Benefits of an Entomopathogenic Fungus, *Metarhizium, for enhancing* Sweetpotato Growth and Sweetpotato Weevil Suppression

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Metarhizium has traditionally been known as an entomopathogenic fungus (EPF) which means it can infect and kill insect pests and has been used as a bioinsecticide in a wide variety of environments (Uma et al., 2008; Sheng et al., 2022). More recently, *Metarhizium* was reported to develop plant growth promoting properties on crop plants including sweetpotato (Gonzales et al., 2022) and improved growth of numerous plant species.

Fig. 1. Sweetpotato weevil infected by a local isolate of *Metarhizium* 'Ko-002' (picture by Wang).

The relationship between *Metarhizium* and the host plant, can be very beneficial for sweetpotato (*Ipomoea batatas*). Sweetpotato is attacked throughout its cropping cycle by the sweetpotato weevil, *Cylas formicarius (Coleoptera: Brentidae)* reducing marketable yields by up to 100% (Reddy et al., 2014) (Fig. 1). Applying entomopathogenic fungi such as *Metarhizium brunneum* F52 (EC, Novozymes Biologicals, Salem, VA), and *Beauveria bassiana* GHA (BotaniGard ES, Laverlam International Corporation, Butte, MT) in combination reduced tuber damage by *C. formicarius*, producing higher yields than spinosad, azadirachtin, or each of these EPF treatment alone (Reddy et al., 2014). Su et al. (1998) also reported a soil in Taiwan naturally infested with *B. bassiana* resulted in no survival of sweetpotato weevils. Most interestingly, researchers have demonstrated that some *Metarhizium* species not only serve as insect biocontrol agents, but also may function as plant growth promoting rhizofungus (Wyrebek et al., 2011; Vega, 2018). González-Pérez et al. (2022) further demonstrated that a volatile organic compound was responsible for enhancing plant growth.

Despite the wide distribution of *Metarhizium* spp. in Hawaii, this microorganism is still not listed as non-restricted microorganism that can be shipped into Hawaii, limiting the use of commercial *Metarhizium* as bioinsecticide in Hawaii (HDOA, 2024). Some isolates of *Metarhizium* act differently even though they are the same species and perform better in certain environments or against some insect pests. Locally isolated *Metarhizium* from Hawaii is likely more adapted to the local environment and better for local growers compared to exotic commercial isolates (Sutanto et al., 2021). This project examined an isolate of *Metarhizium* 'Ko-002' isolated from Koko Head Botanical Garden on Oahu for its ability to 1) improve sweetpotato growth, 2) repel sweetpotato weevils, and 3) aid in the suppression of sweetpotato weevil.

Does Metarhizium improve sweetpotato growth?

Okinawan sweetpotato tuberous roots were cut into 3-cm long pieces each with at least 2 sprouting shoots. Five pieces were randomly selected and coated in a dry Ziploc bag with 'Ko-002' colonized rice grains, and another 5 pieces were placed in a dry Ziploc bag without 'Ko-002' for seven days in darkness at room temperature. Each sweetpotato piece was then planted into a 15-cm diameter clay pot filled with sterile soil and grow in the greenhouse for 2 months. At the end of the experiment, shoot fresh and dry weight were recorded. The experiment was repeated once.

The sweetpotato pieces inoculated with *Metarhizium* 'Ko-002' sprouted and supported green tissue growth while the uninoculated pieces did not show signs of growth at 4 weeks after planting, ranging from 2-7 cm of growth. Whereas that on the uninoculated plants only showed emerging buds on the soil surface (Fig. 2).

Fig 2. Growth of sweetpotato 4 weeks after planting root pieces. A) Not inoculated with *Metarhizium* Ko-002; B) Inoculated with *Metarhizium* 'Ko-002'.

As time progressed, rots were observed on the uninoculated sweetpotato pieces with one piece not exhibit any growth. No rotting was observed in the *Metarhizium* 'Ko-002' treatment. Growth enhancement was less apparent at termination of the experiment with both treatments having similar biomass accumulation. Results were consistent between the two trials. Fresh shoot weights for the inoculated and uninoculated sweetpotato were 25.0 and 21.6 g, respectively ($P > 0.05$, $n = 10$) at 2 months after planting.

Does *Metarhizium* Repels Sweetpotato Weevil?

Dotaona et al. (2017) clearly demonstrated that *Metarhizium* releases volatile chemicals detectable to insects that might be repulsive to sweetpotato weevil (SPW). Thus, we examined if the local isolate ‘Ko-002’ cultured on cooked rice could also repel sweetpotato weevil.

A series of laboratory trials were conducted to establish that SPW were attracted to or repelled by rice cultured ‘Ko-002’. Ten adult SPW were placed in the center (release chamber) of a 2-wing olfactometer (Fig. 3). Three experiments were conducted to examine the preference of SPW provided with two choices. In Experiment 1, the choice was cooked rice or an empty chamber to ensure that rice did not attract the weevils. In Experiment 2, a choice of a sweet potato and a sweetpotato covered with rice without *Metarhizium* was given to ensure that the rice itself would not discourage SPW from feeding. In Experiment 3, SPW were given the choice of sweetpotato with clean rice and sweetpotato mixed with ‘Ko-002’ inoculated rice to determine if *Metarhizium* affected the SPW feeding behavior. Three additional pathways were also provided without connection to any chamber but were sealed by parafilm. The olfactometer was airtight to ensure no volatiles escaped and the connecting tubes were tapered towards the side-wing chambers to minimize volatiles spilling between chambers. The weevils were allowed to move freely for 24 hours after which the number of weevils in each chamber was recorded. Each experiment had 5 olfactometers as replicates. Experiment 1 and 2 were repeated once, whereas Experiment 3 was repeated three times. The results showed that ‘Ko-002’ did not repel nor attract the SPW (Table 1). SPW do not actively avoid sweetpotato with *Metarhizium*.

Fig. 3. Olfactometer used to determine adult sweetpotato weevil attraction and repulsion to volatiles emitted by sweet-potato with or without ‘Ko-002’.

Table 1. Percent of sweetpotato weevils migrating from the release chamber in a 2-winged olfactometer choice test of rice or no rice (Experiment 1), sweetpotato or sweetpotato with rice (Experiment 2), and sweetpotato with rice or sweetpotato with *Metarhizium*-infected rice (Experiment 3).

	Percent of sweetpotato weevils in chamber					
	Release chamber	**No rice**	**Rice**	**Sweetpotato**	**Sweetpotato rice**	**Sweetpotato *Metarhizium* rice**
Experiment 1	50%	30%	20%	-	-	-
Experiment 2	30%	-	-	40%	30%	-
Experiment 3	50%	-	-	-	30%	20%

*Weevil numbers did not differ among chambers in any experiment (P>0.05). Experiment 1 n=10 replicates, Experiment 2 n=10 replicates, and Experiment 3 n=20 replicates.

Does *Metarhizium* 'Ko-002' reduce sweetpotato weevil damage in the Field?

A field trial was conducted at Kualoa Ranch to examine if *Metarhizium* 'Ko-002' amended into the soil as compost amendment could suppress SPW damage in the field. *Metarhizium* compost amendment was prepared by adding 7.5 g of 'Ko-002' cultured on autoclaved rice to 1 kg of millrun and allowed *Metarhizium* to colonize the millrun over 2 weeks in room temperature with frequent hand mixing of the compost. A sweetpotato field trial was established with and without *Metarhizium* 'Ko-002' compost treatment. Each treatment plot was composed of 2 rows of 5 m × 1 m planting bed and replicated in 4 plots. For the *Metarhizium* treatment plots, 184 g/m^2 of the 'Ko-002' millrun compost was incorporated into the soil at sweetpotato planting next to the planting slips. Each treatment plot was planted with 36 slips of 'Simpson' (a white-fleshed sweetpotato cultivar for making chips) sweetpotato. Four months after planting, sweetpotatoes were harvested. Sweetpotato growth at 3 months, tuberous root yield, and sweetpotato weevil damage was recorded at harvest. The number of SPW present in each plot was counted from 10 randomly selected sweetpotato tuberous roots from each plot.

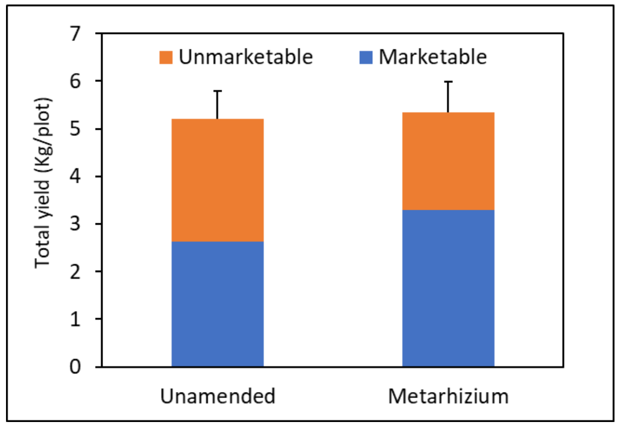

Fig. 4. Total, marketable and unmarketable yield of sweetpotato in *Metarhizium*-amended and unamended plots.

Sweetpotato plants in the *Metarhizium*-amended plots were not taller than the unamended control though they were numerically taller by 7.5 cm at 2 months after planting. Sweetpotato plants in *Metarhizium* plots also appeared more vigorous. Total and marketable yield also did not differ between treatments (Fig. 4). However, yield loss caused by SPW was lower in *Metarhizium*-amended plots than the control ($P \leq 0.05$, Fig. 5A) and the number of SPW recovered from the tuberous roots was also lower in the *Metarhizium*-amended than the unamended control ($P \leq 0.05$, Fig. 5B).

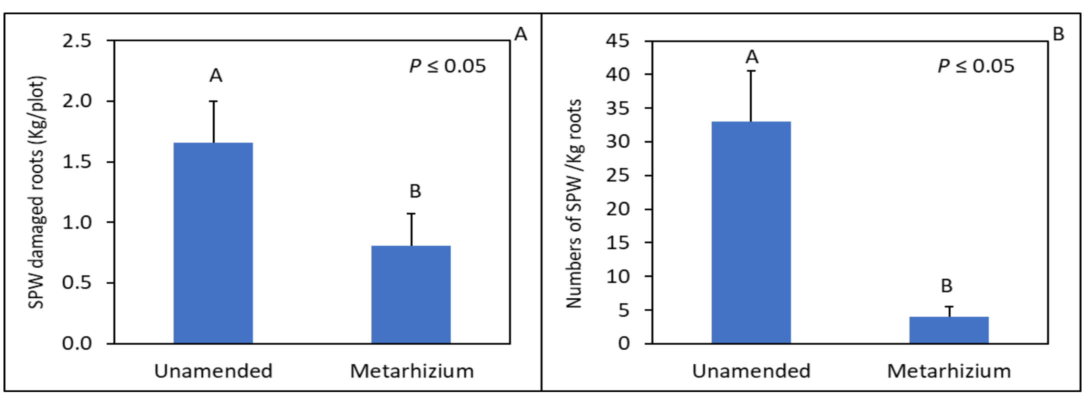

Fig. 5. A) Sweetpotato weevil (SPW) damaged tuberous roots (Kg/plot), and B) population density (SPW/kg sweetpotato roots) in *Metarhizium*-amended and unamended plots. Columns in a graph followed by different letters are different based on Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Discussion and Summary

Although it remains unclear how *Metarhizium* 'Ko-002' stimulates sweetpotato initial growth and reduces the population density of SPW in the tuberous roots, it is encouraging to document that compost made from millrun inoculated with 'Ko-002' reduced SPW damage on sweetpotatoes by 2 folds, and SPW population densities in the tuberous roots by 8.25 folds in the field. Unfortunately, enhancement of initial shoot growth by 'Ko-002' did not result in overall plant health later in the sweetpotato growing period as observed in the greenhouse as well as the field trial. 'Ko-002' also did not demonstrate an ability to repel SPW unlike the highly virulent isolate QS155 of *Metarhizium anisopliae* tested by Dotaona et al. (2017). The lack of increase by 'Ko-002' on overall sweetpotato yield is probably due to the presence of other pests and diseases in the field such as reniform nematodes (*Rotylenchulus reniformis*). More virulent strains of *Metarhizium* should be sought after in Hawaii to repel SPW as this is the primary damaging pest of sweetpotato rendering the roots unmarketable. None-the-less, Metarhizium 'Ko-002' is an effective and useful tool in the development of sustainable pest and soil health management strategies for sweetpotato production in Hawaii. Composting procedure to prepare Metarhizium colonized millrun is still under development (Fig. 6) to improve its consistency and applicability.

Fig. 6. Composting procedure to prepare *Metarhizium* colonized millrun is under development to improve its consistency and applicability.

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Acknowledgements

This project is supported by NIFA OREI (Award 20215130035225), Western Sare (Project# SW22-936) CTAHR Hatch, Multistate (NE2140) and Plan of Work (HAW9048-H, 9034-R, POW 16-964, and POW 22-037). Mahalo to Kualoa Ranch for field support.

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USDA NIFA