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Assessing the host status of banana and other plant species to the enset root mealybug *Paraputo ensete* (Williams & Matile-Ferrero) (Hem.: Pseudococcidae) in Ethiopia

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Ninety backyard gardens in the south-eastern Ethiopian highlands with enset *(Ensete ventricosum* (Welw.) Cheesman), banana and various annual crops were assessed for the presence of enset root mealybugs (*Paraputo ensete* (Williams & Matile-Ferrero)). This study presents the first observation of enset root mealybugs on banana. This pest has until now been exclusively recorded on enset in Ethiopia. In the Dilla Zuria district of the Gedeo zone, southern Ethiopia, infested banana mats of the 'Pisang Awak' (ABB genome) landrace were observed adjacent to infested enset plants in three small-holder backyard gardens. As roots of banana mats and enset plants were overlapping and intertwined, and large numbers of mealybugs were observed on enset roots, possibly representing an overpopulation, the observed mealybugs on banana might have represented a "chance infestation". The smaller size of mealybugs on banana roots might indicate a non-optimal host status of this crop. Experimental choice and no-choice pot trials however provided another indication of the possible host potential of 'Pisang Awak' and of an additional banana cultivar 'Matooke' (AAA-East African Highland). The enset root mealybug was able to fully develop, produce viable offspring and survive on both banana cultivars. Not all investigated banana cultivars presented this host status, and the susceptibility of most *Musa* cultivars remains low.

Key words: Choice trials, corm, crawlers, enset suckers, field survey, *Musa* cultivars, no-choice trials, roots, screenhouse pot trials.

INTRODUCTION

Plant pests and pathogens reduce the yields and quality of agricultural crops, affecting global food security and causing major economic losses (Savary et al., 2017, 2019). The impact of such biotic stress is most severe in developing regions struggling with food security (Savary et al., 2019) and for small-holder farmers who rely on relatively small volumes of yield for their livelihood. In these developing regions, basic information on the distribution of plant pests and pathogens is often limited, especially regarding crops not prevalent in a global setting. Data on pest and pathogen occurrence in terms of climatic and geographical ranges and host range is however critical to predict potential future spread to new regions and hosts (Bebber et al., 2014).

A pertinent example is the occurrence of pests and diseases on enset (*Ensete ventricosum* (Welw.) Cheesman), a major food crop in south and south-western Ethiopia but largely unknown outside of Ethiopia. Although wild enset has a natural distribution spanning eastern, central and southern tropical Africa (Baker and Simmonds, 1953; Lock, 1993; Borrell et al., 2019), its cultivation only occurs in the Ethiopian highlands (Brandt et al., 1997; Borrell et al., 2019) where it is a staple starch food for approximately 20 million people. Enset is a monocarpic perennial herbaceous plant of the Musaceae family, closely related to the *Musa* genus and highly similar in morphology and physiology (Borrell et al., 2019). Contrary to the *Musa* cultivars widely cultivated for its banana fruits, enset is cultivated for its starchy pseudo-stem base, leaf sheaths and underground corm (Borrell et al., 2019). The cultivation of enset is however threatened by a number of pests and diseases. The most severe disease is Xanthomonas wilt of enset (caused by the pathogen *Xanthomonas vasicola* pv. *musacearum*), that by infecting the vascular system damages the harvestable tissue, causes permanent wilting and eventually death (Yemataw et al., 2017). Xanthomonas wilt also affects banana (Yirgou and Bradbury, 1974; Tushemereirwe et al., 2004; Ndungo et al., 2006; Carter et al., 2010).

The occurrence of Xanthomonas wilt on both enset and banana warrants the investigation of *Musa* host susceptibility to other pests and pathogens affecting the domesticated enset crops in Ethiopia to foresee and prevent potential similar situations.

Alongside Xanthomonas wilt, the enset root mealybug (*Paraputo ensete* (Williams & Matile-Ferrero) (Hem.: Pseudococcidae); Figure 1b) forms a major constraint to enset production in Ethiopia (Addis et al., 2008a). Enset root mealybug is an insect pest infesting the roots and corm of enset plants (Addis et al., 2008a), causing a reduced number of roots, dried lateral leaves, reduced pseudostem circumference (Addis et al., 2008a; Azerefegne et al., 2009), retarded plant growth and occasional death, especially under drought stress. The pest was first recorded on enset in 1988 in the Wonago district in Ethiopia (Tsedeke, 1988). It has since been recorded in various districts across the Ethiopian highlands (Addis et al., 2008b) where it has become a serious threat to enset production, especially in the last decade. The dispersal of enset root mealybugs across various sites is mainly attributed to the exchange of infested planting materials between farmers and enset nurseries unknowingly selling infested suckers (Azerefegne et al., 2009). Within a plot, dispersal of the mealybugs to new host plants mostly occurs during the mobile first life-stage (first instar nymphs; 'crawlers'). Mealybugs can also be dispersed during transplanting of enset plants, a practice that is common with small-scale enset farmers. During this practice, plants are uprooted, roots and pseudo-stem trimmed, and replanted at a wider spacing. Other local mechanisms of dispersal include associations with ants (Addis, 2005) and through displacement by water when flooding occurs.

The host range of the enset root mealybug has not been investigated, with a possible broader host range, especially with the closely related banana, being an important threat to wide-spread dispersal of the pest species in Ethiopia and beyond, as has been the case for the bacterial disease Xanthomonas wilt, that affects both enset and banana.

A variety of other crops is often present closeby or within the enset cultivation fields. While enset is often cultivated as a monoculture, intercropping with coffee, vegetables, fruits, root and tuber crops or agroforestry-based systems are common (Abebe and Bongers, 2012; Olango et al., 2014; Garedew et al., 2017). Enset is also often produced in backyard gardens where it is integrated with various other crops (Garedew et al. 2017). While banana plants are generally not intercropped with enset, they do often occur together in these backyards or larger fields (Figure 1a).

In this study, farms and backyard gardens in the Gedeo Zone, which is highly impacted by the enset root mealybug, were surveyed (Addis et al., 2008b). Root mealybug infested enset fields were assessed for the presence of this pest on other plant species (including other perennial and annual crops, trees, shrubs, grasses and woody climbers). Since *Musa* spp. was expected to be the major potential crop species for enset root mealybug infestation in mixed enset-banana backyards, we additionally performed choice and no-choice screenhouse pot experiments in which root mealybugs were added to commonly grown enset and the most widely cultivated banana cultivars grown in Ethiopia. The infestation and

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Figure 1. Enset root mealybugs and their impact: (a) infested enset [single plant on the right] and banana plants [cluster of plants on the left] in the same field; (b) gravid female adult enset root mealybugs with crawlers on an enset root; (c) vials with mealybugs collected from banana (left) and enset (right) host plants; and (d) enset root mealybugs on a banana root of the cultivar Pisang Awak. Photos by Guy Blomme.

survival of the enset root mealybugs on these different potential banana hosts were investigated in these pot trials.

MATERIALS AND METHODS

Enset root mealybug survey

Field surveys were carried out during October to December, 2019 in the Gedeo Zone in the Southern Nations, Nationalities, and Peoples' Region (SNNPR) of Ethiopia, a region situated adjacent to the rift valley, in the humid south-eastern Ethiopian highlands (Figure 2). Three study sites were selected along an altitude gradient with continuous enset cultivation, located in the woredas (districts) of Dilla Zuria and Wonago (two bordering woredas), Yirgachefe and Gedeb. Across these woredas the altitude ranges from 1,600 to 1,900 m above sea level (masl), 1,900-2,040 masl to >2,040 masl, respectively, at Dilla Zuria and Wonago, Yirgachefe and Gedeb. Dilla Zuria and Wonago are dominated by enset-banana-coffee-fruit tree cultivation systems, whilst enset-coffee systems are mainly observed in Yirgachefe. Gedeb is characterized by enset agroforestry. Since the woredas Dilla Zuria and Wonago border the same landscape and farming systems, they are considered as one study area and further referred to as the Dilla Zuria study area. At each of the three study areas, 30 backyard gardens were selected to conduct the enset root mealybug survey. Farm selection was based on a pre-survey to specifically target farms in which banana and other crops (e.g. cabbage, maize, legumes), shrubs (e.g. coffee), trees and herbs were cultivated within or adjacent to the enset crops.

All 90 farmers across the three study areas were interviewed on the presence of enset root mealybugs (local name 'Tsete') in their enset fields and backyard gardens, the specific constraints this pest and other biotic factors cause to enset production, which pest mitigation and control measures they apply, and if they have knowledge on how enset root mealybugs spread within fields, and across fields or villages.

Next, the root systems of the crops or plant species growing in backyards or fields of enset-based farms were investigated to determine the presence of the enset root mealybug. At each farm and if available, 5 mats [that is, a cluster of physically interconnected plants] of each banana cultivar, 5 plants of each

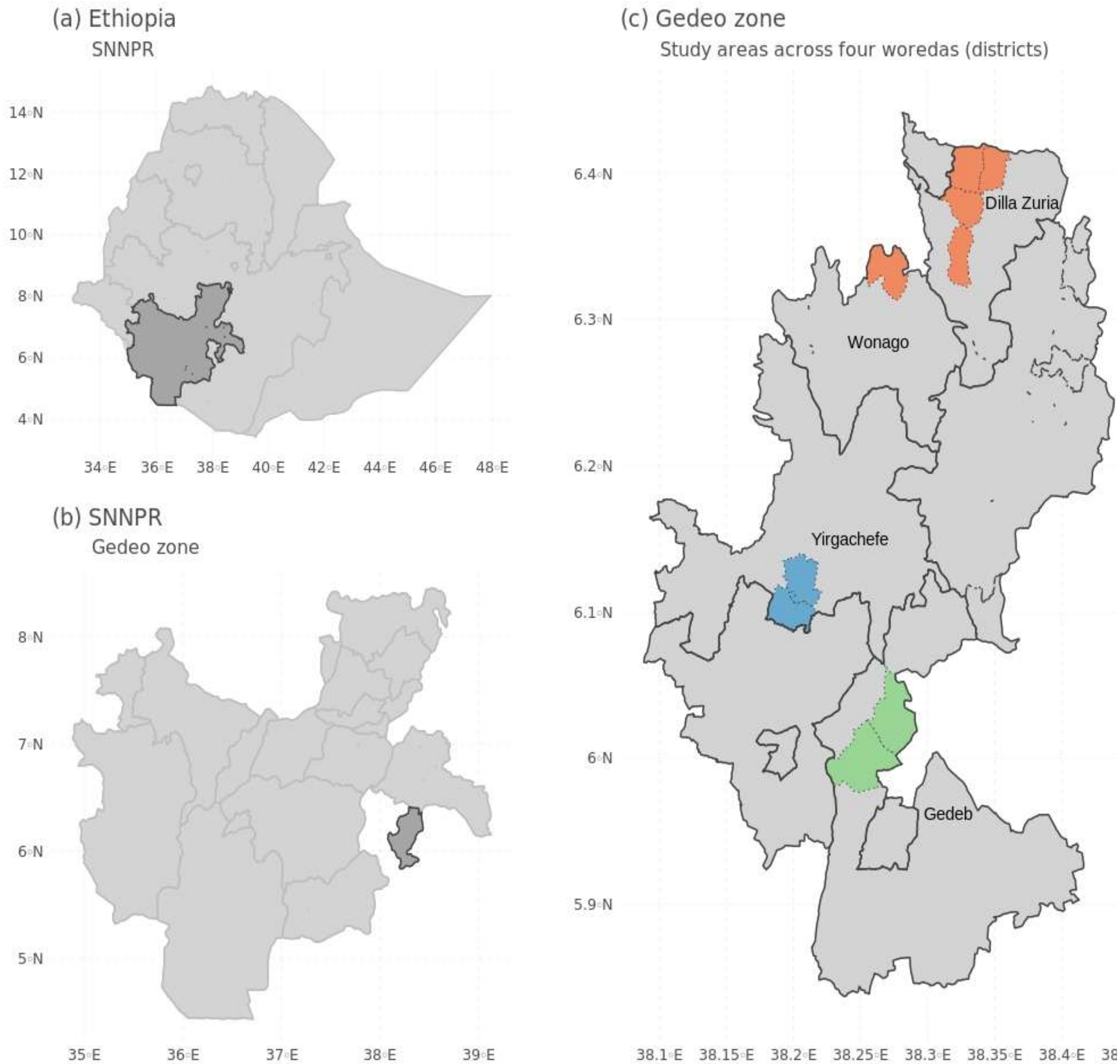

Figure 2. Map indicating the location of the three study sites. All study sites are located in the Gedeo zone in the Southern Nations, Nationalities, and Peoples' Region (SNNPR) of Ethiopia. Within the Gedeo zone the three study sites are situated in the woredas (districts) of Dilla Zuria and Wonago (orange), Yirgachefe (blue) and Gedeb (green).

enset cultivar and 5 plants of any other annual crop, herb, shrub or tree species were assessed. For small plant species, plants were uprooted for mealybug assessment. A non-destructive assessment was conducted for larger plants by which a section of soil was removed to reveal the roots and/or corm and determine the presence of the enset root mealybug.

Recovered root mealybug specimens were collected and stored in vials with 70% ethanol and sent to the IITA research station in Benin Republic for identification. Mealybugs from both banana and enset collections were individually slide mounted and examined under a transmitted-light microscope (Leitz LaborLux S) at a magnification range of 40X to 1000X using the keys by Williams and Matile-Ferrero (1999) and Watson and Kubiriba (2005). Permanent mounts of voucher specimens were deposited in the reference collection at the IITA Biodiversity Center. For enset and banana plants, the distinction on the location of the root mealybug on roots or corm sections was made.

Screen house pot experiments

Pot experiments in which enset root mealybugs were added to enset and banana plants in various configurations were conducted at the Dilla University Botanical and Ecotourism Garden to determine mealybug population dynamics and host suitability. One enset cultivar (local name 'Genticha', dominant enset cultivar in the Gedeo zone) and 4 banana cultivars commonly grown in the Dilla area ('Giant Cavendish' (local name 'Canada Muz rezim'; *Musa* AAA), 'Dwarf Cavendish' (local name 'Dinke Muz'; *Musa* AAA), 'Pisang Awak' (local name 'Feranji Muz'; *Musa* ABB) and 'Matooke' (local name 'Abesha Muz'; *Musa* AAA-East African Highland genome group)) were used in this study. The planting material was sourced from healthy young enset fields and mature banana fields. The enset and banana suckers had an average circumference of 25 cm and an average height of 20 cm after the pseudostem was cut off. Plants were planted in various configurations, as detailed per trial below, in plastic basins filled with 12 kg of soil free of any mealybugs. The soil was sourced from a field that had been free of enset and banana cultivation for at least a year at the time of trial establishment. Drainage holes were made in the bottom section of each plastic pot. The pots were kept under a shade net to reduce direct sunlight and hereby reduce water loss through transpiration, and to prevent excessive water due to the sporadic occurrence of heavy storms. Plants were watered manually after planting and when needed.

Four pot trials were assessed with no-choice and choice planting configurations (Table 3, 'Experimental set-up'). In trial 1, a no-choice trial, each enset and banana sucker (of 4 cultivars) was planted in a separate pot to assess host suitability. In trial 2, a choice trial, one enset sucker and one banana sucker were planted together in one plastic basin to assess host preference of the enset root mealybugs. Four combinations were present, with each of the 4 banana cultivars combined with the same enset cultivar. In each pot, the two plants were spaced with 1 cm in between the corms. In trial 3, a choice trial, one enset sucker was combined with four banana suckers, one of each banana cultivar, into one basin to assess host preference across the investigated enset and banana cultivars. The enset corm was positioned in the middle and surrounded by the four banana corms. No room was available between the plants and all corms physically touched. Trial 4 followed the same configuration as the no-choice trial 1, but differed in the number of mealybugs added and the timing of assessment as addressed here below. All pot configurations were replicated 5 to 12 times, as detailed in Table 3.

Plants were given 2 months to establish in the pots before mealybugs were added. Next, in trials 1 to 3, 10 gravid adult females (that is, offspring producing female mealybugs) of the parthenogenetically reproducing enset root mealybug were applied around the stem of each enset or banana plant. The applied mealybugs were subsequently covered with some loose soil. The mealybug presence was assessed four months later (that is, 6 months after initial planting) on the enset and banana plants through complete plant uprooting.

In trial 4, the plants were given an additional 2 months to establish, after which a higher number of 25 mealybugs were added to each plant and a shorter trial duration of 3 months for assessment of mealybug presence was used. This trial was specifically set up as an auxiliary test to assess the survival of the root mealybugs. As shown by the results (trials 1, 2 and 3), low numbers of mealybugs were found at trial assessment, and therefore additional testing on more developed plants with higher numbers of mealybugs and shorter testing times was performed.

The experimental design of the pot trials initially included the assessment of mealybug infestation effects on enset and banana plant growth characteristics, including control plants without the addition of root mealybugs. However, this aspect was ultimately removed because the number of recovered mealybugs at time of assessment was too small to have any impact on enset or banana plant growth traits. Further screenhouse and also field research is thus needed to assess the impact of root mealybugs on growth and yield of banana.

All analyses were performed using R version 3.6.3 (R core team, 2020).

RESULTS

Survey

Surveying 30 backyard gardens at each study site, enset root mealybugs were found in 23 backyard gardens of Dilla Zuria, in 16 backyard gardens at Yirgachefe, but were absent in Gedeb. Across Dilla Zuria and Yirgachefe, a large variety of 38 enset cultivars was observed which varied in infestation of the root mealybug (Table 1). At Dilla Zuria, 14 different enset cultivars were observed, of which only 2 were not infested by mealybugs, namely 'Gosalo' and 'Filila'. At Yirgachefe, a total of 35 different enset cultivars were recorded, with 21 cultivars having recorded mealybug infestations. Enset cultivars present at both Dilla Zuria and Yirgachefe were generally infested at both sites, namely 'Ado', 'Dimoye Astera', 'Dimoye', 'Bira', 'Shegna', 'Tilako Astera', 'Genticha', 'Harame' and 'Asttere gurecha', with only one cultivar infested in a single site, namely 'Dinke'. Only the enset cultivar 'Filila' was free of mealybugs at the two sites of Dilla Zuria and Yirgachefe. At Gedeb, no mealybug infestation was found. The 8 enset cultivars at Gedeb were however also observed in Yirgachefe, where 6 of these cultivars did show mealybug infestations. The absence of mealybugs on enset at Gedeb was thus related to the study site (highest altitude and lowest temperatures), and not the cultivars.

Besides on enset, the presence of root mealybugs was assessed on a total of 58 plant species found within or adjacent to the enset fields across all sites. The surveyed crop species were composed of trees, shrubs, root crops, vegetable crops, legumes, herbs, grasses and woody climbers (Supplementary Table 1). Root mealybugs were observed on the *Musa* cultivar Pisang Awak in 3 backyard gardens at Dilla Zuria (Table 2 and Figure 1c and d), while this cultivar was not infested in Yirgachefe. The backyard gardens where Pisang Awak was found to be infested showed very high infestations on the enset plants, and rooting zones/roots of both plant species overlapped/intertwined. Mealybugs on Pisang Awak were mainly found on the roots of the banana plants, and not on the corms.

Specimens of the root mealybug collected on enset roots and corms at Dilla Zuria were positively identified as *P. ensete* (Williams & Matile-Ferrero) (Hem.: Pseudococcidae) (Figure 3). The root mealybug specimens collected on Pisang Awak at Dilla Zuria were also positively identified as *P. ensete*, although they were notably smaller in size compared to the specimens found on enset (Figures 1c and 3). The smaller size range of

Table 1. Mealybug presence on various enset cultivars at the three study sites.

Enset cultivars	Mealybug presence at sites		
	Dilla Zuria	Yirgachefe	Gedeb
Ado	Pr	Pr	*
Agena	*	Pr	*
Asttere	*	Ab	Ab
Asttere gurecha	Pr	Pr	*
Bale	*	Pr	Ab
Bira	Pr	Pr	*
Bufe	*	Ab	*
Cochu	*	Ab	Ab
Dabare	*	Ab	*
Dimoye	Pr	Pr	*
Dimoye Astera	Pr	Pr	Ab
Dine	*	Pr	*
Dinke	Pr	Ab	*
Ebisho	*	Ab	*
Filila	Ab	Ab	*
Fuga	*	Ab	*
Ganta	Pr	*	*
Ganticho	Pr	Pr	Ab
Gatira	*	Pr	Ab
Goraroka Asitere	*	Pr	*
Gosalo	Ab	*	*
Harame	Pr	Pr	*
Harenjo	*	Pr	*
Henco	*	Pr	*
Kake	*	Pr	Ab
Karase	*	Ab	*
Korkoro	*	Ab	*
Mike	Pr	*	*
Mundo	*	Ab	*
Nifo	*	Ab	*
Qarase	*	Pr	Ab
Qoshe	*	Pr	*
Shegna	Pr	Pr	*
Tilako Astera	Pr	Pr	*
Toracho	*	Pr	*
Torame	*	Pr	*
Wolamo	*	Ab	*
Xara	*	Ab	*

Enset cultivars not found at a specific site are indicated with an asterisks (*). Presence (Pr) or absence (Ab) of the enset root mealybug is indicated.

the specimens originating on the banana host plants does fit within the size variability recorded by the original description running under *Cataenococcus ensete* namely 2.80 to 4.00 mm long, and 2.85 to 3.70 mm wide (Williams and Matile-Ferrero, 1999).

All other investigated species/crops were free of root mealybugs. Specifically other *Musa* cultivars 'Dwarf Cavendish' ('Dinke Muz'), Cavendish ('Key Muz') and 'Lady Finger' ('Sucrier'), observed at Dilla Zuria, and 'Giant Cavendish', 'Cavendish' ('Canada Muz achir'), and

Table 2. Mealybug presence on various banana cultivars at the three study sites.

Musa cultivars			Mealybug presence at sites		
Common name	**Local name**	**Genome group**	**Dilla Zuria**	**Yirgachefe**	**Gedeb**
Giant Cavendish	Canada Muz rezim	AAA	Ab	Ab	*
Cavendish	Canada Muz acher	AAA	Ab	Ab	*
Cavendish	Key Muz	AAA	Ab	*	*
Dwarf Cavendish	Dinke Muz	AAA	Ab	*	*
Pisang Awak	Feranji Muz	ABB	Pr	Ab	*
Matooke	Abesha Muz	AAA-East African Highland	Ab	Ab	*
Lady Finger	Sucrier	AA	Ab	*	*

Banana cultivars not found at a specific site are indicated with an asterisks (*). Presence (Pr) or absence (Ab) of the enset root mealybug is indicated.

'Matooke', observed at both Dilla Zuria and Yirgachefe, were free of enset root mealybugs (Table 2).

The 30 farmers at both the Dilla Zuria and Yirgachefe study sites reported that enset root mealybugs are the most severe biotic constraint to enset production. The pest is perceived as having a higher impact on yields compared to Xanthomonas wilt of enset and other vertebrate pests like mole rats or porcupines. Some farmers also reported mealybug-infested enset to show retarded plant growth. Farmers at the Dilla Zuria and Yirgachefe sites reported that the enset root mealybugs had been present at the sites since at least 4 years. Farmers had no knowledge on how mealybugs spread from one plant to another or from one village to another.
Specific questions regarding management of the pest and the mitigation of its impact revealed that no control measures are currently applied. Farmers reported that they generally source planting material from relatives or purchase it in nearby markets. Enset sucker purchases and plantings mainly occur during the first small rains in the period April to the end of May. During this period, corms and plantlets are often transported from one woreda to another and from one kebele (peasant association) to other kebeles, thus possibly contributing to pest spread. No pest control measures are applied during planting material preparation, transportation or planting. In addition, no control measures are applied during transplanting, a practice applied by small-scale farmers with limited land, to gradually increase enset plant spacing over time.

Pot experiments

Across all screenhouse pot experiments, the number of adult mealybugs at the end of the trials was very low, even on the enset plants. The enset root mealybugs did not reproduce as effectively in the pots as would be expected in a natural environment.

Within the no-choice or single plant pots (trial 1), root mealybugs were observed on enset and on Pisang Awak as host plants (Table 3). Both on enset and Pisang Awak, the mealybugs did not survive in all replicate pots (5 and 3 pots, respectively), but when present, a similar number of adults (range of 2 to 6 adults) combined with additional crawlers was observed on the enset and Pisang Awak plants. In no-choice trial 4, in which more mealybugs were added and assessed after a shorter time, mealybugs were present in more replicate pots of both enset and Pisang Awak. Additionally, mealybugs survived in similar numbers on the Matooke cultivar. The mealybug population did however not survive on Giant Cavendish and Dwarf Cavendish.

In choice trials 2 and 3, with combined enset and banana host plant configurations, mealybugs were again observed on enset, Pisang Awak and Matooke (Table 3). Low numbers of mealybugs were found in the pots with enset – Dwarf Cavendish and enset – Giant Cavendish combinations, with the few observed mealybugs solely present on enset.

In terms of host preference, no statistical difference could be found on the number of mealybugs observed on the banana cultivars (Pisang Awak and Matooke) with or without the presence of enset. This result is nevertheless likely related to the low number of mealybugs overall.

DISCUSSION

This study provides the first observation of the enset root mealybug *P. ensete* (Williams & Matile-Ferrero) on banana plants. *P. ensete* had until now exclusively been recorded on enset in Ethiopia. In three backyard gardens in Dilla Zuria, the enset root mealybug was observed on the roots of multiple plants of the banana cultivar Pisang Awak ('Feranji Muz'; ABB genome group). As roots/root

Table 3. Screenhouse pot experiments assessing enset root mealybugs host suitability and preference on enset and *Musa* cultivars Pisang Awak, Matooke, Dwarf Cavendish and Giant Cavendish.

Pot configuration	Experimental set-up			Assessment of mealybug presence					
					# of mealybugs found on				
	# pots	# mealybugs added	Duration experiment (momths)	# pots with mealybugs	Enset	Pisang Awak	Matooke	Dwarf Cavendish	Giant Cavendish
Trial 1									
Enset	10	10	4	5	2 - 6 (+)				
Pisang Awak	12	10	4	3		3 - 6 (+)			
Matooke	12	10	4	0			/		
Dwarf Cavendish	12	10	4	0				/	
Giant Cavendish	12	10	4	0					/
Trial 2									
Enset + Pisang Awak	12	20 (10 per plant)	4	5	1 - 5 (+)	2 - 3 (+)			
Enset + Matooke	12	20 (10 per plant)	4	4	2 - 12 (+)		7 (+)		
Enset + Dwarf Cavendish	12	20 (10 per plant)	4	2	2 - 3 (+)			0	
Enset + Giant Cavendish	12	20 (10 per plant)	4	1	3 (+)				0
Trial 3									
Enset + Pisang Awak + Matooke + Dwarf Cavendish + Giant Cavendish	9	50 (10 per plant)	4	7	2 - 3	2 - 3	2	0	0
Trial 4									
Enset	5	25	3	5	4 - 7				
Pisang Awak	5	25	3	3		6 - 9			
Matooke	5	25	3	4			3 - 6		
Dwarf Cavendish	5	25	3	0				/	
Giant Cavendish	5	25	3	0					/

Various planting configurations of each trail is indicated (single planting and various combinations of enset and *Musa* cultivars). For each trial, the experimental set-up is provided, indicating the number of replicate pots (# pots), the number of gravid female adult enset root mealybugs added, and the duration between mealybug additions and mealybug assessments. Results show the number of replicate pots with mealybug presence at the end of the trials, and the number of adult mealybugs found (ranges) on each plant. The presence of additional crawlers is indicated (+).

zones of the banana mats and enset plants were overlapping, and large numbers of mealybugs were observed on the enset roots, possibly representing an overpopulation, the observed mealybugs on banana roots under field conditions might have represented a “chance infestation".

Infestation of the banana cultivar Pisang Awak was only recorded in Dilla Zuria. At Dilla Zuria, several conditions could have aided this spread of *P. ensete* to banana plants. Firstly, environmental conditions proved optimal for *P. ensete* with the district situated within an altitude range of 1,600 to 1,900 masl which led to a high number of backyard gardens with infested enset plants (77% of investigated backyard gardens infested). Previous surveys in Ethiopia have shown that the most severe enset root mealybug infestations were in regions between 1,400 and 2,200 masl (Addis et al., 2008b). In our study, substantial infestation was also recorded in the district of Yirgachefe located between1,900 and 2,040 masl

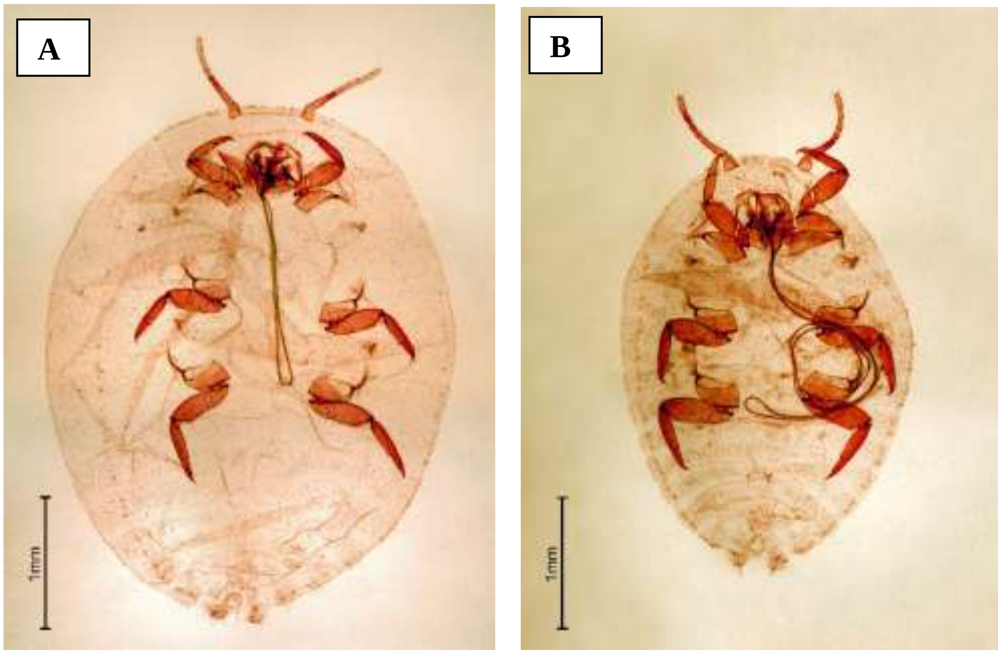

Figure 3. Slide mount picture of the enset root mealybug that was sampled on enset (A) and on the banana cultivar Pisang Awak (ABB genome) (B) in mixed enset-banana backyard gardens at Dilla Zuria, Southern Ethiopia.

(56% of investigated backyard gardens infested) while no infestations were recorded in the district of Gedeb where the altitude extends over 2,000 masl. Secondly, at Dilla Zuria, the high enset infestation numbers were combined with enset-banana-coffee-fruit tree cultivation systems providing a close proximity of the banana plants to its close relative enset. At Yirgachefe, banana cultivation closeby enset cultivation is less predominant, reducing the probability of host transmission. Finally, at Dilla Zuria, the banana cultivar Pisang Awak is frequently cultivated, thus increasing cases of root system proximity with infested enset plants.

The host potential of Pisang Awak was confirmed in the experimental screenhouse choice and no-choice pot trials, as was the susceptibility of a second banana cultivar Matooke (local name 'Abesha Muz', AAA-East African Highland). On both these banana cultivars, *P. ensete* was able to fully develop and produce viable offspring. The adult root mealybugs (as introduced in the trials) have an average life duration of 50 days at 25 ± 3°C (Addis, 2005). Combined with an average development period of nymph to adult of 54 days, the total life span of the enset root mealybug is 94 -113 days (Addis et al., 2008b). This demonstrates that the adults present at the end of the 4-month (trials 1-3) and 3-month (trial 4) infestation periods of the trials were newly developed adults. Additionally, without a suitable host *P. ensete* would suffer starvation and desiccation leading to death. The first-instar nymphs are more prone to starvation, although Addis et al. (2008b) show that all life-stages die within three weeks when exposed to continuous starvation. The presence of adult mealybugs, often combined with crawlers, on Pisang Awak and Matooke when suckers were grown in no-choice/separate pots shows that the mealybugs were both able to survive and successfully reproduce. Overall, offspring production by the mealybugs was very low, even on enset suckers. Similar survival and reproduction values were attained on these banana cultivars compared to the enset suckers. Specifically, for Matooke, mealybugs only survived in no-choice trial 4 in which the plants were already larger at the time of inoculation. In the choice situations, the presence of crawlers still leaves a doubt if it could have been from the enset corms and roots. However, the presence of crawlers at time of assessment in the no-choice conditions is an indication that the insect might be breeding on the banana host.

The observed weak reproductive success across the pot trials is probably to a large extent due to the fact that adult mealybugs had been removed from their initial host plant and transferred to the potted plants. At this developmental stage and after being mechanically removed from their feeding site, they cannot fully settle again and insert their stylet in plant tissues. Thus, the gravid female mealybugs applied to the potted plants would only survive shortly living on their reserves. Under such condition, offspring production is significantly reduced. On the other hand, *P. ensete* has a viviparous habit, that is, offspring hatch directly as crawlers from the female and thus it does not produce egg masses. In addition, breakage of adult female mealybug stylets during mealybug collection (that is when these were removed from infested host corm tissue) in the field occurred. The enset root mealybug's stylet is not retractable, and does not support much axial stretching, the reason why it breaks easily when adult female mealybugs are removed from corm tissues. For example, of 16 field-collected adult mealybugs mounted on slides and examined in the lab, 3 specimens had no stylet, while 9 had only part of it (Figure 3A). Those individuals could certainly not have re-inserted their sucking organ in case they would have been applied to the potted plants. A complete stylet of an adult mealybug has a total length that is about twice the body length of an adult mealybug. In general, the older an individual the lower the likelihood it can feed again following mechanical removal from its original feeding site. It is hence postulated that the gravid female adult enset root mealybugs that were applied on the potted plants, may only have been able to produce a few offspring each before they died. For future research based on pot experiments, we advise a different method. Instead of mechanically removing gravid female adult mealybugs from infested corms in the field and subsequently applying these adult mealybugs to potted plants, 1 cm thick pieces of infested corm surface tissue should be sliced off and these infested corm pieces are then added to the potted plants. This way, the adult mealybugs are not disturbed or removed, and any possible breakage of adult mealybug stylets will be prevented, potentially leading to a higher number of offspring in pot trials. Alternatively, the more mobile young 1st and 2nd instars could be used during pot trial inoculations.

The choice pot trials in which enset and banana suckers were combined also shows that the mealybugs do not have a clear host preference with regards to enset or the Pisang Awak or Matooke cultivars. While the adult mealybugs are not known to be mobile or to actively search for resources, the first and second instar nymph phase ('crawlers') do show this ability and are reported to wander around until they find suitable feeding sites before they settle (Addis et al., 2008b). Nevertheless, even in close proximity the mealybugs did not solely converge on the enset suckers, as both combined infestations or even sole infestation of the banana cultivars was observed in the choice pot trials.

Overall host suitability of *Musa* spp. for enset root mealybugs does however remain low. Only one *Musa* cultivar found in the backyard gardens was infested under natural field conditions and the pot trials demonstrated that the mealybugs could not survive on the cultivars Dwarf Cavendish and Giant Cavendish. The smaller sizes of mealybug specimens found on Pisang Awak in the backyard gardens further indicates that it might not be an ideal host and a "chance infestation" might have occurred due to a mealybug overpopulation on adjacent enset roots. The adoption of non-host plants by pseudococcid mealybugs is relatively frequent and often occurs in situations of over-exploitation of few available hosts (Marohasy, 1997). At the backyard gardens in Dilla Zuria, it is possible that Pisang Awak plants became infested because of the direct vicinity of some heavily infested enset plants, and the occurrence of root system proximity and overlap. Indeed, presence of other infested plants and local pest management strategies applied have a substantial impact on the spread of the pest (Azerefegne et al., 2009).

Further research is needed to investigate the impact of *P. ensete* on physiological and morphological characteristics of potential *Musa* host plants.

Generally, farmers are not aware of how the enset root mealybug was introduced into their backyard gardens, how the pest was spread or of pest management or mitigation techniques. Improved communication to local farmers is critical not only regarding pest management on enset crops, but to prevent occasional mealybug spread to banana plants. Currently the infestation of banana plants is only observed as a result of localized dispersal, possibly representing "chance infestations", at highly infested enset cultivation systems, where banana and enset are grown at very close proximity.

Dispersal in enset fields can be the result of a natural process through active movement of *P. ensete* during the nymphal stages or when adult mealybugs are disturbed (Addis et al., 2008b), through association with ants (Malsch et al., 2001) or displacement by water. Farmers nevertheless also contribute to local dispersal through transplanting of infested enset suckers and the use of contaminated tools. Local management of the pest in infested enset farms is needed to prevent the potential spread to banana plants. A first straightforward approach would be to increase the planting distances between enset and banana plants to ensure there is no overlap between root systems. Additionally, treatment of infested enset planting material before transplanting would reduce local spread. Several methods, or the integration of methods, have been proposed including boiling water treatments, cultural practices, botanical control and the use of insecticides (Azerefegne et al., 2009; Addis et al., 2010; Tadesse et al., 2010a, b; Lemawork et al., 2011, 2018). Specifically, the boiling water treatment is an easily implemented, cheap and highly effective practice to eliminate mealybugs from enset suckers (Lemawork et al., 2018) and could be expected to similarly work on banana suckers. Prevention of the introduction of *P. ensete* into local farms should however be the focus of enset root mealybug management. Logically, a good control of the pest on enset in the first place will prevent any "chance infestation" of bananas or spread to banana plants as alternative hosts.

The main introduction of *P. ensete* is through the exchange of planting material between farmers and the purchase of infested enset suckers from nursery sites (Azerefegne et al., 2009; Addis et al., 2010). Both management at the nursery-level and communication to local farmers will be critical to prevent further dispersal of the enset root mealybug.

CONCLUSION AND RECOMMENDATIONS

Enset and banana are closely related plants in the same family. The enset root mealybugs observed on banana roots in backyard gardens might have represented a “chance infestation”, due to the proximity of banana and enset plants, the intertwining of their root systems and the overpopulation of mealybugs on enset roots. The reduced size of the observed mealybugs on banana roots might reflect an attempt of the pest species to settle on a closely related host. To answer the question on how widespread the root mealybug infestation on banana is, further wide-scale purposive sampling of banana mats (especially the susceptible cultivars observed in the screenhouse study) and enset plants in mixed enset-banana backyards, with various planting densities and crop species proximity and configurations, and high pest infestation levels on enset, would be recommended. Mealybug size differences should also be assessed during the proposed widescale field assessments.

Mature mealybugs were applied in the choice and no-choice screenhouse pot trials. Although limited numbers of mealybugs were observed on both enset and two banana cultivars at the time of plant assessments, the duration of these pot trials only allowed for one new generation of mealybugs to develop. No-choice enset and banana pot trials with much longer time duration, and with the application of crawlers as well as mature enset root mealybugs, would be advisable so that multiple generations of mealybugs would be able to develop and be observed. The proposed field level and screenhouse follow up studies would further elucidate the ability of the enset root mealybug to adapt to banana and would solidify conclusions on host suitability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Table 1. Enset root mealybug presence on various assessed plant species at the three study sites. The common names of the plant species and a description of its growth form are included.

Plant species	Family	Common name	Description	Mealybug presence at sites		
				Dilla Zuria	Yirgachefe	Gedeb
Cucurbita pepo L.	Cucurbitaceae	Field pumpkin	Cultivated annual climber	Ab	Ab	*
Dioscorea bulbifera L.	Dioscoreaceae	True yam	Woody climber	Ab	Ab	*
Dioscorea alata L.	Dioscoreaceae	Purple yam	Woody climber	*	Ab	Ab
Hordeum vulgare L.	Poaceae	Barley	Cereal	*	Ab	Ab
Triticum durum Desf.	Poacea	Durum wheat	Cereal	*	Ab	Ab
Saccharum officinarum L.	Poacea	Sugar Cane	Perennial grass	Ab	Ab	Ab
Zea mays L.	Poacea	Corn, Maize	Cereal	Ab	Ab	Ab
Eleusine coracana (L.)	Poaceae	Finger millet	Cereal	Ab	Ab	*
Lactuca sativa L.	Asteraceae	Lettuce	Green leafy vegetable	Ab	Ab	*
Aframomum corrorima (Braun) Jansen	Zingiberaceae	Ethiopian cardamom, false cardamom, or korarima	Spice	Ab	Ab	*
Allium sativum L.	Alliaceae	Nech Shenkuret, Garlic	Crop	*	Ab	Ab
Amaranthus caudatus L.	Amaranthaceae	Pendant amaranth, tassel flower, velvet flower	Green leafy vegetable	Ab	Ab	Ab
Artemisia abyssinica Sch. Bip. ex A. Rich.	Asteraceae	Artemisia abyssinica	Herb	*	*	Ab
Beta vulgaris var. *esculenta* L.	Chenopodiaceae	Beet	Crop	Ab	Ab	Ab
Brassica carinata A. Br.	Cabombaceae	Ethiopian rape, Ethiopian mustard, and Abyssinian mustard	Crop	Ab	Ab	Ab
Capsicum annuum L.	Solanaceae	Sweet Pepper, Cayenne Pepper, Chili Pepper, Red Pepper	Crop	Ab	Ab	Ab
Capsicum frutescens L.	Solanaceae	Tabasco pepper	Crop	Ab	Ab	Ab
Coriandrum sativum L.	Apiaceae	Coriander	Spice	Ab	Ab	*
Daucus carota L.	Apiaceae	Carrot	Crop	Ab	Ab	Ab
Ensete ventricosum (Welw.)	Musaceae	Enset, False banana	Crop	Pr	Pr	Ab
Lepidium sativum L.	Brassicaceae	Garden cress	Herb	*	Ab	Ab
Lycopersicon esculentum Mill.	Solanaceae	Tomato	Crop	Ab	Ab	Ab
Mentha spicata L.	Lamiaceae	Nana, Spearmint, Common Green Mint	Herb	Ab	Ab	Ab
Ocimum basilicum L.	Lamiaceae	Basil, Sweet basil	Herb	Ab	Ab	Ab
Piper capense L. f.	Piperaceae	Wild pepper	Shrub	Ab	Ab	Ab
Rosmarinus officinalis L.	Lamiaceae	Rosemary	Herb	Ab	Ab	Ab
Ruta chalepensis L.	Rutaceae	Egyptian Rue, Fringed rue	Herb	Ab	Ab	Ab
Zingiber officinale Roscoe	Zingiberaceae	Ginger	Crop	Ab	Ab	*
Phaseolus vulgaris L.	Fabaceae	Common bean	Food legume	Ab	Ab	Ab
Spinacia oleracea L.	Chenopodiaceae	Spinach	Green leafy vegetable	Ab	Ab	Ab
Annona reticulata L.	Annonaceae	Custard apple, bullock's heart	Tree	Ab	Ab	*
Colocasia esculenta (L.) Schott	Araceae	Taro	Crop	Ab	Ab	*
Ipomoea batatas (L.) Lam.	Convolvulaceae	Sweet potato	Crop	Ab	Ab	Ab
Lens culinaris Medikus	Fabaceae	Lentil	Food legume	Ab	Ab	Ab
Musa spp.	Musaceae	Banana	Crop	Pr	Ab	*
Pisum sativum L.	Fabaceae	Pea	Food legume	Ab	Ab	*
Vicia faba L.	Fabaceae	Fava bean, faba bean	Food legume	Ab	Ab	Ab
Lippia adoensis Hochst. ex Walp.	Verbenaceae	Kosseret or Kese	Shrub	Ab	Ab	Ab
Ocimum lamiifolium Hochst. ex. Benth.	Lamiaceae	Dama Kese	Medicinal shrub	Ab	Ab	Ab
Punica granatum L.	Punicaceae	Pomegranate	Shrub	Ab	Ab	Ab

Supplementary Table 1. Contd.

Rhamnus prinoides L'Herit.	Rhamnaceae	Giesho, Buckthorn, Dogwood	Medicinal shrub	Ab	Ab	Ab
Thymus schimperi Ron. (Labiatae)	Lamiaceae	Tosegn, Abyssinian Thyme	Shrub	*	*	Ab
Citrus sinensis (L.) Osb.	Rutaceae	Sweet orange	Tree	Ab	Ab	Ab
Carica papaya L.	Caricaceae	Papaya	Tree	Ab	Ab	Ab
Casimiroa edulis La Llave	Rutaceae	Kazmier, White Sapote	Tree	Ab	Ab	Ab
Catha edulis (Vahl) Forssk. ex Endl.	Celastraceae	Khat	Shrub	Ab	Ab	Ab
Coffea arabica L.	Rubiaceae	Coffee	Shrub	Ab	Ab	Ab
Manihot esculenta Crantz	Euphorbiaceae	Cassava	Crop	Ab	Ab	Ab
Passiflora edulis (Aubl.) Schum.	Passifloraceae	Passion fruit	Woody climber, vine	Ab	Ab	Ab
Prunus persica L.	Rosacaea	Peach	Tree	Ab	Ab	Ab
Psidium guajava L.	Myrtaceae	Common guava	Shrub or small tree	Ab	Ab	Ab
Ricinus communis L.	Euphorbiaceae	Castor bean, castor oil plant	Shrub or tree	Ab	Ab	Ab
Amaranthus graecizans L.	Amaranthaceae	Mediterranean amaranth, short-tepalled pigweed	Green leafy vegetable	Ab	Ab	Ab
Citrus aurantiifolia (Christm.)	Rutaceae	Lime, Key Lime, Mexican Lime	Tree	Ab	Ab	Ab
Malus domestica Borkh.	Rosaceae	Apple	Tree	*	*	Ab
Moringa stenopetala (Bak. f.) Cuf.	Moringaceae	African Moringa, cabbage tree	Tree	Ab	Ab	*
Mangifera indica L.	Anacardiaceae	Mango	Tree	Ab	Ab	Ab
Persea americana Mill.	Lauraceae	Avocado	Tree	Ab	Ab	Ab

Presence (Pr) or absence (Ab) of the enset root mealybug is indicated. Crops not found at a specific site are indicated with an asterisks (*).