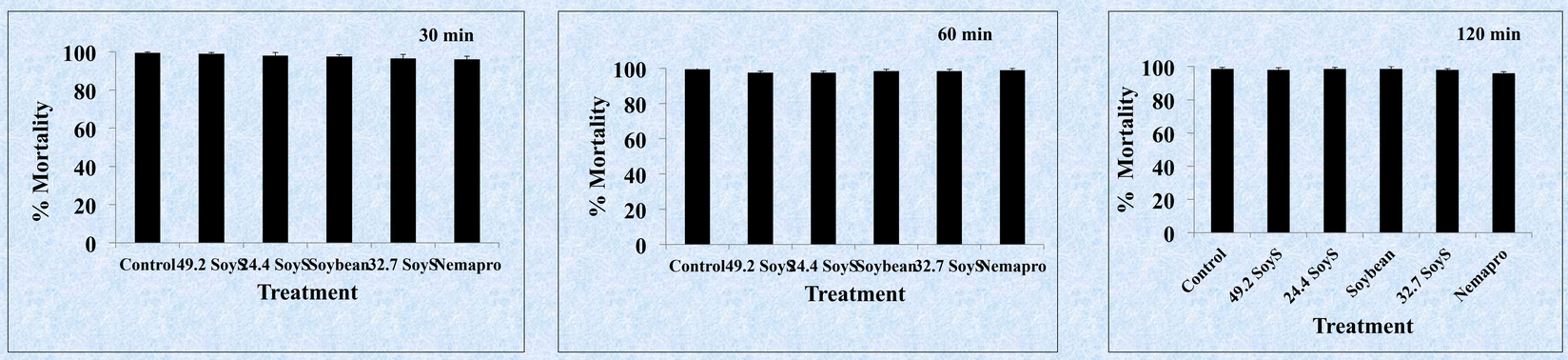

Fig 1.Mortality of *Steinernema carpocapsae* (Sc All strain) nematodes in the exposure to five adjuvants/formulations at three time intervals showed little to no toxicity. All mortality rates were above 93% at all time intervals.

Control = water-only control.

MATERIALS AND METHODS

Objective 1: In a laboratory toxicity assay, five possible protecting adjuvants/ formulations were screened at varying concentrations with the nematode, *Steinernema carpocapsae* (Sc All strain). Three time intervals were used in recording the toxicity of Sc All to four powdered starches (Soybean Oil ratio 46.7% and SoyScreen ratio 49.2%, 32.7% and 24.4%) as well as Nemaprotect. Nemaprotect contains 0.3%Rimulgan, a surfactant based on castor oil and 0.3% xanthan gum. Toxicity was measured by determining nematode survival. Potential treatment differences were analyzed using analysis of variance (ANOVA; alpha = 0.05).

Objective 2: To determine the ability of Barricade gel to be applied as a protective agent when combined with nematodes in a single spray a baseline field test was conducted. Four formulation concentrations were used along with a water-only control; the four formulations included an aqueous solution consisting of nematodes and water, and three different concentrations of barricade plus nematodes (concentrations: 0.5%, 1% and 2%). To determine base-line survivability, of nematodes in the different formulations five time periods were used to measure nematode infection rate: infection immediately after nematode application (a zero time point), and infection 0.25, 0.50, 1 and 2 hours after nematode application. Applications were made outside under field conditions. A set of ten *Galleria mellonella* (wax moth larvae used as a model host) were added to the petri dishes for thirty minutes after each time period. After thirty minutes the *G. mellonella* were removed and placed on sterile 90 mm Petri dishes to be evaluated.; the insects were stored at 25 °C in the laboratory. Infection evaluations were taken 48 hours later by counting the number of live and dead *G. mellonella*. The number of live/dead insects was used to indicate nematode survival and infectivity. Treatment differences were detected through Fischer's Exact Test (alpha = 0.05).

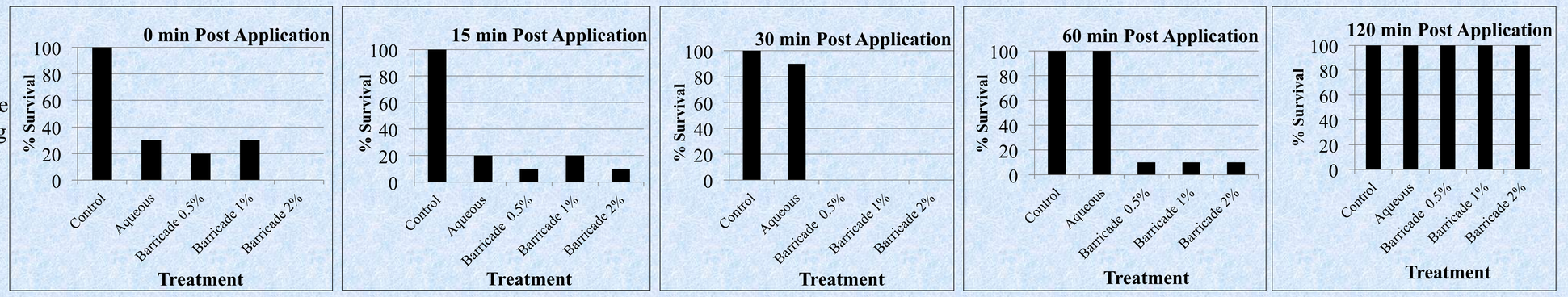

Fig.2 Survival of *Galleria mellonella* (model host) after being exposed to three concentrations of Barricade gel and nematodes at five different time intervals. An aqueous nematode solution (without Barricade) was also a part of the assay. Control= water-only

RESULTS and DISCUSSION

1. Results of the toxicity assay indicated little no toxicity of the nematodes to the five screened adjuvants relative to the control (Fig. 1; $P > 0.05$ ANOVA). This indicates that all of these adjuvants can be used as potential protecting agents against UV or desiccation without harming the nematodes.

2. In the experiment that determined whether Barricade can be applied as a protective agent with nematodes in a single spray , all formulation treatments and the aqueous control caused high levels of *G. mellonella* mortality (i.e. , low survival) at 0 and 15 min post-application (Fig. 2); however, at 30 min and 1 hour only the Barricade treatments caused substantial G. mellonella mortality whereas the aqueous treatment (without Barricade) failed to cause substantial mortality (Fig. 2). At 2 hours post-application all nematode treatments failed to cause substantial mortality. These results indicate that Barricade applied as a single spray at 0.5 to 2% can protect nematodes from UV or desiccation (i.e., compared with aqueous nematodes without Barricade). However, after 2 hours the Barricade formulation's protective ability declines. The combined spray will likely be attractive to growers as a tool to suppress lesser peachtree borers.

3. In future research, the Barricade field test will be repeated. Furthermore, adjuvants (found not to be toxic to nematodes) will be combined with Barricade to determine if additional levels of protection (e.g., beyond 2 hours) can be obtained. Finally, the optimum formulations (combined adjuvants and Barricade) will be tested in field trials to suppress the target pest, lesser peachtree borer.

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