BEAUVERIA BASSIANA ACTIVITY AGAINST LYGUS HESPERUS AT LOW TEMPERATURES

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Abstract

The western tarnished plant bug, *Lygus hesperus* Knight, a key pest of cotton (*Gossypium hirsutum* L.) in production regions of the western U.S., is attacked by the naturally-occurring pathogen, *Beauveria bassiana* (Balsamo) Vuillemin, in the San Joaquin Valley of California. Previous research at Shafter, CA sought *Beauveria* strains that were effective under high summertime temperatures typical of the region. However, the most appropriate use of *Beauveria* against *Lygus* in California cotton may not be as a rescue treatment. Alternatively, *Beauveria* may be useful in efforts to suppress overwintering populations of *Lygus* if strains are available that are highly virulent at temperatures typical of fall and winter seasons. We conducted preliminary laboratory assays of *Beauveria* activity against *Lygus* at temperatures of 55, 65, and 75°F. High levels of infection were obtained with most *Beauveria* isolates at all temperatures tested. Temporal patterns of mortality varied with temperature, and 21 days appears to be an appropriate duration for assessing mortality under the conditions we examined. Results provide a rationale for more detailed examinations of *Beauveria* isolates for low-temperature activity against *Lygus*.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight, is a key pest of cotton (*Gossypium hirsutum* L.) in western production regions of the U.S. Development of improved and ecologically benign management tactics for *Lygus* represents a major component of the overall mission of the Western Integrated Cropping Systems Research Unit (WICSRU). *Beauveria bassiana* (Balsamo) Vuillemin, is a naturally-occurring fungal pathogen that attacks *Lygus* in the San Joaquin Valley of California (McGuire 2002). Previous research within WICRSU sought to select strains or isolates of *B. bassiana* that were effective under the high summertime temperatures typical of the production region (Leland et al. 2005a, McGuire et al. 2005). However, field tests of these strains indicated less impact on population levels of *Lygus lineolaris* (Palisot de Beauvois) (Leland et al. 2005b) or *Lygus hesperus* (McGuire et al. 2006) than was expected at the observed infection levels. Therefore, the most effective use of *B. bassiana* in a management strategy for *Lygus hesperus* may not be as a rescue treatment in cotton.

Effective management of *Lygus hesperus* in cotton is complicated by the mobility of the pest, its wide host range, and by difficulties associated with accurate population estimation. An ecologically-based approach to *Lygus* management whereby populations are reduced by naturally occurring pathogens or other means, before movement into cotton, is conceptually attractive. Development of such a management strategy would require increased knowledge of the dynamics of *Lygus* diapause and overwintering ecology, and identification and development of suitable control tactics, such as a *B. bassiana* isolate that is effective under the relatively cool fall and winter conditions. Our objectives were to conduct preliminary trials to determine appropriate timeframes over which to evaluate the activity of selected *B. bassiana* isolates under low temperature conditions.

Materials and Methods

Experimental Procedure

A series of four preliminary bioassays were conducted. In each assay we used mixed-sex *Lygus hesperus* adults that were 1- to 3-d-old at the time of treatment application. These bugs were obtained from a long-standing laboratory colony maintained on green bean pods and raw sunflower seeds at the WICSRU. Depending on the isolate, *B. bassiana* conidia were either obtained from previously prepared stocks (dried or stored at -80°C in 15% glycerol) or were freshly harvested from colonies grown on Sabouraud dextrose agar (Becton-Dickson, Cockeyesville, MD) supplemented with 0.2% (w/v) yeast extract (Sigma Chemical, St. Louis, MO). One or two days before application the percentage of viable conidia was estimated by culturing an aliquot of each isolate overnight in potato dextrose broth (Sigma) and examining the conidia for germination under a microscope at 400×. Conidia were considered viable if the germ tube was > the diameter of the conidia.

On the morning of treatment application bugs were aspirated into 15-dram vials fitted with foam-lined bottoms. Vials of bugs were held in an environmental chamber at 60°F for 2–4 hrs before treatment. Immediately before the application of each *B. bassiana* isolate, 10 adult *Lygus* were anesthetized with CO_2 and spread in the bottom of a 100 × 15-mm Petri dish lined with filter paper. The plate was then placed within a specially-designed spray chamber used by McGuire et al. (2005), within which 5-ml of the designated conidial suspension (in 0.01% Silwet L77, GE Silicones, Friendly, WV) was applied through a TG 0.4 full cone nozzle (Spraying Systems, Wheaton, IL) at about 20 psi. Immediately after the spray treatment, treated bugs were individually placed into 5-dram plastic vials. Each vial contained a short (≈2–3 cm) section of green bean pod and was closed with a foam plug. Treated bugs were maintained at the assigned temperatures within environmental chambers that also provided a photoperiod of 16:8 (L:D) h. Vials were examined daily to determine mortality, and green bean sections were replaced every 2–3 days.

The first assay was conducted using a stock of dried spores of the isolate referred to as WTPB2 by Leland et al. (2005a). The spores were applied at a final concentration of about 5.6×10^6 viable conidia ml^{-1}. Temperature treatments were 55, 65, and 75°F. An untreated control (CO_2 anesthetized bugs) was also included and mortality was monitored for 23 days.

The second assay used previously frozen spores of the isolate 38-06 (McGuire et al. 2005), which were applied at a concentration of 10^6 viable conidia ml^{-1}. Other procedures were the same as for the first assay, except that mortality was monitored for 28 days.

The third assay used the isolate 56-06 (McGuire et al. 2005) which was freshly harvested, and GHA, an isolate derived from a commercial formulation (Mycotrol, Emerald BioAgriculture, Lansing, MI), which was previously frozen. Both isolates were applied at a concentration of 10^7 viable conidia ml^{-1}. Other procedures were the same as for the earlier assays, except that mortality was monitored for 23 days.

The fourth assay incorporated eight *B. bassiana* isolates but only two temperatures (55 and 65°F). In addition to three of the isolates included in previous assays (WTPB2, 56-06, and GHA), isolates included 3769, NI6, NI8, and NI9 (Leland et al. 2005a) and 17-41 (McGuire et al. 2005). All isolates were applied at a concentration of 10^7 viable conidia ml^{-1}. The experiment also included two controls, one of bugs that were anesthetized only, and one in which anesthetized bugs were also sprayed with 5 ml of 0.01% Silwet L77. Mortality was monitored for 26 days. In addition, bugs that died were retained for four days at their assigned temperatures to determine the incidence of sporulation. On the day each bug died, the foam plug closing the vial was replaced with a plastic cap. Green bean sections were left in the vials to maintain high levels of humidity.

Statistical Analysis

The data from each assay were examined by survival analyses using the LIFETEST procedure of SAS (SAS Institute 2002). For the first three assays, survival functions of the various treatments, including the controls, were examined for differences. A subsequent analysis for each *B. bassiana* isolate compared survival functions among temperature treatments. PROC LIFETEST was similarly used to analyze data from the fourth assay, except comparisons among isolates excluded both control treatments (for which no mortality was observed). In all analyses, statistical differences were declared on the basis of the log-rank χ^2 statistic, which places more weight on larger survival times.

Results and Discussion

Results of the first two assays indicated the *B. bassiana* treatments reduced *Lygus* survival regardless of the isolate or temperature, despite a control mortality of 40% by day 23 at 55°F in the first assay (assay 1, WTPB2, $\chi^2 = 8.87$ to 19.00, df = 1, $p < 0.01$, Fig. 1; assay 2, 38-06, $\chi^2 = 5.98$ to 6.36, df = 1, $p = 0.01$, Fig. 2). Comparisons among temperature treatments within isolates indicated a significant temperature effect for isolate WTPB2 in assay 1 ($\chi^2 = 34.08$, df = 2, $p < 0.01$; Fig. 1b). In this assay, mortality from *B. bassiana* infection occurred more slowly at 55°F that at the other temperatures. A similar mortality response to temperature was not demonstrated in the second assay (isolate 38-06; $\chi^2 = 1.20$, df = 2, $p = 0.55$; Fig. 2b). A demonstrable temperature effect in the second assay was probably lacking because isolate 38-06 did not produce complete mortality of treated *Lygus* at any temperature, and differences in mortality among temperature treatments were relatively small at the longest survival times.

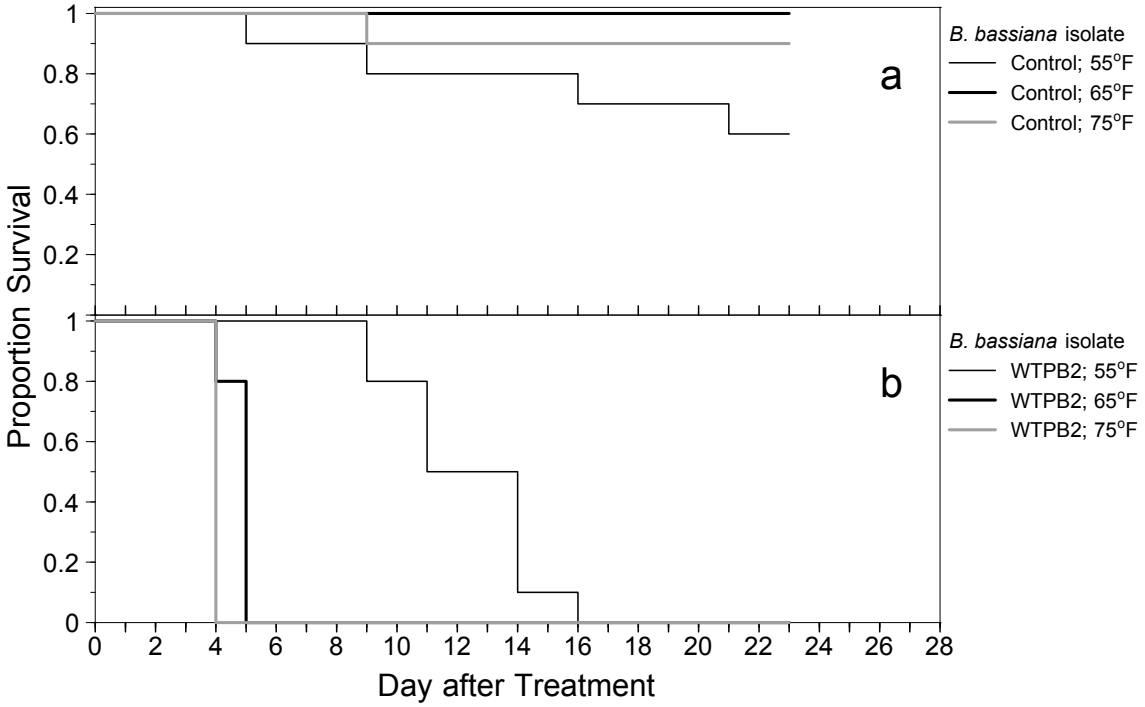

Figure 1. Survival functions for *Lygus hesperus* adults at three temperatures and a) not treated (control) or b) treated with isolate WTPB2 of *Beauveria bassiana*.

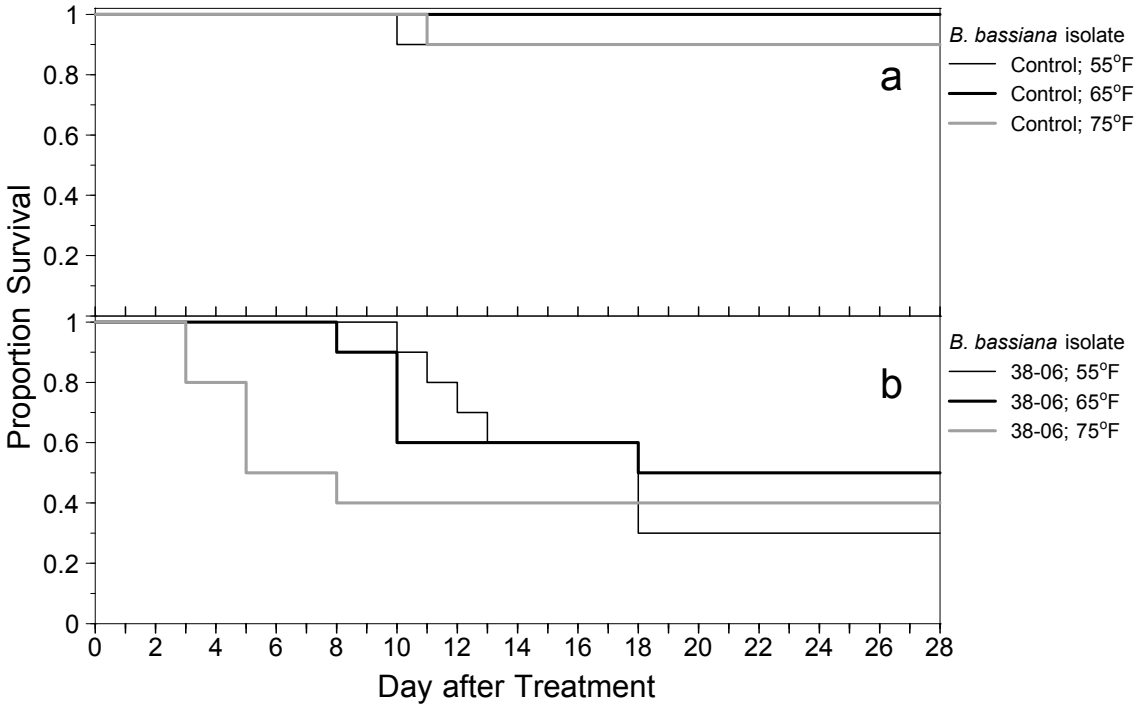

Figure 2. Survival functions for *Lygus hesperus* adults at three temperatures and a) not treated (control) or b) treated with isolate 38-06 of *Beauveria bassiana*.

In the third assay differences in *Lygus* mortality were again apparent in response to *B. bassiana* treatment (55°F, χ^2 = 34.17, df = 2, $p < 0.01$; 65°F, χ^2 = 30.69, df = 2, $p < 0.01$; 75°F, χ^2 = 32.72, df = 2, $p < 0.01$; Fig. 3). At 55 and 65°F, isolate 56-06 tended to produce higher levels of mortality than did isolate GHA. Differences between the two isolates were less apparent at 75°F, when isolate 56-06 appeared to produce mortality slightly earlier than did isolate GHA, but both isolates killed all of the treated bugs by day 13 after treatment. Also, isolate 56-06 tended to produce mortality of *Lygus* adults earlier at 65 and 75°F than at 55°F (χ^2 = 22.61; df = 2; $p < 0.01$). For isolate GHA, mortality tended to occur earlier and levels of observed mortality increased with increasing temperature (χ^2 = 25.90, df = 2, $p < 0.01$).

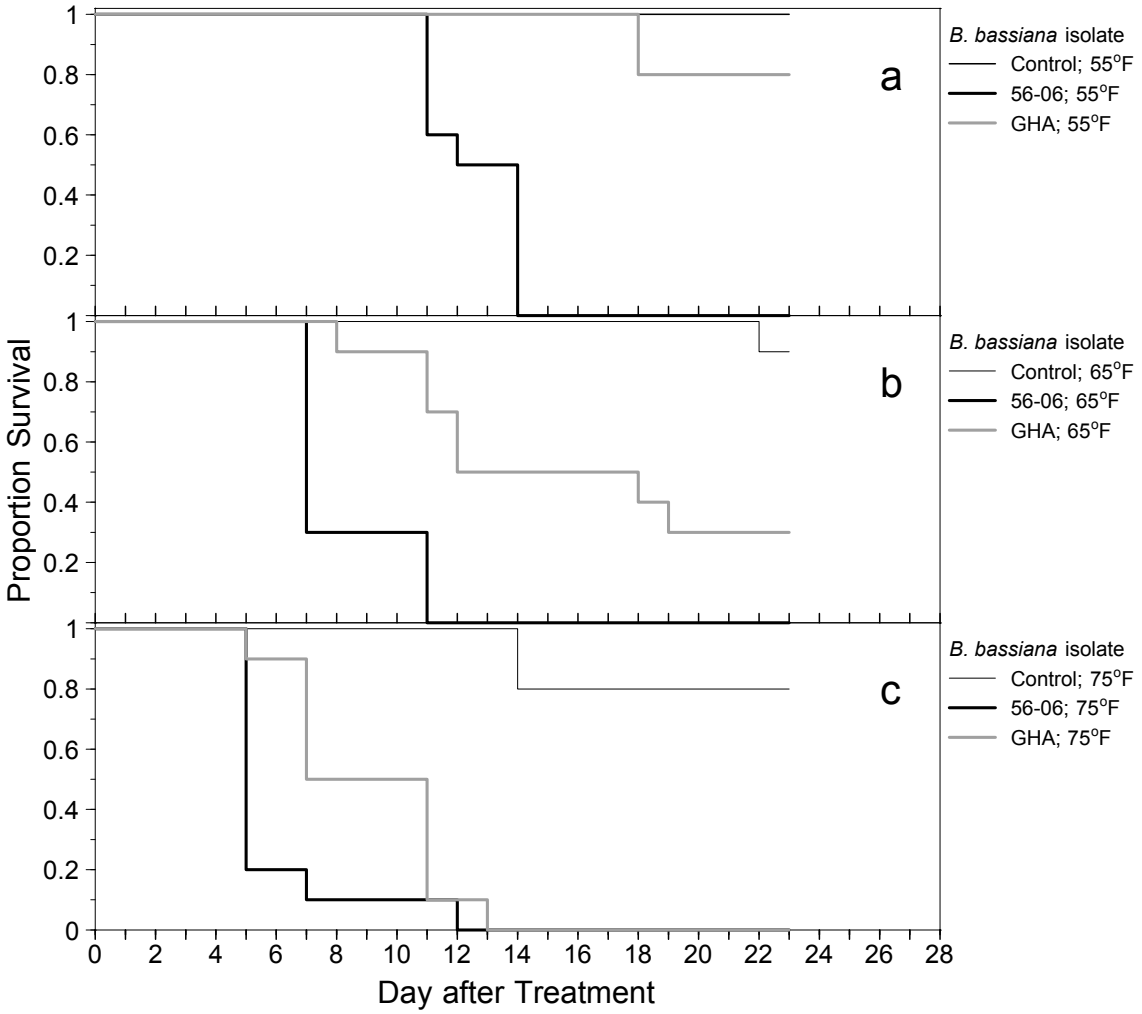

Figure 3. Survival functions for *Lygus hesperus* adults untreated (control) or treated with *Beauveria bassiana* isolates 56-06 or GHA at a) 55°F, b) 65°F, or c) 75°F.

Finally, in the fourth assay 17-41 was the only isolate that did not produce complete mortality at 55°F, and sporulation was observed on only 87.5% of the dead *Lygus* for that isolate. The only other isolate for which sporulation was observed on <90% of cadavers was GHA at 65°F (70%). Of those combinations of isolate and temperature that produced complete mortality, isolates 17-41 and GHA appeared to be among the slowest to do so. However, differences among isolates in this trial were relatively subtle, and the analyses did not indicate significant differences in the survival functions of the various isolates at either temperature (55°F, χ^2 = 8.47, df = 7, p = 0.29; 65°F, χ^2 = 11.98, df = 7, p = 0.10; Fig. 4). When isolates were examined for the effects of temperature, all except GHA (χ^2 = 1.53, df = 1, p = 0.22) produced mortality significantly earlier at 65° than at 55°F ($p < 0.01$; Fig. 4).

Failure to detect a temperature difference for GHA was probably caused by a combination of the overlapping survival functions for this isolate at the two temperatures for days 13–15 after treatment, and the emphasis on later survival times of the log-rank χ^2 statistic.

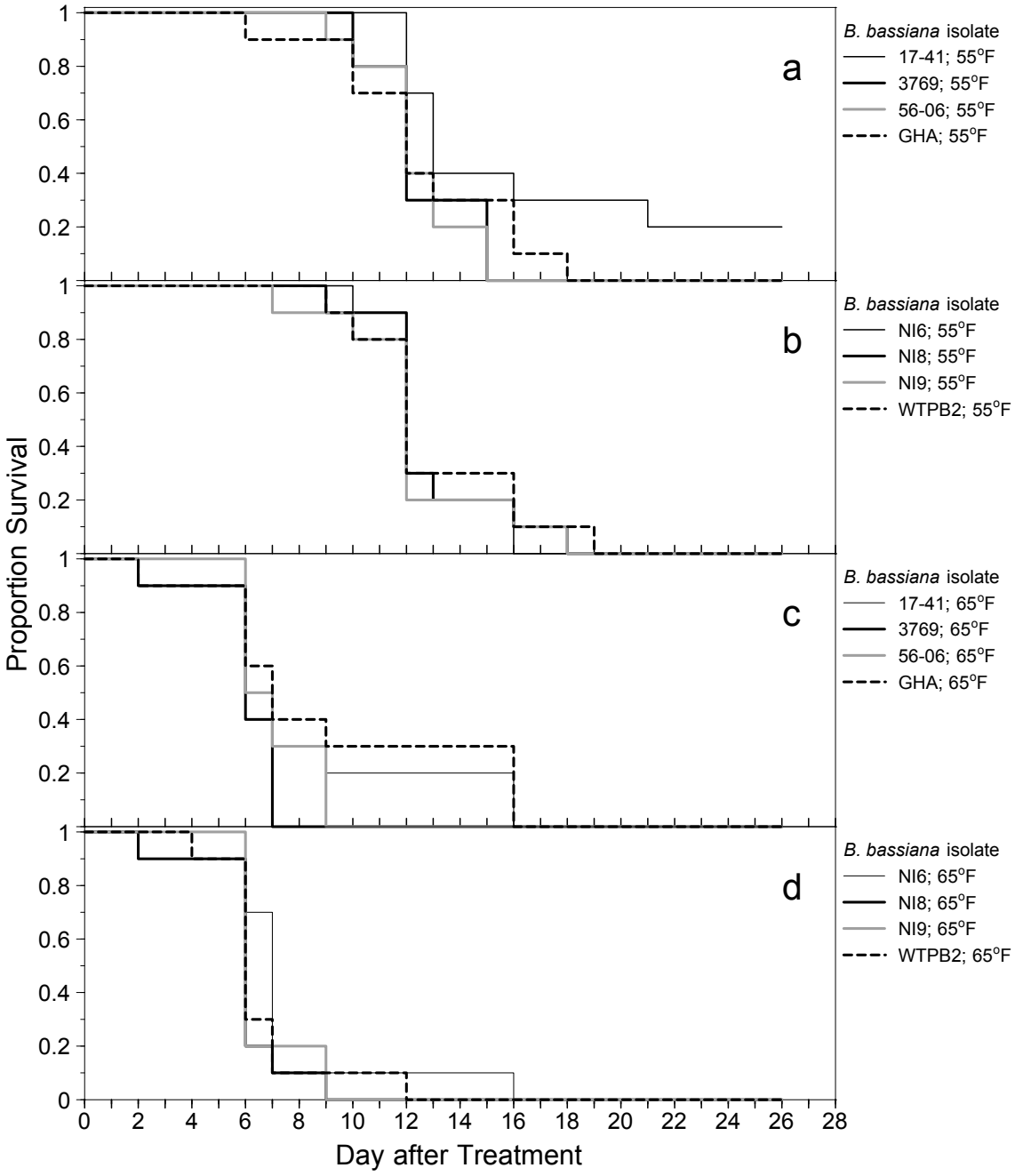

Figure 4. Survival functions for *Lygus hesperus* adults corresponding to eight *Beauveria bassiana* isolates at a, b) 55°F or c, d) 65°F.

In summary, these preliminary results suggest at least some differences existed in the mortality responses of *Lygus hesperus* adults to tested *B. bassiana* isolates. Therefore, screening of additional isolates, especially those collected from field insects during the fall and winter periods, may result in the identification of isolates with high levels of virulence at low temperatures. In addition, differences in *B. bassiana* activity between temperatures of 55 and 65°F

appeared to be larger than corresponding differences between 65 and 75°F. Given the observed responses to temperature, in tests conducted under similar conditions a post-treatment monitoring period of 21 days should be adequate to detect meaningful differences in *B. bassiana* activity.

Acknowledgments

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