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Role of macadamia insect flower visitors on nut set, retention and yield in central Kenya

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ABSTRACT

Aim: The aim of this study was to evaluate the contribution of macadamia insect flower visitors on nut set, retention, nut-in-shell and kernel yields.

Materials and Methods: This study was conducted at Macadamia Research Centre, in Kandara, Murang'a for three seasons from August 2020 to May 2022. Nut set and nut retention was assessed by purposively selecting twelve trees that were in full bloom at the study orchard. On each of the twelve trees, racemes at bud stage were randomly selected and labeled on four branches of the same size, at a height of 1 to 1.5 metres from the ground.Racemes were then bagged during the day with mesh nets, others bagged at night and some bagged throughout the flowering season excluding all insects, while some racemes were left unbagged, thus having unlimited flower visitation by insects.

Results: There were significant differences on nut-in-shell yield (mass) ($P \leq 0.05$) in flowers that were unbagged (68.23 ± 4.03), bagged during the day (61.50 ± 3.51), bagged at night (6.53 ± 1.05) and those that were bagged throughout (4.45 ± 0.95). Racemes that had flower visitors fully excluded,resulted in low nut-in-shell production.The initial nut set, retention and ultimately the nut-in-shell yields were significantly increased in flowers that were left unbagged thus insects had unlimited access.

Conclusion: It was concluded that Macadamia nut set, retention and ultimate yield is highly reliant on pollen transfer which is mediated by insect flower visitors that forage during day time.

Keywords: Central Kenya, insect flower visitors, Macadamia, nut-in-shell yield.

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Introduction

Pollinator-dependent crops make up 75% of the main types of food crops grown throughout the world and some of the most expensive and nutritious foods (Eilers *et al*., 2011; Chaplin-Kramer *et al*., 2014; Garibaldi *et al*., 2022; Potts *et al*., 2016a). The degree to which pollinator-dependent crops rely on insect pollinators varies greatly; for example, *Macadamia integrifolia* yield can be up to 185 % greater following insect pollination (Grass *et al*., 2018) whereas oilseed rape can receive an 18% yield boost when pollinated (Bommarco *et al*., 2012), strawberry yields can be increased by over 70% (Hodgkiss *et al*., 2018). Pollination is an important ecosystem service for plant reproduction, and insects account for 85 % of all pollen transfer (Gill *et al*., 2016; Potts *et al*., 2016b Kamper *et al*., 2021).

Insect pollination accounts for the greatest share of the single most crop production (Klein *et al*., 2007). Globally, 87.5 % of angiosperm production depends on pollination (Ollerton *et al*., 2011).

Macadamia (*Macadamia integrifolia* Maiden and Betsche and *Macadamia tetraphylla* Johnson and their hybrids) is the main lucrative nut crop grown for its edible kernel that has great nutritional benefits and is native to Australia (Trueman, 2013). The main countries where its currently grown are Australia, Hawaii, Kenya, and South Africa (Quiroz *et al.,* 2019). Macadamia has pendant racemes that are 10 - 20 cm long with 100 - 300 protandrous flowers, which are self-incompatible (Trueman, 2013). Macadamia profusely flowers, but yields are low as only 3 % of the initial flowers mature to form nuts due to, among other physiological factors, pollination deficits (Howlett *et al*., 2019). Most of the nuts abscise within the first two months after nut set (Lavi *et al*., 1996; Howlett *et al*., 2015). Nut yields may be improved by addressing factors that lead to nut dropping, such as pollination (Howlett *et al*., 2015). Insects are the main agents of pollen transfer in macadamia, and the number of visits by various insect species influences both the quantity and quality of macadamia nut yields (Khalifa *et al*., 2021). There are diverse invertebrates such as bees, wasps, beetles, and butterflies that forage for nectar or pollen in macadamia flowers (da Silva *et al*., 2020). However, the honey bee (*Apis mellifera*) is dominant and present in all the main areas where there is cultivation of macadamia (Evans *et al*., 2021). Although *A. mellifera* is the most abundant macadamia flower visitor, there are other bee species that contribute to macadamia pollination. Pollination by more than one bee species, such as honey bees, carpenter bees, stingless bees, feral bees, and social and solitary bees, results in better pollination (Klein *et al*., 2003; Khalifa *et al*., 2021).

The transfer of pollen in macadamia is mainly animal-mediated, as the stigma is small in size and has sticky pollen, demonstrating minimal possibility of wind pollination, thus, macadamia greatly benefits from insect visitation for pollination, with bees being the most abundant visitors globally (Howlett *et al*., 2015, Kamper *et al*., 2021). According to Delaplane *et al*. (2013), the exclusion of flower visitors has been recognized as a technique for measuring agricultural pollinator dependency. Studies on pollinator dependency in a variety of crops, including macadamia nuts, have been conducted using mesh bags (Grass *et al*., 2018). Exclusion of flower visitors from macadamia flowers has been demonstrated to result in a reduction of nut set and yields as low insect flower visitors or insect activity in a macadamia orchard may result in insufficient pollen transport and hence poor pollination (Anders *et al*., 2023, Tavares *et al*., 2015). Flowers left with unlimited insect visitor access have been shown to increase by 304% and 23%, nut set and nut-in-shell yields, respectively (Anders *et al*., 2023). The abscission and a lack of nut development following flowering are signs of insufficient ovule fertilization (Trueman *et al*., 2022). The pollination of macadamia mainly happens during the day, so nocturnal insects such as bats and flying insects have little impact on the pollination of macadamia blossoms (Heard, 1993; Blanche *et al*., 2006). Wind could be a contributor to pollen transfer in macadamia (Tavares *et al*., 2015). However, no one has attempted to distinguish the contribution of wind-borne macadamia pollen from pollen transferred by other agents, despite the possibility that wind is a particularly significant agent of pollination on macadamia (Blanche *et al*., 2006). Although most experiments have not taken wind pollination into account, Urata (1954) argued that the role of windblown pollen in macadamia pollination should not be discounted. Blanche *et al*. (2006) quantified the initial fruit or nut set as a sign of successful pollination. Production of self-pollinated nuts has been shown to decrease due to gametophytic incompatibility that inhibits pollen tube growth (Trueman, 2013). There is no information on how flower visitors influence nut set, retention and yields (nut-in-shell and kernel) of macadamia in Kenya.

The lack of knowledge on insect flower visitors and their contribution in macadamia orchards in Kenya, presents a huge gap in assessing the impact of pollinator guilds on macadamia production. To improve the nut yield of macadamia, this study highlights the effects of insect flower visitors' presence or exclusion through bagging on nut set, retention, and yield. Specifically, this study assessed (i) effects of insect flower visitors on macadamia nut set in Murang'a county, (ii) effects of insect flower visitors on macadamia nut retention in Murang'a county (iii) effects of insect flower visitors on macadamia nut-in-shell and kernel yields in Murang'a county. This knowledge is intended to form the basis for macadamia pollination and can be utilized to improve nut yields in Kenya.

Materials and Methods

Field experimentson the effect of flower visitors on nut yield was conducted for three seasons between August 2020 and May 2022, in a homogenous macadamia orchard at Kandara Macadamia Research Centre in Murang'a county within central Kenya. The study area was located between 0º59ˈ43.9˝S, 37º03ˈ31.0˝E and 1º00ˈ00.7˝S, 37º03ˈ39.2˝E, in East Africa. Kandara is a sub-county within Murang'a county that has deep and well drained red or brown nitosols soils (Jaetzoldet al., 2006). Total monthly rainfall and mean temperature data of the study area ranged from 1.2 to 254.2 mm and 17.4 to 22 °C, respectively. The study area has four weather seasons namely; cold season that occurs during the months of June, July and August, dry (January, February and September), short rain

(October, November and December, and long rain (March, April and May).

Study crop

Macadamia integrifolia was the main species grown in the orchard, which is an evergreen tree that grows to a height ranging between 12.5 to 16.0 metres and with the base width of lower branches ranging between 3.2 and 6.6 metres. In central Kenya, the macadamia trees bloom throughout the year with months of August, September and October having dense flowering whereas January, February, March, April, May, June, July, November and December having sparse blooming. The dense and sparse flowering patterns were described by percentage blossoming, where flowering less than 50 % was regarded as sparse and 50 % and above flowering was termed as dense, during the survey.

Assessment of nut set and retention

To assess the effect of insect flower visitors on macadamia nut set and nut retention, twelve trees were randomly selected at the study orchard. On each of the twelve trees, inflorescences at the bud stage were randomly selected and labeled on four branches of the same size, at a height of 1 to 1.5 metres from the ground. The branches were selected when trees were in full bloom and with inflorescences located on the outer side of the canopy. One inflorescence was selected per branch. All the selected inflorescences, at the bud stage, were then bagged with fine mesh nets up to when the flowers matured. The mature inflorescences were then bagged with a fine mesh net measuring 30 centimeters by 15 centimeters (Fig. 1a and b) that was then strengthened with a wire frame to avoid bruising the florets and secured with a wire tie. This standardized procedure was repeated on each of the twelve trees for four experimental treatments namely; (i) bagged during the day from seven am to six pm, in order to exclude insect flower visitors during the day; (ii) bagged at night from six pm to seven am, in order to exclude insect flower visitors at night; (iii) bagged throughout the flowering period to exclude all insect flower visitors from accessing the macadamia flowers; and (iv) not bagged throughout the flowering stages in order to provide unlimited insect flower visitors access to the macadamia flowers. Nut set was recorded after fertilization, twenty-one days after flower abscission, and this experiment was conducted three times, in the months of August, 2020 and August 2021. Nut retention on all treatments was observed one month before harvesting or eight months (October 2021 and March 2022) after the nut set experiment.

Assessment of nut-in-shell yield

One month before harvesting, immediately after the nut retention experiment, specifically in October 2021 and March 2022, mature nuts were bagged with a recyclable bag. This was to allow fully matured nuts to drop into the bag for assessment of nut yield. This experiment was repeated for each of the four treatments described in the nut set experiment. In the experiment, each of the four treatments was assigned a bag with a different colour namely: (i) blue for bagged during the day, (ii) white for bagged at night, (iii) green for bagged throughout, and (iv) red for the unbagged flowers. Yield was assessed by recording the weights of individual nuts-in-shell and kernels that were collected at the end of the successful protocol for each of the four treatments on nut set and nut retention (Fig 2a and b).The macadamia nuts harvested from each treatment were immediately de-husked at the study site and transported to a laboratory at the department of Plant Science and Crop Protection at the University of Nairobi, where they were dried at 38°C for seven days in an oven. The weight of the individual dried nuts-in-shell was then measured to the nearest 0.01g using a digital scale, while length and width were measured using a vernier calipers.

Assessment of kernel yield

The nuts were thereafter cracked manually using an improvised nut cracker, and the weights of individual kernels were taken using a digital scale to the nearest 0.01 g, and length and width were measured using a vernier calipers.

Data analysis

To compare the effects of bagging, unbagging, bagging throughout, and bagging flowers at night on the mean nut set, retention, nut-in-shell, and kernel weight, pollination treatment was used as a fixed effect. Means of nut set, retention, nut-in-shell weight, kernel weight, and nut-in-shell length, width, and kernel length and width were subjected to analysis of variance (ANOVA). To test for significant differences, the means were separated using Tukey's test.The data analyses were computed using R statistical software (R Development Core Team, 2018).

Fig. 1: Fully bloomed macadamia tree (a) and bagged inflorescence (b) in Kandara, Murang'a County in August 2020.

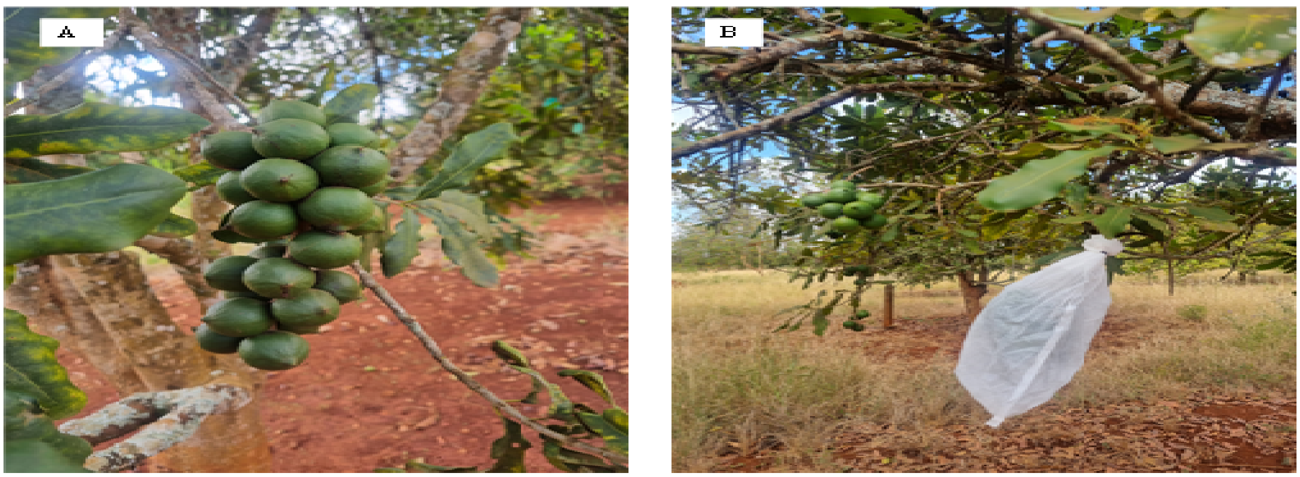

Fig. 2: Mature macadamia nuts (a) and bagged mature nuts before harvest (b) in Kandara, Murang'a County in March, 2021.

Results and Discussion

Nut set

Flower visitors had an effect on macadamia nut set in all three seasons (Table 1). Unbagged flowers had the highest mean ± SE number of nuts set (13.67±1.13) whereas those that were bagged throughout had the least (1.31±0.21). There was a significant difference among the four treatments (unbagged, bagged at night, bagged during the day, and bagged throughout) (Table 1) in the mean ± SE number of nuts set per flower (F=50.14, p value <0.0001). Flowers that were bagged throughout had a significantly lower nut set compared to other pollination treatments (Table 1).

Nut retention

Flower visitors had an effect on macadamia nut retention, with flowers that had unlimited visitation (unbagged throughout) having significantly higher nut retention than those that were bagged at night, bagged during the day and those that were bagged throughout. There was statistical difference among the four groups in mean number of nut retention (F=50.14, p value <0.0001) (Table 2).

Nut-in-shell and kernel yields

Mean nut-in-shell weight differed significantly among the four treatments (unbagged, bagged at night, bagged during the day and bagged throughout) (F=71.89, p<0.0001). There was statistical significance of mean kernel weight among the four treatments (unbagged, bagged at night, bagged during the day and bagged throughout) (F=74.39, p<0.0001) (Table 3).

Nut-in-shell, kernel length and width

There were no statistical differences (P≤0.05) among the four treatments (unbagged, bagged at night, bagged during the day and bagged throughout) in nut-in-shell length and width as well as kernel length and width (Table 4).

Table 1: Mean macadamia nut set 21 days after anthesis in Kandara, Murang'a County in three seasons (August 2020-May 2022)

	Nut set in 3 seasons		
Treatment	Sept 2020	Feb 2021	Oct 2021
Unbagged	14±1.14a	18.08±1.04a	20.25±1.45a
Bagged at night	12.83±0.55b	17.25±1.25b	19.67±1.26b
Bagged during the day	3.5±0.51c	2.42±0.53c	3.17±0.42c
Baggedthroughout	1.25±0.3d	2.08±0.24d	2.17±0.43d
p value	<0.0001	<0.0001	<0.0001

Means ±SE followed by the different letter(s) within the same column are significantly different at P≤0.05.

Table 2: Mean macadamia nut retention before harvest in Kandara, Murang'a County in three seasons (August 2020-May 2022)

	Nut retention in 3 seasons		
Treatment	Sep-20	Feb-21	Oct-21
Unbagged	7.33±0.93a	6.75±0.66a	7.33±0.54a
Bagged at night	6.5±0.72b	6.33±0.57b	6.83±0.71b
Bagged during the day	0.75±0.18c	0.91±0.29c	0.67±0.18c
Baggedthroughout	0.58±0.15d	0.5±0.19d	0.33±0.14d
p value	<0.0001	<0.0001	<0.0001

Means ±SE followed by the different letter(s) within the same column are significantly different at P≤0.05.

Table 3: Mean macadamia nut-in-shell and kernel weight (g) in Kandara, Murang'a County in three seasons (August 2020-May 2022)

	Nut yield (3 seasons)	
Treatment	Nut in shell weight (g)	Kernel weight (g)
Unbagged	68.23 ± 4.03a	21.25 ± 1.24a
Bagged at night	61.50 ± 3.51b	19.52 ± 1.07b
Bagged during the day	6.53 ± 1.05c	2.03 ± 0.34c
Bagged throughout	4.45 ± 0.95d	1.47 ± 0.31d
p value	p<0.0001	p<0.0001

Means ±SE followed by the different letter(s) within the same column are significantly different at P≤0.05.

Table 4: Mean length (mm) and width (mm) of nuts-in-shell and kernels in unbagged, bagged at night, bagged at day and bagged throughout inflorescences in Kandara, Murang'a County in three seasons (August 2020-May 2022)

Treatment	Nut-in-shell length	Nut-in-shell width	Kernel length	Kernel width
Unbagged	23.31a	22.06a	16.32a	12.7a
Bagged at night	23.76a	22.21a	16.25a	13.06a
Bagged during the day	22.75a	21.41a	15.63a	13.84a
Baggedthroughout	23.14a	21.21a	15a	12.14a

Means ±SE followed by the different letter(s) within the same column are significantly different at P≤0.05.

Macadamia pollination is mediated by insect flower visitors and their presence significantly affected the nut set, retention, nut-in-shell and kernel yields. Insect flowervisitors are essential for successful production of macadamia nuts. Nut set happens after the pollen is transferred from the anthers by insect flower visitors that forage macadamia flowers to a receptive stigma which leads to fertilization and development of the nuts. In this study, exclusion of flower visitors through bagging of macadamia racemes resulted in reduced nut set which supports the findings that insect visitors contribute to nut set (Anders *et al.*, 2023; Evans *et al.*, 2021). Flowers that were bagged during the day and the ones bagged throughout, excluding the flower visitors had few nuts setting per raceme. This indicated that pollen transfer happens during the day and is majorly mediated by insect flower visitors. Racemes that were left open with visitors having unlimited access and the ones bagged only at night had higher nut set indicating that flower visitors that foraged during the day contributed largely to nut set, and there were minimal nocturnal pollen transfer activities. Exclusion of flower visitors from macadamia flowers has been demonstrated to result in decreased nut set and yields (Anders *et al.*, 2023, Tavares *et al.*, 2015). Initial nut set is an indicator of possible expected yields if other agronomic aspects as nutrients, weather and pests are well addressed. The findings concur with Olesen *et al.* (2011) and

Trueman (2013) who showed that macadamia pollination is animal mediated and insect pollinators significantly influence nut set.

After nut set, to realize yields, the nuts must be retained on the trees and develop into mature nuts. Successful pollination results in more nuts being retained upto maturity as the ones that are not adequately pollinated drop off (Wallace *et al*., 1996). Flowers that had unlimited insects access (unbagged) and those that were bagged at night had more nuts being retained to maturity. This indicated that they were sufficiently pollinated therefore minimal abscission after nut set (Tavares *et al*., 2015), as insufficient pollen transfer causes poor nut retention. The racemes bagged throughout and those that were bagged during the day, denying insects access resulted to very low nuts being retained to maturity. Previous studies have shown that inadequate pollination can lead to low nut retention in macadamia trees (Penter, 2007, Holwett *et al*., 2015; Holwett *et al*., 2019). On the other hand, when there was adequate pollination, macadamia nut retention was usually high. This was because the fertilized flowers develop into healthy nuts that were able to remain on the tree until they reach maturity (Huett, 2004, *Tavares et al*., 2015). Therefore, it was important for macadamia growers to ensure that their trees are well-pollinated in order to maximize nut retention. This can be achieved by providing suitable habitats for pollinators and by managing the orchard environment to ensure optimal conditions for pollination, such as adequate moisture and temperature levels.

Macadamia flower visitors contributed to increased yield of nuts-in-shell and kernels. Racemes that were unbagged or bagged at night, thus had unlimited access by diverse flower visitors produced more nuts-in-shell and kernels than those that were either bagged during the day or those that were bagged throughout the flowering period. Studies have shown that the yield of macadamia nuts and kernels was positively correlated with the flower visitor species present in the orchard (da Silva Santos *et al*., 2020; Trueman *et al*., 2022). When there were more species visiting the flowers, more pollen was transferred, leading to larger and heavier nuts and kernels (Khalifa *et al*., 2021; Reddy *et al*., 2022). Adequate pollination is necessary for the maximum yield of macadamia nuts and kernels. This was because each flower needs to be pollinated in order to develop into a nut, and the more nuts that are produced, the greater the potential yield. The quality of the nuts and kernels produced was also affected by pollination (Herbert *et al*., 2019). When the flowers were adequately pollinated, the nuts develop evenly, and the kernels were well-filled, leading to better yields (Howlett *et al*., 2015). Inadequate pollination, on the other hand, can result in poor-quality nuts that were small or malformed, or that have low kernel-to-nut ratios.

Studies have shown that when macadamia flowers are adequately pollinated, the resulting nuts tend to be larger and more uniform in size (Evans *et al*., 2021; Grass *et al*., 2018). This was because adequate pollination leads to the fertilization of more ovules within the nut, which can result in the development of larger and more fully-formed kernels.

Conclusions

Insect flower visitors play a critical role in determining the yield of macadamia nuts and kernels through pollination. Adequate pollination is necessary for the optimal macadamia nut yields, and growers should take steps to ensure that their orchards are well-maintained to provide suitable habitats for diverse insect flower visitorsto enhance adequate allogamy.

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