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APPLICATION OF A NEW STRAIN OF METARHIZIUM ANISOPLIAE (FUNGI IMPERFECTI) AS A MEANS OF BIOLOGICAL CONTROL AGAINST THE COCONUT LEAF HISPID BRONTISPA LONGISSIMA (COLEOPTERA: HISPIDAE) IN SAMOA

Karl J. Marschall[1], Agnes M. Vargo[2], and Sipaia Fatuesi[2]

[1] K.J. Marschall Samoa, Ltd., Biological Laboratories, Vaoala, Apia, Western Samoa.

[2] Land Grant Program, American Samoa Community College, Pago Pago, American Samoa.

American Samoa Community College Land Grant Program
P.O. Box 2609, Pago Pago, American Samoa 96799

ABSTRACT *Brontispa longissima* (Gestro), coconut leaf hispid (CLH), is a serious pest of coconut palm, an important staple crop in Samoa. In 1980, a naturally occurring strain of *Metarhizium anisopliae* var. *anisopliae* was found by the senior author on field collected specimens of *B. longissima* at Vailoa-Tai in American Samoa. A laboratory strain of the pathogen was subsequently developed and spores were mass produced and packaged. In 1982, the spores were used successfully in Mulifanua, Western Samoa, to suppress *B. longissima* populations. In 1984, *M. anisopliae* spores were used in the Manua Islands of American Samoa to control *B. longissima* on 5 - 6 month old coconut trees. A follow-up survey in American Samoa in 1988 indicated that the CLH population was significantly lower in those areas where the fungal spores had been used as compared to untreated areas. In addition, observable *B. longissima* damage was negligible in treated areas. Survey of areas adjacent to initial *M. anisopliae* applications revealed the fungus was present on dead beetles in coconut trees up to 1 km from the initial source of application.

The coconut leaf hispid (CLH), *Brontispa longissima* (Gestro), is a small beetle native to Southeast Asia. It lives between the folded leaves of coconut palms and feeds on the surface layer of leaf tissue. In the early 1970's *B. longissima* was accidentally introduced to Tutuila, American Samoa, and to Upolu, Western Samoa in 1980. In contrast to its native home where CLH is of little economic significance, it became a devastating pest in the Pacific islands. CLH is particularly devastating as it destroys young palm trees. In November 1980, during a search for pathogens of *B. longissima* on Tutuila, a few CLH beetles were found with fungal infections in palm trees on the coast near Taputimu Farm, Vailoa-Tai, American Samoa. They were subsequently taken to the commercial laboratory of the senior author at Vaoala, Upolu, Western Samoa. The fungus was propagated and identified as *M. anisopliae* var. *anisopliae*, a cosmopolitan, entomopathogenic fungus with a wide insect host range. Strains affecting beetles tend to be rather host specific. Toxicological tests indicated that it had no detrimental effect on nontarget organisms. After the fungal spores contact the host's integument, they grow and penetrate the host's haemocoel, multiply, and kill the host. Eventually they grow through the cuticle to the outside, producing an abundance of conidiospores on the carcass, thus allowing for spread of the disease to surrounding beetle populations.

Laboratory trials were conducted to determine virulence, host range and potential of mass producing *M. anisopliae* under limited technical conditions in Samoa. The effect of this new strain was determined in the field and an efficacious rate of 0.045 g or 3×10^9 spores per palm tree was established. Spore weight was subject to variations of up to 20 percent due to drying and other environmentally determined variables.

This study evaluated the effects of spraying a naturally-occurring strain of *Metarhizium anisopliae* var. *anisopliae* in the control of *B. longissima* on a large scale coconut palm plantation in Western Samoa; and determined any differences between *M. anisopliae* treated and untreated sites on two islands of American Samoa.

Methods and Materials

Western Samoa. After suitable rearing methods had been developed and disease virulence verified under field conditions, a combined research and practical biological control program commenced in July 1982 at the 60-ha Mulifanua Plantations of Western Samoa Trust Estates Corporation, (WSTEC), on Upolu.

Conodiospores were reared on artificial media so that they could be separated from the medium and hyphae before weighing and counting. Small plastic bags were filled with 2.5 g of spores, which was the amount calibrated to fill a hand-operated 17 L capacity knapsack sprayer for application to 0.4 ha of 50 to 60 palms. Each palm crown was sprayed with an approximately 300 cc spore suspension. Spores were mixed with ordinary kitchen detergent and water, and then sprayed into the unopened leaflets of the central shoot with a single-jet nozzle.

Treatments were applied to survey plots in July, September, December 1982, and June 1983. In total, over 7,500 three to five-year-old trees were treated on the plantations. The first treatment in July 1982 was done partly with *M. anisopliae* and partly with dicidex trichlorfon. Dicidex was applied because it was desirable to compare its effect with the biological control agent, and because not enough spores were available at that time to treat all endangered palms. Dicidex was applied in concentrations of 0.2 and 2.0 percent in water with detergent.

Using a small ladder, checks for *M. anisopliae* infected beetles in palm trees were made immediately before the first fungus application and counts were made about 2 weeks or more after spraying to allow for the week-long incubation period of the disease. Palm trees were checked to determine the effect of the treatment. Folded palm leaflets were carefully opened so that *B. longissima* stages would not fall out. CLH larvae, pupae, and adult beetles found within were counted and diagnosed. Only dead insects with clear signs of *M. anisopliae* infection (i.e., either green spores or white hyphae) were recorded as dead.

Because beetle damage to the leaf surface is difficult to assess, trees surveyed were grouped into 3 categories: no damage (0 percent), medium damage (less than 50 percent of leaf surface affected), and heavy damage (greater than 50 percent of leaf surface affected).

American Samoa. In 1984, a CLH outbreak occurred in American Samoa on the Manua Islands of Ofu and Olosega. These islands have a combined area of 1270 ha and are about 100 km east of Tutuila, the main island of American Samoa. Ofu and Olosega are only 70 m apart and connected by a bridge. Fronds of 5 - 6 month old coconut palm trees in the Toaga area of Ofu were almost totally brown and wilted due to heavy CLH infestation. In the village of Olosega on the island of Olosega, 5 - 6 month old palm trees were similarly infested. Following the methods used in Western Samoa, all palm trees (approximately 50) were sprayed with spores of *Metarhizium anisopliae* var. *anisopliae*. At two similarly infested sites, the Ofu village and Ofu airport, 5 - 6 month old coconut trees were left as untreated controls. A follow-up survey was conducted in 1988 to assess the outcome of this single spraying and to compare the CLH population at the four sites.

Results and Discussion

Western Samoa. At the beginning of the trial in early 1982, the coconut replanting area under consideration was at the brink of destruction because of high *B. longissima* infestation. Trees were heavily damaged (most young leaves were brown and wilted), and it was thought that most would die. No *M. anisopliae* infection was detected in the pre-treatment counts in the Mulifanua area.

Counting *B. longissima* stages in palm crowns was a formidable task. Ninety-five crowns were checked, most of them 6 to 10 times each. A total of 17,965 individual insects were counted. Results from 36 crowns are presented here. In some trials the number of beetles became too low and too erratic after treatment to yield meaningful statistical data.

Because only dead beetles with clear signs of infection were recorded, our mortality estimates due to *M. anisopliae* infection were probably low. This was because both living CLH beetles and dead beetles without visible fungal infection were recorded as non-infected by the disease. In the dry months of July to November 1982, the number of dead *B. longissima* beetles without *M. anisopliae* symptoms was about one-third of the total number of dead beetles (Fig. 1). During the wet months of December 1982 to April 1983 most dead insects exhibited *M. anisopliae* symptoms. The infection process did not appear to be influenced by the humidity in contrast to the fungus growth outside the dead body which was humidity influenced.

In dicidex-treated trees, less than 40 percent CLH mortality was recorded using the 0.2 percent solution. Ninety-five percent mortality was found in the 2 percent dicidex concentration treatment. In the pathogen-treated trees, *M. anisopliae* caused between 15 to 40 percent mortality (Fig. 2).

However, one month post-treatment, the CLH population regained its previously high numbers in the dicidex-treated palms whereas in the *M. anisopliae* treatment, the population remained significantly below the pre-treatment figures. This phenomenon was consistently observed and corroborated by observations from other plantations where dicidex was applied. Additionally, this insecticide had a deleterious effect on honey bees and other pollinating insects whereas the fungus exclusively attacked the target pest. After the initial trials, dicidex was not used again.

After the initial application of *M. anisopliae*, the CLH population remained relatively low although only low levels of *Metarhizium* infection were observed (Fig. 2). In no case were numbers of the pests found in the treated trees in the range of the pre-treatment figures (Fig. 1). After each application, the beetle population dropped drastically and later slowly increased, possibly due to *B. longissima* beetles immigrating into the treated area from surrounding untreated palms. The palms recovered in 6 to 8 months with less than 10 percent lost with damage levels generally decreasing throughout the survey period after introduction of the pathogen (Fig. 2).

CLH counts were made within a block of untreated trees in the treated area. Two months after the initial microbial application, no *M. anisopliae* infected beetles were found in the untreated trees (Fig. 3). However, six months later (January 1983) *M. anisopliae* incidence in the untreated control block (22 percent) was similar to the treated blocks and remained about this level until observations were terminated.

Since 1983, replanting areas in Mulifanua have been regularly treated every 3 to 4 months with *M. anisopliae* spores. CLH is still present, but at low levels.

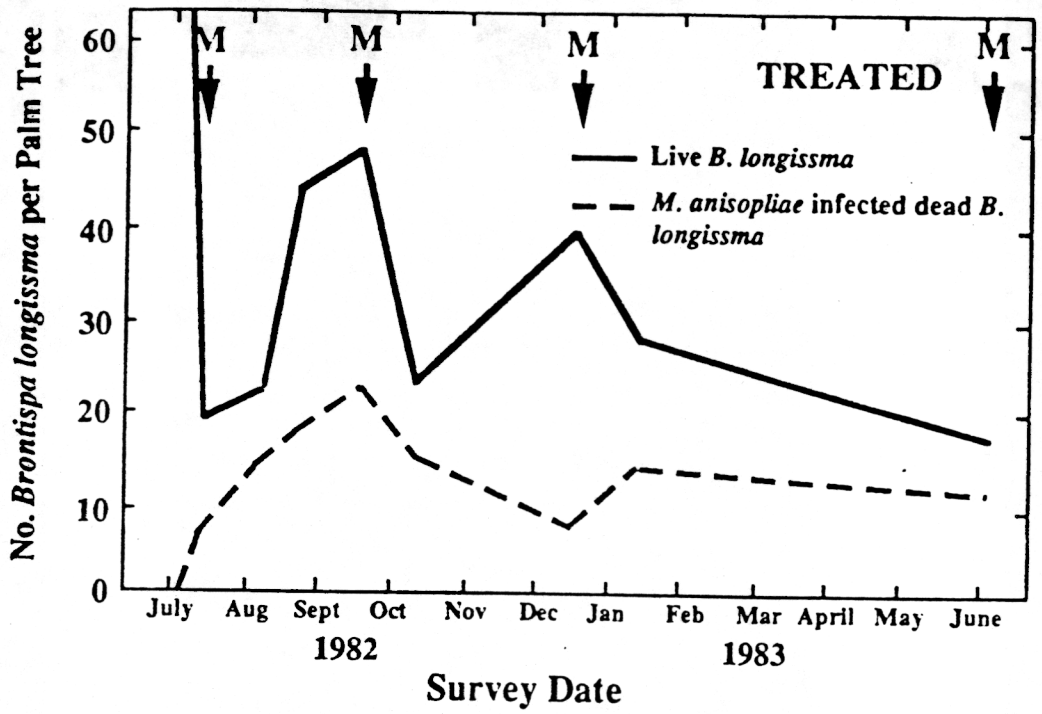

Fig. 1. Changes in mean numbers of *Brontispa longissima* larvae and adults per palm tree in Western Samoa after application of *Metarhizium anisopliae*, and the incidence of the pathogen at various times after microbial application. Arrows with "M" indicate spraying with spores.

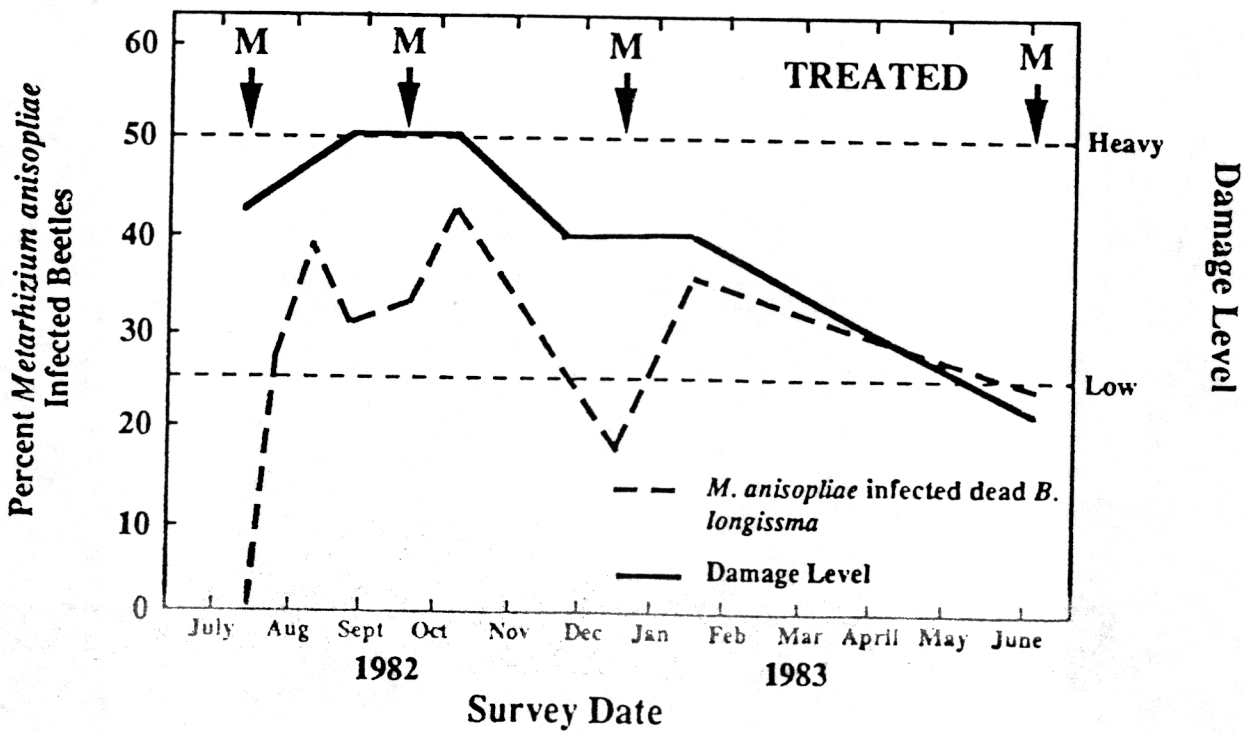

Fig. 2. Mean percentage of *Brontispa longissima* per palm tree which died from confirmed *Metarhizium anisopliae* infections, and palm damage level in relation to pathogen applications in Western Samoa. Arrows with "M" indicate spraying with spores.

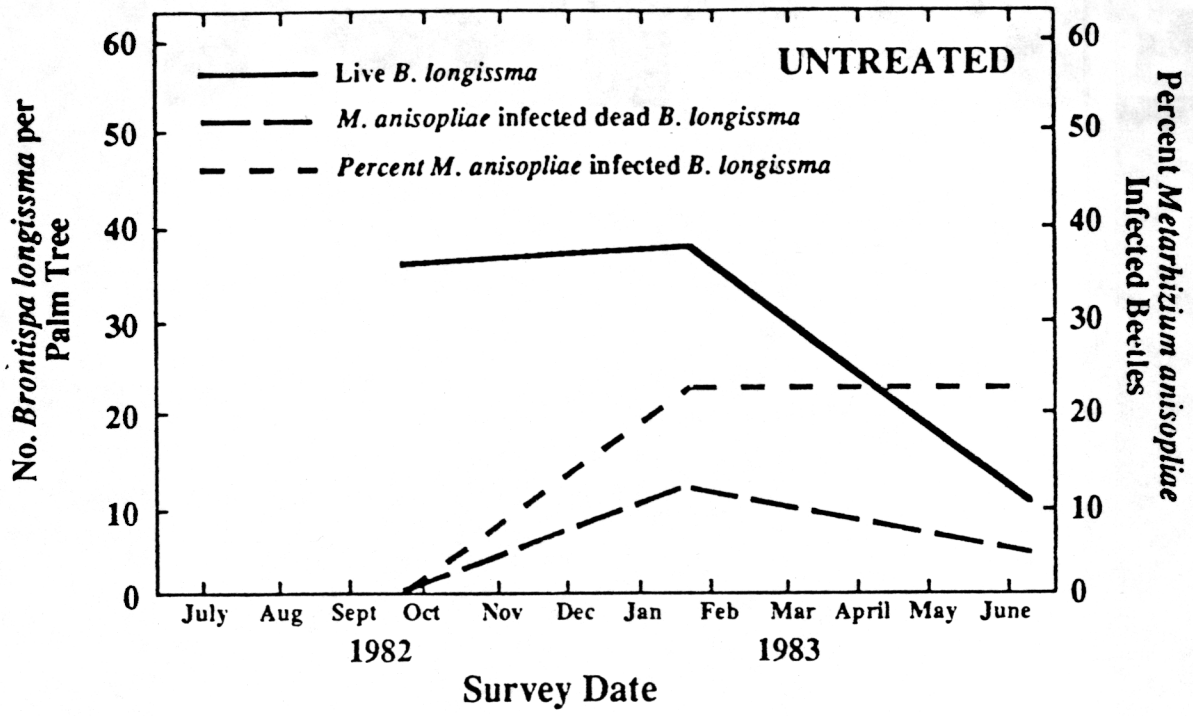

Fig. 3. Mean numbers of live and *Metarhizium anisopliae* infected dead *Brontispa longissima* larvae and adults per palm tree in untreated controls, and the percent *B. longissima* dead from *M. anisopliae* infection in Western Samoa. Data was obtained from 10 palm tres with a total of 950 *B. longissima* evaluated.

Proof of the effect of effective biological control by the pathogen is the beautiful growth of once wilted palm trees and their exceptionally high nut yield. This treatment is now also applied to other replanting areas.

American Samoa. On Ofu and Olosega, there was a noticeable difference between *M. anisopliae* treated and untreated sites with palm fronds visibly more green and full in *M. anisopliae* treated sites. In a statistical comparison (Kruskal-Wallis) between treated and untreated sites, the CLH levels were significantly lower at treated sites ($P < 0.05$). Rarely was more than 1 beetle found per leaflet at treated sites as compared to 5 - 7 beetles per leaflet at untreated sites.

While making counts, it was noted that two varieties of coconut palm were present. They differed in petiole coloration with a reddish variety and a greenish variety called 'Malaysian Dwarf' and 'Samoan Tall', respectively. There were significantly more *B. longissima* in the Malaysian Dwarf variety ($P < 0.01$).

Sampling palm trees on either side of the initially treated sites showed that the *M. anisopliae* fungus had dispersed from the treated sites. Dead CLH beetles, covered with the fungus, were found on trees up to 1 km from treated sites.

Conclusions

Metarhizium anisopliae var. *anisopliae* was used effectively in Samoa in the control of *B. longissima* in both large-scale plantations (WSTEC, Western Samoa) and in isolated stands on smaller islands. (Ofu and Olosega, American Samoa) While varietal differences of palms play some part in CLH incidence, *M. anisopliae* treated palms had consistently low beetle populations which appeared to be maintained below economically significant levels.

Acknowledgement

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