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Natural occurrence of *Diadiplosis megalamellae* (Barnes) in mealybugs on roses in Kenya

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Over the last decade there has been an increasing adoption of Integrated Pest Management on rose farms in Kenya. As a consequence, there has been a rise in secondary pests on rose plants, including in particular the citrus mealybug *Planococcus citri* (Risso). On cut flowerrose farms in Kenya, the presence of the predatory midge *Diadiplosis megalamellae* (Barnes) (Diptera: Cecidomyiidae) was observed. Therefore, a survey was carried out to quantify the occurrence of *D. megalamellae* and the association with mealybug infestations in commercial cut flower rose crops in Kenya. Four farms in four different regions of Kenya and eight rose varieties were surveyed. The midge *D. megalamellae* was present on farms located in Naivasha, Nairobi and Thika, but was absent in Nanyuki region. The midge *D. megalamellae* was found mainly in *P. citri* mealybug colonies and, although in much lower numbers, in the long tailed mealybug *Pseudococcus longispinus* (Targioni Tozzetti) colonies. The number of mealybugs was positively correlated with the number of number of *D. megalamellae* larvae suggesting increased multiplication of the *D. megalamellae* when the pest is present in larger numbers. The number of mealybugs increased with an increase in altitude at which a rose farm was located but there were no *D. megalamellae* present at the high altitude farm. The reasons for differences in mealybug population between farms is discussed along with further work needed, however, as an indigenous Kenyan predator, this midge offers potential for mealybug biocontrol on rose farms in Kenya.

Key words: Pseudococcidae, Cecidomyiidae, biological control, predatory midge.

INTRODUCTION

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera:Pseudococcidae), is a highly polyphagous pest that was initially associated with citrus. By now their host range has been reported to include at least 27 different plant families, including economically important indoor ornamentals, vegetables, and fruits (Tingle and Copland, 1988; Gill et al., 2013). This pest increasingly occurs in greenhouse ornamentals and is becoming a prominent problem in cut flower rose crops (Messelink, 2014). With the increasing adoption of Integrated Pest Management (IPM) and the reduced application of broad-spectrum pesticides, *P. citri* has become a major pest in rose crops in Kenya. Plants infested by *P. citri* exhibit yellow, distorted, and wilted leaves, premature leaf drop and stunted growth (Hill, 2008). This deformation leads to reduced photosynthesis and thereby to reduced yield.

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Roses are intensively grown and the high nutrient dosages supplied, especially high nitrogen, are known to increase mealybug pressure in a variety of species (Hogendorp et al., 2006). Female mealybugs are wingless, unlike males, and can produce five or more generations per year (Hill, 2008). Eggs of *P. citri* are laid in female ovisacs that can contain 300 eggs at a temperature of 18°C (Copland et al., 1985). Depending mostly on temperature, mealybugs have three nymphal stages lasting 16 to 38 days to develop to adults. Mealybugs are covered with white waxy particles and secrete honeydew. This is the main reason for the low efficiency of pesticides as they are unable to penetrate through the waxy layers (Al-Ali, 1969).

The importance of biological control of mealybugs has long been recognized and there has been strong interest in biological control solutions in the horticultural industry in Kenya for over a decade (Wainwright and Labuschagne, 2009). In Europe, three biocontrol agents are used against mealybugs, namely the ladybug *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and the endoparasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). Both *C. montrouzieri* larvae and adults feed on mealybugs. *C. montrouzieri* larvae are covered with white wax threads, causing them to resemble mealybugs. The adults can fly and find new mealybug colonies, while larvae find their prey by physical contact. In Kenya, *C. montrouzieri* was introduced in 1924 and has established (Booth and Pope, 1986). Despite numerous commercial trials in ornamentals in recent years *C. montrouzieri* is not used as a biological control agent in Kenya. This is mainly due to the high production costs and because it cannot be used as a preventive measure, as adult *C. montrouzieri* will disperse when mealybug population is low. The adult lacewing *C. carnea* is not predacious, but the larvae feed ferociously on many pests. In Europe, America and Asia, it is a common predator of aphids (Hemiptera: Aphididae) and can be used to control mealybugs (Rashid et al., 2012). The lacewing *C. carnea* is not indigenous to Kenya, and at the moment it is not commercially available for mealybug biological control in Kenya. The endoparasitoid *A. pseudococci* is a specialist parasitoid of *Planococcus* and *Pseudococcus* species mealybugs. The parasitoid lays one egg in a mealybug, the hatched larvae will feed on the mealybug whilst developing. The parasitoid *A. pseudococci* is indigenous in Kenya (Tanga et al., 2015), but is not commercially available in Kenya. However, since it is indigenous and used as a biocontrol agent in other parts of the world, it has the potential to be exploited as a suitable biocontrol agent in Kenya.

In addition, the authors recently found the predatory midge *Diadiplosis megalamellae* (Barnes) (Diptera: Cecidomyiidae) feeding on *P. citri* and on *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) in Kenya. Cecidomyiids are commonly known as gall midges because the larvae of many species feed within plant tissues inducing the formation of noticeable galls. Moreover, the family also includes several less-noticeable genera such as *Diadiplosis,* whose larvae are predators of organisms such as scale insects (Hemiptera: Coccoidea) (Gagné, 1994). In a recent study Hayon et al. (2016) collected five gall midge species using mealybug sentinel traps in Israel: *Diadiplosis donaldi* (Harris, 1968), *Diadiplosis multifila* (Felt), *Dicrodiplosis manihoti* (Harris), *Lestodiplosis* species, and *Trisopsis tyroglyphi* (Barnes). Two species in the Cecidomyiidae family that are currently commercialized as biocontrol agents are *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) and *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae), which are used against aphids and tetranychid mites, respectively (Harris, 2004). The midge *D. megalamellae* is an indigenous species, only collected in tropical Africa (East Africa, Zaire, and West Africa) and preys on both *Planococcus* and *Pseudococcus* mealybug species (Harris, 2004). At the moment, little information is available on the predatory midge *D. megalamellae* (Barnes) occurrence in Kenya or its ability to control mealybugs. As emphasized by van Lenteren (2012), the most critical phases in any biological control programme are the steps where selection of natural enemies takes place. As an initial step in the development of a biocontrol strategy for mealybugs in roses, a survey was conducted in commercial rose crops to quantify the occurrence of *D. megalamellae*. This research provides evidence of the potential this predatory midge may offer for the control of mealybugs in roses.

MATERIALS AND METHODS

To determine the occurrence of *D. megalamellae* in cut flower roses in Kenya, a multiple location survey was conducted in the four major cut flower growing regions: Thika (Farm A), Nairobi (Farm B), Naivasha (Farm C), and Nanyuki (Farm D), in June 2018 (Figure 1). Most rose cut flower farms in Kenya are located at altitudes between 1,500 and 2,200 m so large areas of Kenya do not produce roses. Each farm, consisting of two greenhouses (100 × 100 m^2) with two rose varieties, were observed for mealybug infestation and predatory midge occurrence and sampled for the study (Table 1). To quantify *D. megalamellae* numbers, 92 rose plants that had mealybugs were sampled randomly in the greenhouse, the mealybugs were carefully brushed off by using a camel brush, placed into a vial (30 ml) and taken to the laboratory (Real IPM, Kenya). The mealybug colonies were removed and the number of *D. megalamellae* eggs, larvae and pupae were recorded (Figure 2). In addition, the altitude and mealybug species were recorded for each farm.

The identification of *D. megalamellae* (Barnes, 1939) was undertaken by Keith M. Harris (Private communication, 2017) based on a sample of adults (24 males and 10 females) and larvae (>40 third and earlier instars) (Harris, 1968).

Statistical Analysis

Data obtained from the surveys, region altitude, mealybug

Figure 1. Kenyan counties in which mealybug samples were collected:Kiambo (Farm A), Nairobi (Farm B), Nakuru (Farm C), Laikipia (Farm D).
Source: Adapted from Lewis (2016).

Table 1. Mean number of mealybug colonies, number of midge eggs, larvae and pupae in these mealybug colonies on four different rose farms in Kenya. Mealybug species and farm altitude in metersare also shown.

Farm	Mealybug species	Mealybug colonies (Mean ± SE)	Midge eggs (Mean ± SE)	Midge larvae (Mean ± SE)	Midge pupa (Mean ± SE)	Farm altitude (m)
Farm A	*P. citri*	3.81 ± 0.24^{a}	0.17 ± 0.07^{a}	0.68 ± 0.10^{b}	0.31 ± 0.07^{c}	1,525
Farm B	*P. longispinus*	7.84 ± 0.78^{b}	0.00 ± 0.00^{a}	0.05 ± 0.03^{a}	0.01 ± 0.01^{a}	1,973
Farm C	*P. citri*	9.40 ± 0.59^{c}	0.42 ± 0.15^{b}	2.38 ± 0.26^{c}	0.14 ± 0.04^{b}	1,905
Farm D	*P. citri*	10.74 ± 0.84^{c}	0.00 ±0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2,335
$F_{(3,732)}$ value	-	21.05	5.89	64.93	11.70	-
P value	-	<0.001	<0.001	<0.001	<0.001	-

Means within each column followed by the same letter are not significantly different (P>0.05). SE: Standard error.

species, and number of *D. megalamellae* eggs, larvae, and pupae, were recorded in Microsoft Excel spreadsheets, and subjected to analysis of variance with a General Linear Model (GLM). Regression analysis of the number of mealybug number (independent variable) against the number of *D. megalamellae* larvae (dependent variable) was undertaken with the exclusion of

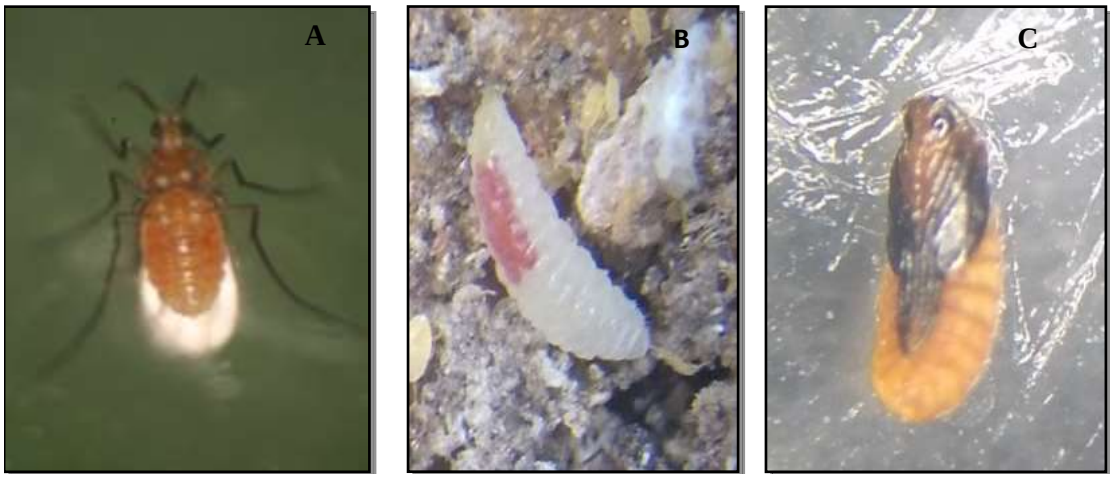

Figure 2. *Diadiplosis megalamellae* (Barnes) life cycle stages. (A) Adult (ventral view); (B) Larva (dorsal view), (D) Pupa (ventral view).

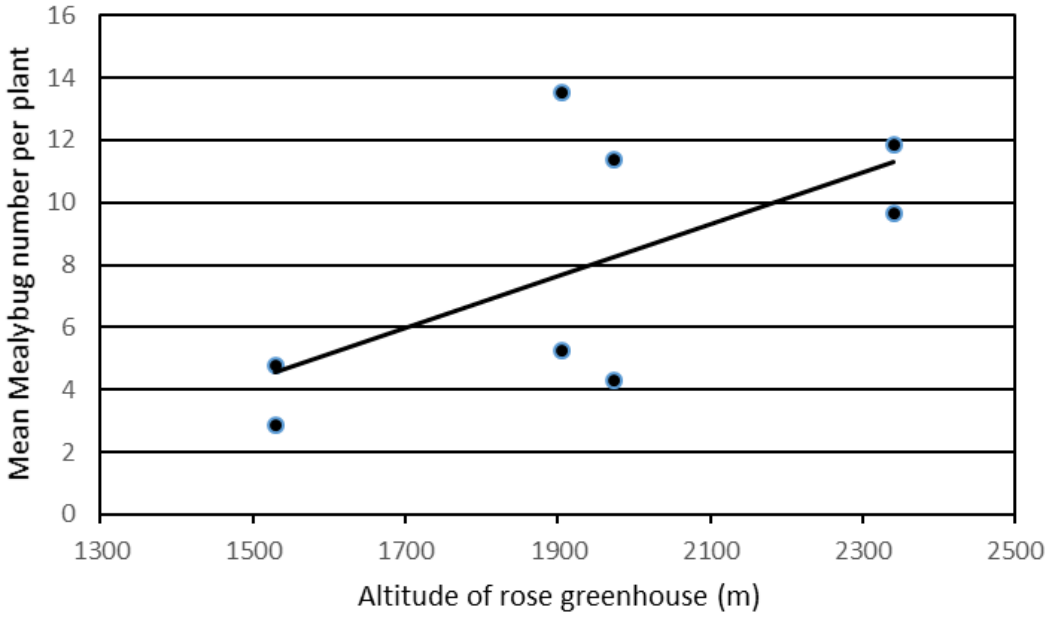

Figure 3. Linear regression of mean citrus mealybug colony number per rose plant against altitude at which the rose farm was located. $Y = 0.0083x - 8.213$; $R^2 = 0.392$; $F_{(1,7)} = 3.86$; $P = 0.097$.

farm D where no *D. megalamellae* was observed. An additional regression analysis of mealybug against the larvae against farm altitude was undertaken using the mean data from all varieties and locations (Figure 3). All analysis was performed using Genstat software and means separated by LSD-test and Tukey's test at $P \leq 0.05$.

RESULTS

On Farm A, C, and D, the mealybug *P. citri* was observed, whilst on Farm B the mealybug *P. longispinus* was found feeding on rose plants. The number of mealybug colonies was significantly different between farms ($P<0.001$). The highest number of mealybug colonies per plant were recorded on Farm D, and the least on Farm A (Table 1). The highest number of midge eggs and midge larvae was observed on Farm C, whilst the highest number of midge pupae was found on Farm A (Table 1). On Farm D, no *D. megalamellae* eggs, larvae and pupae were observed.

The number of mealybug colonies present on each farm was also influenced by rose variety ($P<0.001$). The highest mealybug colony number was found on Madam Red, whereas the lowest mealybug colony number was

Table 2. Mean number of mealybug colonies on infested rose plants and the number of midge eggs, larvae and pupae in these mealybugcolonies on eight rose varieties.

Rose variety	Farm	Mealybug colonies (Mean ± SE)	Midge eggs (Mean ± SE)	Midge larvae (Mean ± SE)	Midge pupa (Mean ± SE)
Nightingale	Farm A	2.87 ± 0.25^{a}	0.12 ± 0.06^{a}	0.71 ± 0.14^{b}	0.41 ± 0.13^{c}
Aqua	Farm B	4.28 ± 0.56^{a}	0.00 ±0.00^{a}	0.10 ± 0.05^{a}	0.00 ±0.00^{a}
Proud	Farm A	4.75 ± 0.38^{a}	0.23 ± 0.12^{a}	0.65 ± 0.14^{b}	0.21 ± 0.07^{b}
Pegasso	Farm C	5.26 ± 0.35^{a}	0.04 ± 0.02^{a}	1.04 ± 0.26^{c}	0.12 ± 0.05^{ab}
Maritim	Farm D	9.64 ± 0.77^{b}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
Sweet Sara	Farm B	11.39 ± 1.36^{bc}	0.00 ±0.00^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
Confidential	Farm D	11.84 ± 1.48^{bc}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
Madam Red	Farm C	13.53 ± 0.95^{c}	0.79 ± 0.29^{b}	3.72 ± 0.39^{d}	0.15 ± 0.07^{a}
$F_{(7,728)}$ value		21.75	5.74	47.10	5.9
P value		<0.001	<0.001	<0.001	<0.001

Means within each column followed by the same letter are not significantly different (P>0.05). The respective farms where these varieties were grown are indicated as A-D. SE: Standard error.

found on Nightingale (Table 2). The number of midge eggs per colony was significantly higher on variety Madam Red compared to midge egg number on the varieties Nightingale, Proud and Pegasso. However, the number of midge eggs found on Nightingale, Proud and Pegasso varieties was not significantly higher than Aqua, Maritim, Sweet Sara, and Confidential varieties that had no midge eggs (P > 0.05).

On variety Madam Red a significantly higher number of midge larvae was found than on Pegasso, Nightingale and Proud varieties. On variety Aqua, the number of midges did not differ from the varieties Maritim, Sweet Sara, and Confidential, that had no midge larvae (P > 0.05).

The number of pupae was higher on variety Nightingale compared to the varieties Proud and Pegasso. However, the number of pupae on variety Proud did not differ from variety Pegasso, although the number of midge pupae on Pegasso did not differ from the remaining varieties including those that did not have any midge pupa (Table 2).

The regression analysis of the number of mealybug number (independent variable) against the number of *D. megalamellae* larvae (dependent variable) (y = 0.058x + 0.626, R2 = 0.0414, n=552, P < 0.001) showed there was a positive but weak relationship. The number of mealybugs increased with an increase in altitude at which a rose farm was located (Figure 3). At a lower altitude, a lower number of mealybugs was found, whereas at a higher altitude a higher number of mealybugs was present.

DISCUSSION

The predatory midge *D. megalamellae* may serve as a potential predator for the control of mealybugs in roses in Kenya. There was a significant positive correlation between the number of mealybug and the number of midge *D. megalamellae*. As the midge is feeding on the mealybug this relationship is to be expected. In addition, a high number of *D. megalamellae* pupae was associated with a lower number of mealybugs present on the farm. There was also a relationship between the number of mealybugs present and the altitude of the farm (Figure 3). It is expected that at a lower altitude, temperatures and mealybug growth are higher, in line with the study of Laflin and Parrella (2004) that studied the mealybug number on roses in California. However, in our study at higher altitude, where temperatures are lower, a higher number of mealybugs occurred. Under controlled conditions, the development of mealybugs in relation to temperature follows a sigmoid curve (Laflin and Parrella, 2004). However, extrapolation of such laboratory results to the farm is not straightforward, as other factors such as humidity, temperature over time, could well be as important. In addition, absence of *D. megalamellae* at the highest altitude (farm D) could have played a role in this outcome.

The population of mealybugs varied significantly between the four farms surveyed. The reasons for this are probably multifactorial but could include the management practices on each farm, differences in susceptibility of rose varieties to mealybugs, the chemicals applied, the climatic conditions and the presence of natural enemies. The presence of *P. longispinus* mealybugs on farm B further complicates the understanding of what factors influence mealybug and midge populations. Nymphs of *P. longispinus* mealybugs hatch immediately upon oviposition, which has led some observers to conclude mistakenly that female *P. longispinus* mealybugs are viviparous (Goolsby 1994). Consequently, the midge *D. megalamellae* may not be a suitable predator for long tailed mealybug; hence, the very

low presence of the midge on farm B. However, Charles (1981) does report *Diadiplosis koebelei* (Diptera: Cecidomyiidae) preying on *P. longispinus.* Future studies could test the suitability of *P. longispinus* mealybugs as prey for *D. megalamellae* and *D. koebelei*.

The presence of mealybugs on cut flower roses is a relatively recent development. It is slightly surprising that the predatory midge *D. megalamellae* has migrated into commercial crops of cut flower roses that have citrus mealybug in a period of just a few years. Furthermore, our survey has demonstrated that this is not a one-off event as the midge is present in three distinct geographical rose growing areas of Kenya, Thika, Naivasha and Nairobi. It may be possible to supplement the predation by the midge with augmentative applications in order to achieve biological control of citrus mealybug in cut flower rose production in Kenya. Hayon et al. (2016) pointed out that the advantages of predatory Cecidomyiidaeare to effectively locate mealybugs even in cryptic places, and that they appear to be less susceptible to intraguild predation than other natural enemies. Additionally, they suggest that predatory Cecidomyiidaeare not deterred by ants that guard colonies of mealybugs. The observation that *D. megalamellae* lays its eggs in the egg mass under the waxy layer of the citrus mealybug offers the predatory midge protection against conventional pesticides being used in rose cut flower production.

To conclude, the predatory midge *D. megalamellae* may be an important biocontrol agent for citrus mealybugs. However, there is little fundamental knowledge on the biology of this predatory midge and on its role in restraining mealybug populations and its potential in biocontrol programs. More information on its prey range, sensitivity to climate conditions and insecticides, is required for their successful deployment as a biocontrol agent in Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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