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Epizootics of entomopathogenic fungi at overwintering sites of *Oebalus mexicana* Sailer (Hemiptera: Pentatomidae) from Western Mexico

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Abstract

Background: *Oebalus mexicana* Sailer (Hemiptera: Pentatomidae) is an important pest of sorghum in Central-Western Mexico. In addition to damaging the grain, it is a vector of the panicle blight, *Fusarium moniliforme*. *Oebalus mexicana* hibernates at > 2200 m above sea level (masl), and its control in these sites is through entomopathogenic fungi (EPF). Their effectiveness could be misperceived as natural epizootics at hibernation sites. To characterize fungal epizootics associated with hibernating adults of *O. mexicana*, during 2019 and part of 2020, a study was established in the Zináparo hill, Michoacan, Mexico. Three sampling strata were defined—two at low (2219 masl) and high levels of elevation (2351 masl), and another one at intermediate elevation (2244 masl) and soil humidity most of the year. Under the leaf litter, live arthropods and corpses with and without mycosis were recorded every 2 weeks; associated EPF were isolated and identified. Soil humidity, temperature, and RH under the litter were recorded.

Results: Six genera of fungi were identified, with *Beauveria* being the most prevalent. Mortality of *O. mexicana* in the field was > 80%, with a high correlation (r = 0.85 - 0.97) with mortality due to fungi (> 65%). The humidity of soil and litter created a suitable environment for the development of epizootics. The highest prevalence was registered at the elevations of 2244, 2351, and 2219 masl, respectively. More than 15 families of arthropods were registered, several of them with the presence of mycosis. Their permanence as alternate hosts contributed to the persistence of the fungi. Field and laboratory evidences suggested that the frequent flights of *O. mexicana* favoured the self-dissemination of EPF. Thus, more than 37% of live individuals with inoculum and subsequent infection and death were recorded.

Conclusions: The EPF disease at overwintering sites of *O. mexicana* was enzootic, and under favourable conditions it can become epizootic. The high levels of natural control in hibernation sites suggest that additional control measures for *O. mexicana* are unnecessary; on the contrary, the conservation of these sites as reservoirs of EPF is proposed for a sustainable use in the management of this and other pests.

Keywords: Sorghum, Oebalus mexicana, Insect overwintering, Epidemics, Beauveria

Background

The "brown sorghum-head stink bug", Oebalus mexicana Sailer (Hemiptera: Pentatomidae), has become one of the main sorghum pests in many states of Central-Western Mexico, where each year private and public resources are dedicated to dealing with the pest (Salazar et al. 2002). In addition to direct damage, the

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insect can be a vector of *Fusarium moniliforme* fungus, an important pathogen of the sorghum panicle (Martínez et al. 2005). The stink bug hibernates for more than 9 months in the surrounding hills at altitudes of 2200–2700 masl, where it seeks refuge under the leaf litter of *Quercus* spp. trees (Salazar et al. 2002). This behaviour has prompted control campaigns against the insect, with a focus on its adult stage at overwintering sites, which supplements control strategies used by crop producers (Galván and Marín 1995).

Conventional control of this pest has been through chemical products, a widely criticized strategy that has been declared unsustainable because of its negative effects on the environment, and the eventual development of resistance by the pest (Altieri et al. 2015). A sustainable strategy is biological control, in which entomopathogenic fungi (EPF) have been one of the tools recently used to control hibernating stink bugs in Central-Western Mexico (Salazar et al. 2002). However, there is little knowledge about the biology and ecology of the bug in overwintering sites. Preliminary observations at hibernation sites northwestern of Michoacán, México, revealed fungi-infected corpses of O. mexicana. These observations should be documented through planned epidemiological studies. There are scarce epidemiological studies of EPF in hibernating insects around the world (Mustu et al. 2011), but there are no known related studies in Mexico.

Microbial control of insects as applied epidemiology should rest on ecological-epidemiological studies that allow understanding the effect of environmental factors on the development of specific entomopathogens (Mora-Aguilera et al. 2017). Thus, it is of utmost importance to develop studies on the entomopathogens in overwintering sites of the stink bug O. mexicana. Questions such as: which are the associated species of fungi, which are the survival strategies of the entomopathogens during the year, which are the alternate hosts of the fungi at overwintering sites, and what is the effect of abiotic factors on the development of epizootics, among others, must be elucidated. At overwintering sites of O. mexicana in Northwestern Michoacán, natural epizootics of EPF may occur in adults of the insect; and its temporal progression is determined by the species and strain of the fungus, the edaphoclimatic conditions, and the species and behaviour of hibernating insects.

The objective of the study was to characterize the natural epizootics of EPF associated with hibernating adults of *O. mexicana* in the Zináparo hill, as a representative site of Northwestern Michoacan, Mexico.

Methods

Study site

The Zináparo hill, in the municipality of the same name, is part of the Neovolcanic Belt and is located in the Northwestern region of the State of Michoacán, México. It belongs to the Sierras and Bajios Michoacanos subprovince and it is part of the important agricultural basin of Lerma-Chapala. It is located at more than 2500 masl, with a temperate sub-humid climate in summer, with 700–1000 mm of rainfall annually. The predominant soil type is of the Phaeozem (INEGI 2009) and the tree cover is formed mainly by *Quercus castanea*, *Q. gentryi*, and *Q. obtusata* (Arizaga et al. 2009).

It was verified that no control measures had been taken against *O. mexicana* at the study site, either with chemicals or with EPF. During 2019–2020, no control actions were taken either.

Sampling

During the year 2019 and part of 2020, every 2 weeks, samplings were performed at overwintering sites of O. mexicana, previously selected through a preliminary sampling. The area is located on one of the eastern slopes of the northern area of the hill. The sampling was stratified based on the terrain slope, at one low elevation (2219 masl) and one high elevation (2351 masl). A third site was included as a control, which, in contrast to the other two, showed an intermediate elevation (2244 masl), constant soil humidity during most of the year, and direct and permanent insolation (without shade, but with leaf litter coverage). GPS (GPSmap 60CSx, Garmin®, Taiwan) was used to determine the altitude and geographical coordinates of the sites were determined through. The lowest elevation is at 20. 1380° N, 102.0181° W; the highest elevation at 20.1378° N, 102.0178° W; and the intermediate elevation is at 20.1380° N, 102.0178° W.

The sampling method was based on that of Southwood (1978), which consists of the absolute sampling method called sampling per habitat unit. Samples transversal to the hillside slope were taken in an area of 30×30 cm (replica); 3 repetitions for each of the elevations at 2219 and 2351 masl, and 2 for the elevation at 2244 masl.

Once the site was selected, the litter was carefully removed and the number of individuals was recorded using a manual multiple-tally counter (The Denominator Company®, Woodbury, CT, USA). Live and dead specimens, with or without mycosis, were recorded, both of O. mexicana and other species of stink bugs and arthropods. When the number of live insects was high, the stink bugs on the litter were shaken inside transparent self-sealing zipper bags of 20×15 cm, onto which a grid had been previously drawn to make the counting easier. The bags

were placed over white card paper and photographs were taken immediately for counting later on a computer screen. The samplings started at 9:00 h and ended around 15:00 h, depending on the population density of the bugs.

On every date, samples of live stink bugs ($n \le 10$), and corpses with and without mycosis were taken for laboratory tests; the rest of them were returned to the site and covered with litter. The relative humidity (RH) and temperature of the bugs' hibernation microenvironment were estimated using a digital hygrothermograph (Thermotraker®, Taiwan) placed beneath the leaf litter. In addition, at each sampling, a sample of soil per elevation level was taken, and its humidity content was determined. The samples were weighed before and after drying inside a stove. The humidity percentage was obtained through the difference.

Fungi prevalence and persistence

Field samples of live stink bugs and recent corpses (both without mycosis) were incubated at controlled conditions (25 ± 2 °C and 12:12 light/darkness) for the eventual development of EPF. The corpses were sanitized with a solution of sodium hypochlorite (8%), and placed on a slide which was placed, in turn, on a crystal triangle; both inside Petri dishes with distilled water in order to favour the development of mycosis. The live stink bugs were placed inside Petri dishes with a paper towel moistened with sterile water (6 ml), and sealed hermetically. The corpses recorded daily were incubated separately for the development of possible mycosis.

The recorded fungi were isolated in a Sabouraud dextrose agar+yeast extract culture medium (SDA+YE) and their identification into genuses was made through dichotomous keys and specialized literature (Humber 1998; Zimmermann 2008).

Analysis of results

The analysis was based on the biological knowledge of the species and the use of descriptive statistics, and an analysis of variance (ANOVA) was performed to compare the soil moisture and the development of mycosis between the elevation levels. Finally, a linear correlation analysis was performed to determine the relationship between the mortality of *O. mexicana* in the field and estimated infection by EPF. The SAS statistical program was used (SAS Institue 2012). The identification of the family (up to species in a few cases) of the arthropods associated with *O. mexicana* was confirmed through keys and specialized bibliographic material (Panizzi and Grazia 2015).

To estimate fungal prevalence (i.e. insect mortality) in the field, data from corpses with mycosis in the field and that developed in corpses incubated in the lab were

used. With both data, field mortality by EPF (corpses with sporulation + corpses without sporulation) was estimated. The following equations were used:

- (a) InF = (MyF) (100)/Li + MyF
- (b) InL = (MyL) (100)/MyL + NMyL
- (c) EIC = (DFNMy)(InL)/100 N
- (d) EMo = (MyF + EIC) (100)/Li + MyF + EIC

where InF=Incidence of mycosis in the field (%), $MyF=N^{\circ}$ of mycosed corpses in the field, Li=live stink bugs. InL=Incidence of mycosis in the laboratory (%), $MyL=N^{\circ}$ of mycosed corpses in the laboratory; $NMyL=N^{\circ}$ of non-mycosed corpses in the laboratory. EIC=Estimation (N°) of fungi infected corpses in the field, DFNMy=Dead in the field with no mycosis. EMo=Estimated mortality (%) by fungi in the field.

Results

Behavior of Oebalus mexicana

The stink bug, O. mexicana was active during its hibernation period. Once the sun appeared at the study site (which varied with the time of the year), it was normal to see stink bugs flying and landing frequently at different sites. Mean counts of up to 406 individuals/90 cm² under the oak litter of the species Q. obtusata were registered. However, O. mexicana was not always found under the litter. At least during February, March, May, and June (02/28, 03/14, 05/09, 06/06), the stink bug came out of its refuges under the leaf litter and formed large aggregates similar to bee swarms. They were observed on trunks and branches of Acacia panulata, Quercus sp., and Camarostaphylis polifolia (Figs. 1 and 2A). On some dates (04/27, 05/9, 05/23), specimens on shoots and young leaves of Quercus sp. and Prunus *capuli* were observed in the process of feeding (Fig. 2B).

On August 1st a considerable decrease in the population of bugs was recorded, but it was not until August 15th, 2019, that virtually no live specimens were recorded at the overwintering site. Once again, starting on October 10th, the first live specimens were recorded at the overwintering sites, but it was not until November 9th, that large bug populations were recorded once again on the hill (Fig. 1).

The first populations were observed in constant flight and several specimens were recorded on oak leaves, presumably in the feeding process. According to the above and considering the emigration in the first days of August and the immigration during the first days of October, the stay of *O. mexicana* at the overwintering site on the Zináparo hill, lasted around 9.5 months.

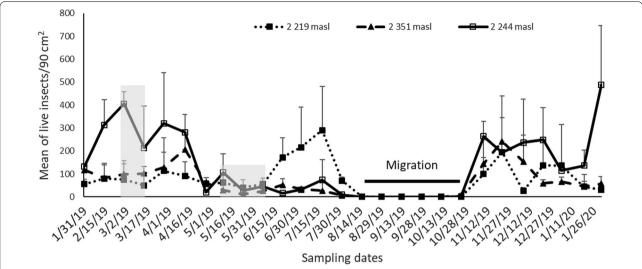


Fig. 1 Seasonality of populations of *Oebalus mexicana* in hibernation sites of the Zináparo hill, Michoacán, México. 2019–2020. The lines over the points indicate the standard deviation (n = 3). Gray bar indicates swarm periods

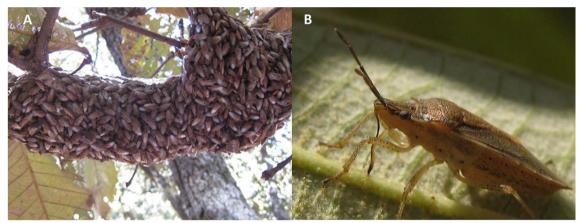


Fig. 2 Behavior of *Oebalus mexicana* in hibernation sites. **A** Swarms of bugs on branches of *Quercus* sp. **B** Adult feeding on a *Quercus* sp. leaf. Zináparo hill, Michoacán, México. 2019–2020

Records and prevalence of entomopathogenic fungi

A wide diversity of EPF associated with arthropods at the overwintering sites was recorded, with the greatest prevalence of *O. mexicana*. The genera *Beauveria*, *Isaria*, *Hirsutella*, *Metarhizium*, and *Cordyceps* were recorded in that order of frequency (Fig. 3). Sporulation of the latter 3 was recorded in the field only under the highest humidity levels (i.e. during the rain season) or under laboratory conditions. In the genus *Lecanicillium*, only one individual was incubated in the laboratory.

A trend of lower mortality of *O. mexicana* (with and without mycosis) was observed at the site of 2219 masl, compared to 2351 masl. The mortality of this sampling level was similar and on some dates higher (80.2%) than

that registered (71.5%) in the intermediate elevation (2244 masl), where it always maintained moisture in the soil. The highest mortality occurred in May, the period with the highest temperature and lowest RH of the microenvironment (Fig. 4A, C).

However, until May 23, the site with the highest registration of corpses with mycosis in the field had the intermediate elevation (2244 masl) and the highest soil moisture (>50%), with intermittent registrations that increased even during periods of low RH and high temperature. With the first rains (starting from June 6) the increase in corpses with mycosis was evident in the 3 sampling sites; thus, on June 26th, the maximum registration of corpses with mycosis (58.9%) occurred, which



Fig. 3 Entomopathogenic fungi associated with *Oebalus mexicana* at hibernation sites in the Zináparo hill, Michoacán, México. **A** *Beauveria*, **B** *Metarhizium* in an alternate host in the field; **C** *Isaria*, **D** *Hirsutella* and, **E** *Metarhizium* in *O. mexicana* developed under laboratory conditions. 2019–2020

was registered at the site with intermediate elevation. Of the other two sampling sites, only the one located at 2351 masl reached levels of mycosis similar to those recorded at the intermediate elevation. On the contrary, at the site of 2219 msnm the levels of mycosis registered in the other 2 sampling sites were never reached. The highest level of corpses with mycosis coincided with a combination of high RH (85.9% to >100%) and low temperature in the microenvironment (under the litter; Fig. 4B).

Although altitude did not affect the development of mycosis in the field, a trend of greater development of mycosis was observed at elevation levels that tended to have higher soil moisture, with statistical differences only for the intermediate elevation level (Fig. 5).

When corpses without mycosis in the field, and those that later developed mycosis in the laboratory, were incorporated into the formula (mycosis in the laboratory+mycosis in the field), the results were different. First, higher mortality caused by fungi (>65%, May 23) was observed, which was maintained at the site with intermediate elevation. However, the trends were similar between the 3 elevation levels; for example, in mid-March, the highest level of infection (36.48%) was

recorded at the lowest altitude site (Fig. 6). Second, unlike what was observed in Fig. 4B, the infection levels increased in the periods of lower relative humidity (31.9% RH) and higher temperatures (24.3 $^{\circ}$ C).

A close correlation was recorded between the total mortality of *O. mexicana* in the field and the estimated mortality due to EPF for altitudes of 2219, 2244, and 2351 m (r=0.85, r=0.97, and r=0.79, respectively).

Alternate hosts

In addition to *O. mexicana*, other arthropods were present at the overwintering sites. Six orders of insects were recorded, distributed in more than 15 families, with more than 18 apparent species, and some of them were identified as species. Members of the order Hemiptera, with 8 families and 15 apparent species, stood out. In addition, the centipede *Scutigera* sp. (Chilopoda: Scutigeromorpha: Scutigeridae) was recorded (Table 1). There were phytophagous and predator species recorded, but no species predating on *O. mexicana* or another species was recorded. An agglomerate of stink bugs of different species stood out, although *O. mexicana* was predominant.

The arthropods associated with *O. mexicana* were present during most of the year, together with *O.*

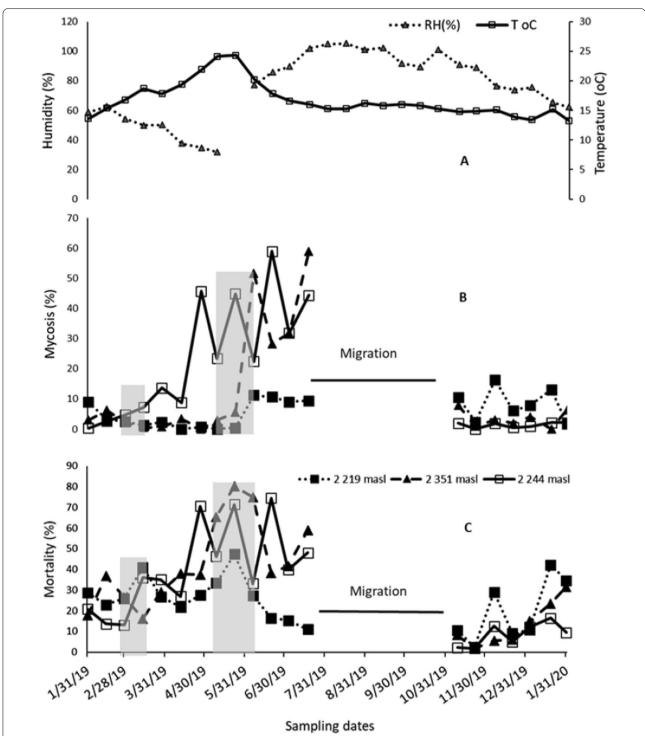


Fig. 4 Mortality (mycosed + non-mycosed (**C**), and mycosed corpses in field (**B**) of *Oebalus mexicana*, and **A** Relative humidity (RH) and temperature in the microenvironment of the overwintering sites of the Zináparo hill, Michoacán, México. 2019–2020. Gray bar indicates swarm periods

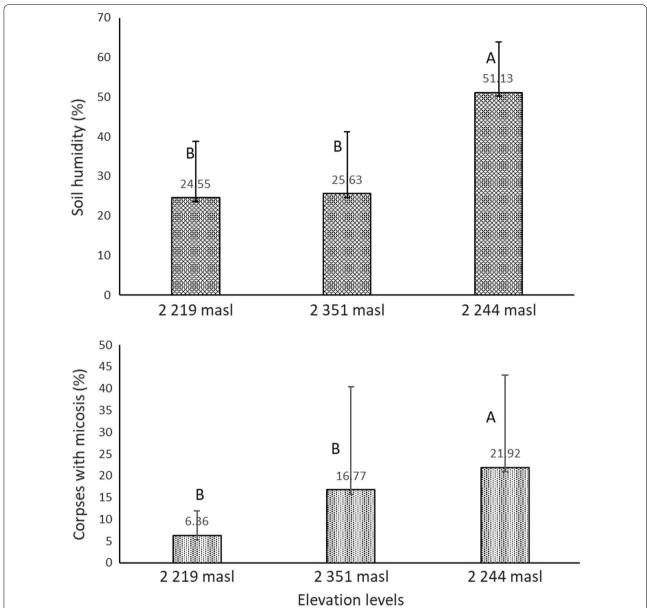


Fig. 5 Corpses with mycosis in the field and its relationship with the soil moisture recorded at the indicated elevation levels. Bars within each graph with the same letter do not differ statistically (Tukey, 0.05). The lines on the bars correspond to the standard deviation

mexicana; in fact, species of stink bugs and other arthropods were recorded after the emigration of the brown sorghum-head stink bug to the agricultural valleys, e.g. the *Arhaphe argata* bug (Bliven, Largidae) and the centipede *Scutigera* sp. Mycosed corpses were recorded frequently in the different groups associated with *O. mexicana*, including specimens of Chilopoda. Recorded fungi species corresponded to the genus *Beauveria*, and, to a lesser extent, *Metarhizium* (Fig. 3B), and only in one specimen, the genus *Lecanicillium* was recorded.

Discussion

The results of this research desmonstrate for the first time that at least 6 genera of EPF were associated with *O. mexicana* at an overwintering site representative of Northwestern Michoacán, Mexico. There are few studies on the EPF of stink bugs in the world (Mustu et al. 2011). However, only the one developed by Mustu et al. (2011) is related to the species *Aelia rostrata* Boh. (Hemiptera: Pentatomidae), an important wheat pest in Europe. Just like *O. mexicana*, it hibernates in mountains under the litter of species of *Quercus*. Different species of fungi

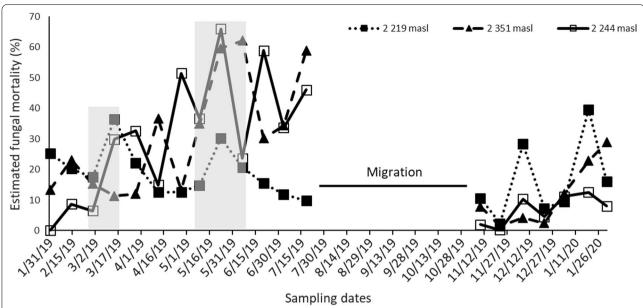


Fig. 6 Estimated mortality of *Oebalus mexicana* based on corpses with mycosis in the field + corpses with mycosis in the laboratory, obtained from hibernation sites in the Zináparo hill, Michoacán, Mexico. 2019–2020. Gray bar indicates swarm periods

Table 1 Arthropods associated with the stink bug *Oebalus mexicana* in hibernation sites of the Zináparo hill, Michoacán, México. 2019–2020

Order	Family	Apparent species	Habit	Biological state
Hemiptera	Pentatomidae	5	Ph	A
	Coreidae	4	Ph	А
	Scutelleridae	1	Ph	А
	Largidae ^a	1	-	Α
	Reduviidae	1	Р	N, A
	Aradidae ^a	1	-	А
	Alydidae ^a	1	-	А
	Cicadellidae	1	Ph	А
Lepidoptera	_	-	Ph	L, A
Coleoptera	Chrysomelidae ^a	_	Ph	А
	Carabidae	1	Р	L, A
	Coccinellidae ^a	_	Р	А
	Tenebrionidae	1	-	-
Hymenoptera	Vespidae ^a	_	Р	А
	Apidae ^a	_	Po	А
Dermaptera ^a	_	_	-	Α
Orthoptera	Acrididae		Ph	А
Scutigeromorpha (Chilopoda)	Scutigeridae	Scutigera sp.	Р	А

A adult, N nymph, L larvae, Ph phytophagous, P predator, Po pollinator

cause important epizootics in hibernating adults; among them, *Isaria farinosa* and *B. bassiana*; the latter was the most prevalent genus, similar to what was found in this study. In addition, adults of other hibernating stink bugs

(Scutelleridae) were infected by the genera *Isaria* and *Verticillium* (= *Lecanicillium*) (Abdulhai et al. 2010).

With these results, it can be argued that EPF is present during the whole year at the overwintering sites of

^a Species not identified due to decomposition of their tissues

O. mexicana and that the disease could be catalogued as enzootic in type; under favourable conditions, they can develop important epizootics (Shapiro-Ilan et al. 2012) as it was recorded in the present study. The development of epizootics of entomopathogens is the result of complex pathogen—host—environment interactions (Mora-Aguilera et al. 2017).

The absence of records of corpses with mycosis of *Metarhizium, Isaria*, and *Hirsutella* in the field during the period of drought does not mean that those species do not cause mortality in *O. mexicana*; rather, this suggests that the environmental conditions did not favour the saprophytic development (sporulation) of those fungi. For example, corpses without mycosis collected during the dry season, but later incubated under favourable conditions $(25\pm 2~^{\circ}\text{C}, \text{RH}>90\%)$, developed the growth of those genera.

There is a general idea that EPF require humidity of >90% for their development (Tanada and Kaya 1993). Various studies, however, have shown that the humidity required for germination and infection is typically lower. Thus, an isolate of *B. bassiana* (Bb-M) in Jiquilpan, Michoacan, Mexico, required 63% of humidity to infect and kill larvae of *Galleria mellonella* L (Lepidoptera: Pyralidae) (Suárez-Núñez et al. 2017); while Padmini and Padmaja (2010) found that out of 30 evaluated isolates of *B. bassiana*, most of them were inhibited in their mycelial development and sporulation at 91.3% of humidity, while 60% of humidity was optimal for the development and sporulation of the isolates.

The traits of the overwintering site, such as permanent leaf litter coverage, ensured favourable microenvironments (e.g. relative humidity) for infection, sporulation, persistence, and development of epizootics by fungi; on one hand, it worked as a humid chamber and on the other hand, as a protection against UV rays during the periods of more insolation (e.g. leaves falling from trees). This could explain the development of mycosis with a relative humidity of <40% during months with no precipitation. Temperatures recorded under the leaf litter fluctuated between 13.3 and 24.3 °C, conditions within the favourable limits for the development of EPF (Tanada and Kaya 1993).

In addition to the above, the behaviour of the host probably also contributed; e.g., the aggregation of individuals as a common characteristic in hibernating insects (Leather et al. 1993) is also one of the main aspects that favour the development of epizootics (Inglis et al. 2001). For *O. mexicana*, mean counts of up to 406 individuals/90 cm² were recorded under the leaf litter, but up to 22,000 individuals/m² have been reported (Salazar et al. 2002). Under these conditions, the probability of transmission and infection by fungi is high. In addition,

the frequent flight observed during the stay of the stink bug at the overwintering site suggests that *O. mexicana* showed an active hibernation period, such as was noted for other hibernating species (Leather et al. 1993). Given that hibernating populations of *O. mexicana* are only composed of adults, all the individuals are capable of flight and this favours the self-dissemination of EPF. High percentages of mycosis (>37%) recorded in live individuals in the field and incubated in the laboratory suggest so. It is known that self-dissemination of entomopathogens is one of the main strategies for their dispersal and transmission (Bayerstock et al. 2010).

Part of the behaviour of *O. mexicana* was that on some dates, the insect fed on some tree species. Because of the position of the stylet, it was inferred that they were in the feeding process. In insects of the Pentatomidae family during the feeding process, the labrum draws away from the buccula and the stylet is extracted from the groove of the second segment of the labium; and, together with the labrum, they align with the rest of the segments of the rostrum (Esquivel 2019), as was observed in *O. mexicana*. The fact that the insect can feed at overwintering sites could explain, in part, its long period (>9 months) of stay in those places.

Finally, *O. mexicana* was not the only host of EPF. A large diversity of groups of insects and other arthropods were recorded as being infection by fungi. It is known that the greather the diversity of hosts, the larger the prevalence and dispersion of fungi, favouring the development of epizootics (Pell et al. 2010). During the study, some species of stink bugs were permanent residents of the overwintering sites and functioned as alternate hosts to the entomopathogens, even after the emigration of *O. mexicana* towards the agricultural valleys.

The results of the present study are of fundamental importance for integrated pest management. The importance of the overwintering sites of the brown sorghumhead stink bug should be reassessed; on one hand, as a reservoir of pests that require control at any moment (i.e. O. mexicana); and on the other hand, to consider them as sites for the conservation of natural enemies (e.g. EPF). The second perspective seems more rational, since O. mexicana is active as a pest only during the development of the sorghum grain, before its hardening (Salazar et al. 2002). According to the principles of integrated pest management, insects should be considered pests—that is, deserving control—only when their populations reach an economical threshold (Metcalf and Luckman 1994).

It is obvious that at the overwintering sites (> 9 months) *O. mexicana* does not cause damage, but also, EPF are important biotic regulators of the stink bugs at these same sites, with mortality levels superior to 60%. There is already high natural control of the stink bug at

overwintering sites. If other biotic and abiotic factors of mortality were added, mortality could be superior to 80%.

Conclusions

The disease caused by EPF at overwintering sites of O. mexicana is of the enzootic type, and under favourable conditions, it can become epizootic. The fungi involved were Beauveria, Isaria, Hirsutella, Metarhizium, Cordyceps, and Lecanicillium, with a higher prevalence of the first. Soil humidity and leaf litter were key abiotic factors for the development of epizootics, while the active behaviour of O. mexicana at overwintering sites contributed to self-dissemination of the fungi. Arthropods associated with O. mexicana contributed, as alternate hosts, to the persistence of the fungi. The natural high mortality (>80%) of O. mexicana at overwintering sites, including that caused by EPF (>60%), suggests that additional control measures are not needed; on the contrary, it is proposed to conserve overwintering sites as EPF reservoirs for integrated and rational management of O. mexicana and pests in general.

Abbreviations

ANOVA: Analysis of variance; SDA: Sabouraud dextrose agar; YE: Yeast extract; InF: Incidence of mycosis in the field (%); MyF: No of mycosed corpses in the field; Li: Live stink bugs; InL: Incidence of mycosis in the laboratory (%); MyL: No of mycosed corpses in the lab; NMyL: No of corpses with no mycosis in the laboratory; EIC: Estimated number of corpses infected by the fungi in the field; DFNMy: Dead in the field with no micosis; EMo: Estimated (%) mortality by fungi in the field.

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Authors' contributions

HCM developed and coordinated the project, participated in samplings, identification of the associated organisms, data analysis and writing. JMR participated in samplings and isolations and culturing of fungi. CCO collaborated in fungi isolation. MOE collaborated in determining humidity in soil samplings. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

Not applicable for that section.

Consent for publication

Not applicable for that section.

Competing interests

The authors declare that they have no competing interests.

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References

- Abdulhai M, El-Bouhssini M, Jamal M, Trissi AN, Sayyadi Z, Skinner M, Parker BL (2010) *Beauveria bassiana* characterization and efficacy vs. Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Pak J Biol Sci 13:1052–1056. https://doi.org/10.3923/pjbs.2010.1052.1056
- Altieri MA, Nicholls CI, Gillespie M, Waterhouse B, Wratten S, Gbèhounou G, Gemmill-Herren B (2015) Crops, weeds and pollinators understanding ecological interaction for better management. FAO, Rome. https://www.fao.org/3/i3821e/i3821e.pdf. Accessed 28 Nov 2021
- Arizaga S, Martínez-Cruz J, Salcido-Cabrales M, Bello-González MA (2009)

 Manual de encinos michoacános. Instituto Nacional de Ecología, México.

 https://www.researchgate.net/publication/328432683. Accessed 21 Sep 2021
- Baverstock J, Roy HE, Pell JK (2010) Entomopathogenic fungi and insect behavior: from unsuspecting hosts to targeted vectors. Biocontrol 55:89–102. https://doi.org/10.1007/s10526-009-9238-5
- Esquivel JF (2019) Stink bug rostrum length vs. stylet penetration potential. Entomol Exp Appl 167:323–329. https://doi.org/10.1111/eea.12782
- Galván F, Marín A (1995) Manejo integrado de la chinche café del sorgo (*Oebalus mexicana*) en el Bajío, México. Manejo Integrado Plagas 35:33–39
- Humber RA (1998) Entomopathogenic fungal identification, APS/ESE workshop. USDA-ARS Plant Protection Research Unit, Ithaca
- INEGI (2009) Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Zináparo, Michoacán de Ocampo Clave geoestadística 16109. http://www3.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/16/16109.pdf Accessed 21 Sept 2021
- Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents. CABI International, Bristol
- Leather SR, Walters KF, Bale JS (1993) The ecology of insect overwintering. Cambridge University Press, New York
- Martínez JL, Pescador A, Lezama R, Rebolledo O, Molina J, López M, Betancourt A (2005) Asociación de la chinche apestosa *Oebalus mexicanus* con el hongo *Fusarium moniliforme* causante del tizón de la panoja del sorgo, en la Ciénega de Chapala, Jalisco, México. Av Investig Agropecu 9:1–7. https://www.redalyc.org/articulo.oa?id=83709104. Accessed 21 Sept 2021
- Metcalf RL, Luckman WH (1994) Introduction to insect pest management, vol 101. Wiley, Hoboken
- Mora-Aguilera G, Cortez-Madrigal H, Acevedo-Sánchez G (2017) Epidemiology of entomopathogens: basis for rational use of microbial control of insects. Southwest Entomol 42:153–169. https://doi.org/10.3958/059.042.0116
- Mustu M, Demirci F, Kocak E (2011) Mortality effects of *Isaria farinosa* (Holm.) and *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) on *Aelia rostrata* Boh. (Hemiptera: Pentatomidae). Turk J Entomol 35:559–568
- Padmini PCP, Padmaja V (2010) Impact of different relative humidities on in vitro growth and sporulation of entomopathogenic fungal isolates of *Beauveria* species. Int J Pharm Biol Sci Arch 1:355–359. https://doi.org/10.1007/s12639-013-0408-0
- Panizzi AR, Grazia J (eds) (2015) True bugs (Heteroptera) of the neotropics, vol 2. Springer, Dordrecht
- Pell JK, Hannam JJ, Steinkraus DC (2010) Conservation biological control using fungal entomopathogens. Biocontrol 55:187–198. https://doi.org/10.2307/1308603
- Salazar E, Garciducieñas R, Salas MD (2002) Utilización del hongo entomopatógeno *Beauveria bassiana* para el control de la chinche del sorgo *Oebalus mexicana* en sus sitios de invernación. Rev Prot Veg No. 2122
- Institue SAS (2012) SAS for personal computers version 9.3. SAS Institue, Carey Shapiro-Ilan DI, Bruck DJ, Lacey LA (2012) Principles of epizootiology and microbial control. In: Vega FE, Kaya HK, Tanada Y (eds) Insect pathology. Academic Press, San Diego
- Southwood TRE (1978) Ecological methods with particular reference to the study of insect populations. Chapman and Hall, London

Suárez-Núñez J, Cortez-Madrigal H, García-Munguía AM (2017) Epidemiology of *Beauveria bassiana* in controlled populations of *Bactericera cockerelli1*. Southwest Entomol 42:1041–1056. https://doi.org/10.3958/059.042.0423 Tanada Y, Kaya HK (1993) Insect pathology. Academic Press, London Zimmermann G (2008) The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontr Sci Technol 18:865–901. https://doi.org/10.1080/09583 150802471812

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