



Developmental biology of Bean Leaf Beetle (*Ootheca mutabilis*) on common bean

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Abstract. Common bean (*Phaseolus vulgaris* L.) is a major source of food and income for people in sub-Saharan Africa. Attack by Bean Leaf Beetles (*Ootheca* spp. Chev.) is one of the major causes of yield loss to the crop, causing up to 48.9% grain yield loss. Despite the damage, there is limited information on the biology, including preferred oviposition sites and development, of *Ootheca* spp. This information provides strategic entry points to potential control interventions. The objective of this study was to investigate the oviposition and development duration of *O. mutabilis*, which is the most abundant and damaging species of *Ootheca* on common bean in Uganda. A greenhouse study was conducted during 2019, using the common bean variety, NABE 16. Parameters monitored included type of oviposition sites, egg cluster size, and morphological changes and development duration for each stage. The beetles preferred to lay clustered eggs than singly, and oviposited in crevices or cover surface, and near plant stalks or roots, which protects the eggs and ensuring the neonates easily find their food. Development of *O. mutabilis* took 120 days with the larval stage lasting the longest; followed by pupae and eggs at 75, 24 and 22 days, respectively. This long biological cycle of the beetle goes beyond that of most common bean varieties of the bush type, implying that juvenile stages of the beetles remain in the soil after bean harvest. As a management strategy, we recommend rotation with non-host crops such as maize in order to break the biological cycle of the beetles, and open tillage to expose the beetles in the soil to extreme weather and natural enemies. Further studies will be needed on the fertility, longevity and diapause mechanisms.

Key words: Beetle larvae, duration, morphology, oviposition, *Phaseolus vulgaris*

Introduction

Common bean (*Phaseolus vulgaris* L.) is a very important legume crop to the people in sub-Saharan Africa (SSA) (Siddiq and Uebersax, 2013; Laroche *et al.*, 2016). Dry common beans are a source of cheap dietary protein and minerals such as iron and zinc for over 100 million people in Africa; thus providing nutrition security (Buruchara *et al.*, 2011; Rodríguez De Luque and Creamer, 2015).

In Uganda, common bean constitutes 45% of the protein intake and provides 25% of the total dietary calorie intake (Kilimo-Trust, 2012). Apart from nutritional security, common bean is a source of income, especially for the rural communities. Unfortunately, the level of production of the crop in Uganda is still very low. National yield statistics are still stagnated in the range of 440 to 600 kg per hectare (Laroche *et al.*, 2016). Elsewhere in SSA, mean productivity reaches 7,100 kg per ha in Mali

(FAO, 2022). Biotic factors, especially insect pests, are among the most devastating constraints to common bean production in SSA (Buruchara *et al.*, 2011; Beebe *et al.*, 2012). Bean Leaf Beetles (BLBs), *Oothea* spp., are some of the major insect pests that attack common bean, leading to yield loss (Halerimana *et al.*, 2021). *Oothea* spp. are endemic in Africa and comprise 13 species, of which *O. mutabilis* Sahlberg, 1829, *O. bennigseni*, and *O. proteus* are known agricultural pests in Africa (Kortenhaus and Wagner, 2010).

Adult beetles cause damage by eating away leaf lamina; whereas subterranean larvae feed on lateral roots and nodules leading to severe plant damage (Buruchara *et al.*, 2010). This damage leads to reduced photosynthetic area and low plant vigour (Paul *et al.*, 2007). Apart from causing physical shoot and root damage on common bean, the beetles mechanically transmit bean common mosaic virus in common bean (Abate and Ampofo, 1996). The resultant loss caused by *Oothea* spp. on common bean is about 76,000 metric tonnes per year in eastern Africa (Wortmann *et al.*, 1998). In Uganda, yield loss due to *Oothea* species is estimated to range from 5.8 to 48.9% depending on level of infestation (Halerimana, 2019).

Despite its dominance and impact on production of common bean, little is known about the biology of *O. mutabilis* regarding preferred oviposition sites and development biology. The biology of *O. mutabilis* was first described by Ochieng (1978) on cowpea in Nigeria, dating back four and half decades and a lot may have changed due to adaptation of organisms to climate change. Similarly, the described life cycle does not explain the erratic occurrence of adult beetles on common bean in Uganda. This hinders development of strategic control interventions, which can leverage from these entry points. Therefore, the objective of this study was to investigate the oviposition and development of *O. mutabilis*, on common bean.

Materials and Methods

Study site

This study was conducted in the screenhouse and insect laboratory at the National Crops Resources Research Institute (NaCRRI), located in Namulonge village, [latitude: 0.53° and longitude: 32.62°, altitude: 1150 meters above sea level (masl)], Busukuma subcounty, Wakiso district in Uganda. This location was selected because of availability of research infrastructure for the study.

In the screenhouse, average daily minimum and maximum air temperature, and relative humidity (RH) were 19.1 and 34.7 °C, and 34.9 and 82.8%, respectively. The minimum, average and maximum soil temperatures were 16.9, 26.8 and 36.6 °C, respectively. The RH in the laboratory ranged from 65 to 80%, and average daily minimum and maximum temperature was 18.6 and 26.3 °C, respectively. All laboratory lighting requirement was limited to natural illumination, through windows at 12:12 hr photoperiod.

Source and sex identification of adult beetles

Adults of *O. mutabilis* were collected from common bean fields in Manibe sub-county [latitude: 3.09° and longitude: 30.98°], in Arua district in north-western Uganda, during the month of April, 2019. This was the time the beetles first appeared during 2019A season. The adults were hand-picked from the plants, placed in ventilated plastic containers and kept in cooler boxes to prevent over heating during transportation.

The collected beetles were provided with bean leaves for food and transported to the laboratory. The beetles were categorized in the laboratory by sex into separate cages. Males of *O. mutabilis* have

double grooves on the margin of last abdominal sternite anterior to the anal opening (Fig. 1) and depression where the endophallus lies (Grobbelaar, 2008).



Figure 1. External structures of *Ootheca mutabilis*: (A and B) dorsal view of adult; (C) ventral view of abdomen of male beetle showing double grooves of last sternite (black arrows) and depression (white arrow); and (D) ventral view of abdomen of female.

Culturing beetles and egg collection

In order to culture the stock of beetles used for subsequent biology studies, 150 females from the field collection, were placed into three rearing cages measuring 50 cm x 50 cm x 60 cm. The cages were made of frames of plastic pipes and Nylon cloth ('Organza') with one side stitched with a zip-lock to allow opening. Each cage had ten pots comprising a 750 ml capacity of disposable plastic cup with two common bean plants (var. NABE 16) at V3 stage (i.e., with three trifoliolate leaves). The bean plants were grown in a soil potting mixture of black soil: sand: manure at 3:1:1 ratio. The beetles freely roamed in the cage and laid at different oviposition sites and pots of their choices.

In order to avoid contamination with soil and soil-borne pathogens, eggs used for larval development were collected in oviposition containers in the laboratory. Sixty mating pairs from the field collections were enclosed in 20 oviposition cylindrical plastic containers similar to those used by Costa *et al.*, (2014), but plugged with a plant shoot on one end. Bean shoots to provide food for the beetles, were plugged in the drilled hole using cotton. The shoot was changed after two days or whenever the leaves were all eaten by the beetles (Khan *et al.*, 2009). Moistened filter paper was placed on the lid of an inverted top container to prevent desiccation of eggs laid by beetles.

Determining egg clustering and preference to oviposition

In order to determine the oviposition site preferences and their influence on egg cluster sizes, the beetles in the cages were allowed five days to roam inside the cages. After this period, three pots were selected at random from each cage and examined for presence of eggs, site of oviposition, number of eggs laid and size of clusters. A cluster included two or more eggs were in physical contact (Paul *et al.*, 2015). The oviposition site was recorded as either '1 = plant stalk and protruding root', '2 = bare soil surface', '3 = covered soil surface and crevices', or '4 = underside of fallen debris'. The debris were dry leaves of common bean that fell off the plant to the soil surface in pots. For easy handling, the clustered eggs were separated using distilled water. A soft camel hair brush was used to dislodge eggs for easy counting (Minja *et al.*, 2003).

Egg development duration and morphology

Egg development, which marked the time from oviposition to end of hatching, was studied in the greenhouse starting with eggs laid by beetles in the cages. Four pots, from the cages above, with egg batches consisting of 20 to 50 eggs, were incubated in a greenhouse. The eggs were checked daily until all eggs hatched or no new larvae emerged for five consecutive days (Khan *et al.*, 2009).

To collect data on egg morphology, a sample of eggs were picked to determine egg diameter and to record colour changes. The eggs were measured under dissecting microscope (Optika SZM-1LED®) fitted with a graduated eye piece (Grobbelaar, 2008).

Larval and pupal development

The development of larvae and pupae of *O. mutabilis* was studied on potted common bean plants, under greenhouse conditions. During infestation, a total of 1,280 neonates were introduced at the base of the plant. A total of 1,280 neonates were introduced at the base of the plants, grown in 64 pots (20 larvae per pot). Each pot contained two common bean plants at V2 stage (Minja *et al.*, 2003). In order to ensure proper growth of potted bean plants and development of larvae, the infested pots were watered regularly with tap water to keep the soil moist but avoided waterlogging. The pots were perforated at the bottom to drain excess water. A nylon cloth was laid at the bottom of the pots to prevent the larvae from escaping through the drainage holes.

In order to obtain data on the age of larvae and pupae, four pots were randomly sampled every week (Ampofo *et al.* 2001). The sampled pots were emptied on black polyethene mat and checked for surviving and any newly formed life stages (larvae, pupae or adults), according to Minja *et al.* (2003). Sampling was repeated weekly until only adults were recovered, corresponding of 16 weeks after infestation.

In order to determine the instar stages, head capsule widths (HCW) of the larvae recovered during weekly sampling were measured using a graticule in a stereo microscope (Optika SZM-1LED®). Head capsule is a keratinized structure and exhibits a linear relationship between discrete instar stages during larval growth and development (Joda, 2019).

The HCW was the ultimate parameter used to determine larval instars, following the Brooks-Dyar's rule (Floater, 1996). The rule describes HCWs in successive stages as a regular geometric progression for the discrete increase in size of insect. The larvae from sampled pots were immobilized by immersing them in 70% (v/v) (ethanol/water), which also maintained the bodies flexible enough to be measured (Riedell *et al.*, 2011). In addition to HCW, body lengths of larvae were also determined.

Adult longevity

To determine adult longevity, 16 apparently healthy (without any visible physical injuries) emerged beetles (ten females and six males) recovered from the larval and pupal development experiments described earlier were used. The beetles were fed on freshly harvested bean leaves, which were changed every two days (Arionget, 2012). To obtain data on longevity, the number of days each adult lived was recorded.

Data analysis

Chi-square goodness of fit was performed for egg cluster size parameters for the four sites. The null hypothesis tested was that; the same number of eggs and clusters would be found at each of the four oviposition sites examined. To determine the number of larval instars, a histogram was constructed to visually inspect frequency peaks for the HCW. The corresponding instars were, thereafter, determined from the frequency distribution (Riedell *et al.*, 2011).

Later, a linear regression model was fitted for the natural log of HCW ($\ln Y$) and larval instars as final check to verify the instars (Floater, 1996). It was assumed that during development, increase in size of highly sclerotized body parts such as HCW of larvae, is predictable and regular by a relatively constant factor, 'b' (Floater, 1996). Thus, the linear equation is, *viz*:

$$\ln Y = \alpha + bX$$

Where: Y is the HCW; X is number of instars; and a and b are constants.

The instars were correctly determined if there was no significant deviation from the straight regression line. After delineating larval instars, their means and standard errors for HCW were calculated; followed by Analysis of Variance (ANOVA) using GenStat version 14 statistical package (VSN International, 2011). Means generated were separated using the Tukey's Honest Significant Difference (HSD) to verify significant differences at $p < 0.05$ level of significance.

Results

Egg clustering and oviposition sites preference

The adult females laid a total of 1,043 eggs, of which 3.5% (37) were singles and 96.5% (1,006) were laid in clusters. The number of clustered eggs was significantly different ($p < 0.01$) at different oviposition sites. Females laid most of the eggs on covered surfaces and crevices; followed by plant stalk and protruding roots (Fig. 2, see magnified inserts), bare soil surface, and underside of fallen debris or dry leaves (Table 1). In terms of size of egg clusters, the largest were laid in covered surfaces; followed by plant stalks; while smallest clusters were laid on the bare soil surfaces.

Egg morphology

Changes were observed in egg morphology from the time the eggs were laid (Fig. 2) to time of hatching. Freshly laid eggs were oval, translucent and golden yellow; but became spherical and transparent towards hatching. The eggs changed from golden yellow to pale brown or tan. The shell also became transparent and the head and anal plate were visible a day before hatching. At this stage, larvae could be seen wriggling inside the shell.

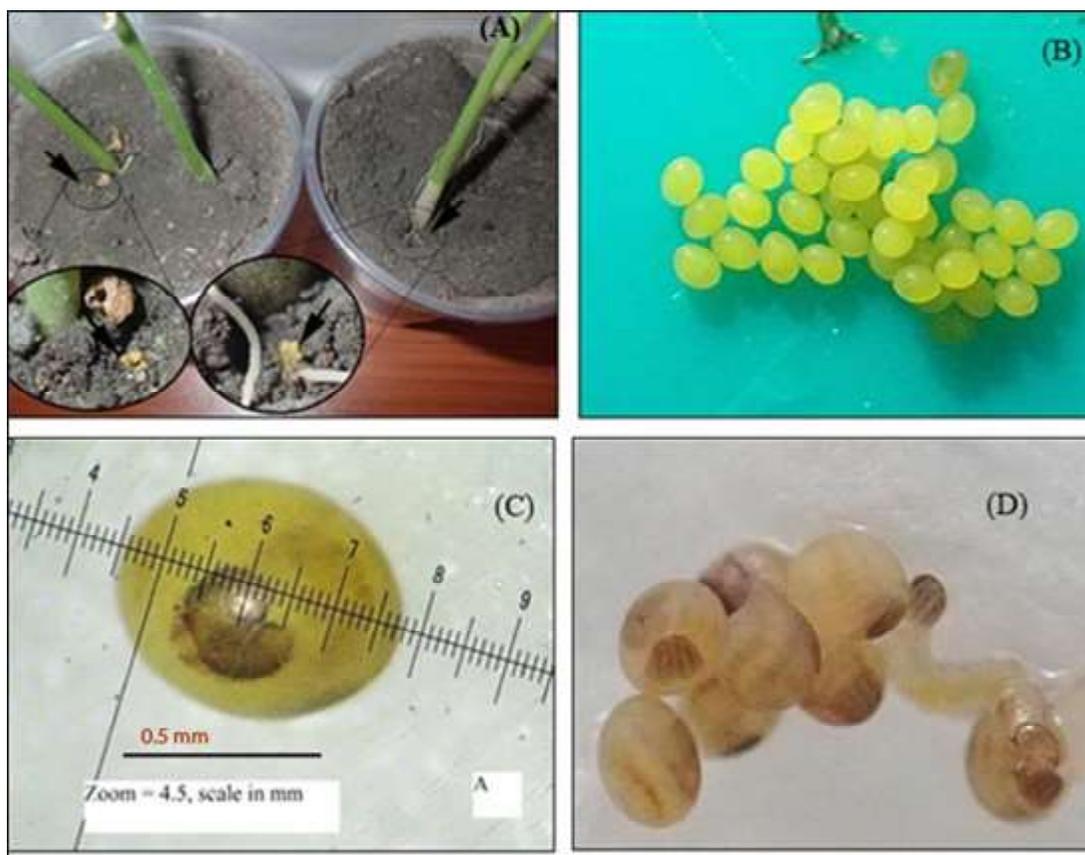


Figure 2. Oviposition site and morphology of eggs of *Ootheca mutabilis*: A – oviposition near plant stem and protruding roots to the surface (inserts show magnified images) on potted plants in screenhouse; B – Freshly laid eggs in cluster as seen on surface of oviposition chamber in laboratory; C – Magnified transparent egg with visible jaws and head of larva as seen under microscope; and D – Cluster of eggs about to hatch.

Table 1. Pearson’s Chi-square goodness of fit for total eggs laid in clusters and their size at four different sites after five days of oviposition by females of *Ootheca mutabilis* in the screenhouse

Oviposition sites	Total number of eggs			Mean cluster size		
	Observed	Residual	Expected	Observed	Residual	Expected
Plant stalk and protruding roots	308	3.56	251.5	17.0	1.25	12.6
Bare soil surface	233	-1.17	251.5	4.8	-2.19	12.6
Covered soil surface and crevices	319	4.26	251.5	21.2	2.43	12.6
Underside of fallen debris	146	-6.65	251.5	7.4	-1.47	12.6
Total	1,006	0	1,006	50.4	0	50.4
Degrees of freedom		3			3	
χ^2 values		76.43 ***			14.44**	

Figures followed by (***) and (**) are significant at 99% and 95% confidence interval, respectively

The average size of the longest and shortest diameter of newly laid eggs was 0.65 ± 0.007 mm (range: 0.60 to 0.73) and 0.45 ± 0.012 mm (range: 0.40 to 0.67 mm), respectively.

Number of larval instars

The frequency distribution of HCW for the larvae revealed three distinct clusters (Fig. 3), signifying three instars. Head capsule width significantly differed ($p < 0.05$) among the three larval instars. The mean HCW for first, second and third instar larvae of *O. mutabilis* were 0.27, 0.39 and 0.59 mm, respectively. On verification of the number of instars, regression analysis for \ln (HCW) and the corresponding instars revealed a near perfect straight line (Fig. 3B), with an estimated equation:

$$\ln Y = 0.387X - 1.694; R^2 = 0.968.$$

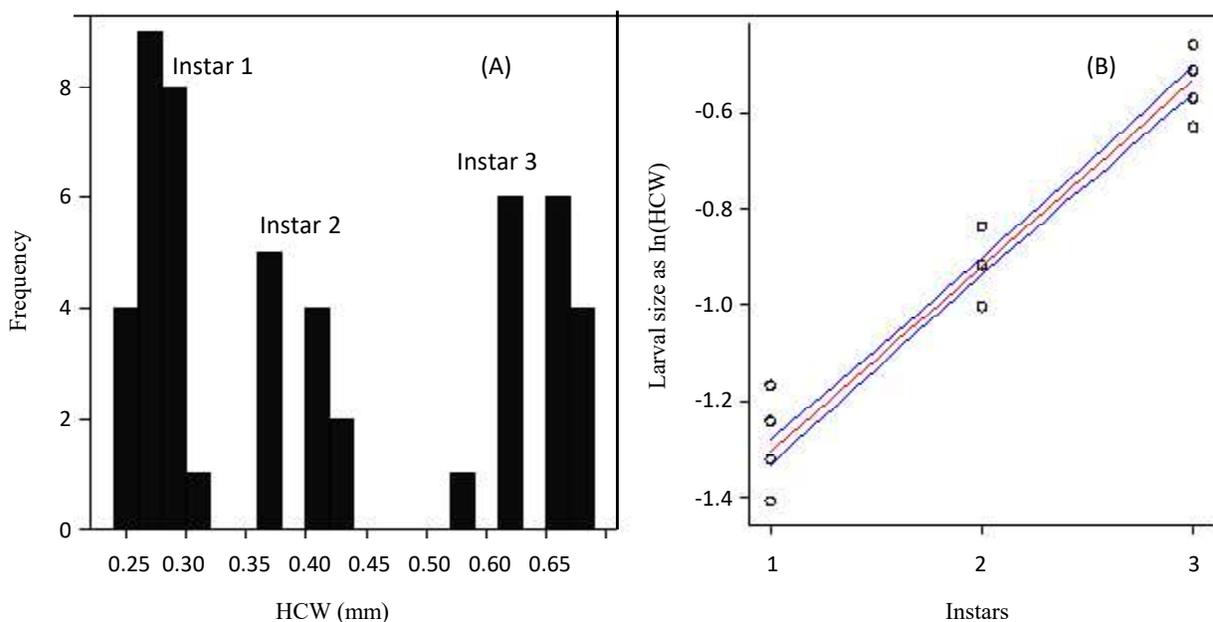


Figure 3. Frequency distribution of head capsule diameter (mm) of *Ootheca mutabilis* larvae reared in the greenhouse: A) showing three peaks representing three instars; and B) Regression line for natural log of head capsule width showing near-perfect straight line verifying the three larval instars.

Larval and pupal morphology

The morphology of the larvae and pupae of *O. mutabilis* are presented in Figure 4. Day-old larvae were tan, and changed to white towards their first moult. The mature second and third instar larvae were pale yellow to cream, with pale to dark brown head and anal plate (last tergite at tail region). The head and anal plate were sclerotized, and usually of the same colour.

Larvae had six thoracic legs (prolegs) and a single clasper just below the anal plate (Fig. 4C), used for movement. When fully mature, third instars underwent reduced feeding and movement in preparation for pupation. At this stage, third instar larvae compressed their body length and formed a C-shape, curving at the region between the abdomen and thorax (Fig. 4E). This stage is herein referred to as pre-pupa. The pre-pupae and pupae were enclosed in soil moulded into earthen cells.

The body sizes of the life stages of *O. mutabilis* are presented in Table 2. Second instar larvae are three times the length of the first instar larvae. Third instars were 1.5 times longer than second instars. The body for adult females was longer than for males, but their length did not differ significantly.

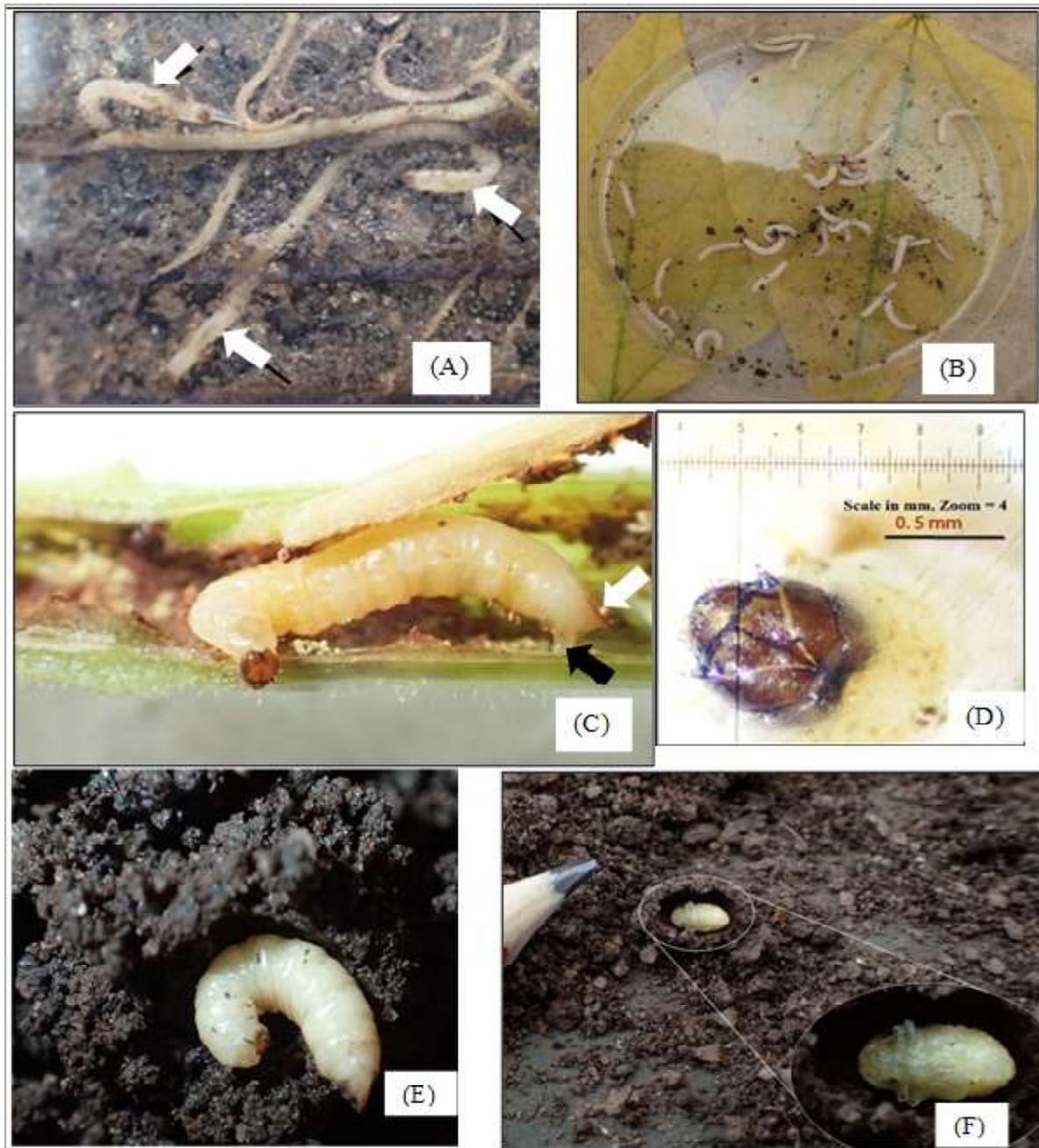


Figure 4. Morphology of larvae and pupae of *Ootheca mutabilis* reared in the screenhouse: A) Larvae feeding on root of common bean plant visible in transparent pot (white arrows); B) Larvae recovered from soil; C) external parts of third instar larvae showing anal clasper (black arrow) and plate (white arrow); D) keratinized HCW under microscope at magnification (x40); E) the C-shaped pre-pupa forming in earthen cell; and F) pupa formed in earthen cell.

Development duration of life stages

The development of the beetles from eggs to adult occurred in the soil. Development duration from eggs to adults took approximately 120 days; egg incubation, larval, and pupal stages lasted for 22, 75, and 24 days, respectively (Fig. 5). The first, second and third instar, and pre-pupa stages lasted for 22, 18 and 24, and 10 days, respectively (Table 3). Immediately after emergence, the neonates migrated to the root zone and started feeding on the root hairs.

Table 2. Body sizes of life stages of *Ootheca mutabilis* reared on common bean under screenhouse conditions

Life stage	Mean ± SEM	Range
Total Length (mm) ^w		
First instar	1.7 ± 0.02	1.6–1.9
Second instar	5.5 ± 0.23	4.1–6.8
Third instar	8.4 ± 0.44	5.8–12
Pupae	5.5 ± 0.07	5.4–5.6
Female adult	6.8 ± 0.29	6.0–7.3
Male adult	6.3 ± 0.04	6.2–6.3
Pronotum width (mm) ^ø		
Pupae	1.8 ± 0.15	1.5–2.0
Female adult	3.3 ± 0.05	3.2–3.4
Male adult	2.9 ± 0.00	2.9–2.9

^w Total length of adult females and males was measured from mandible to posterior tergum

^ø pronotum width was measured as the dorsally-lateral diameter of the pronotum of adult female and males. Pupae pronotum width represents the longest diameter at thorax region

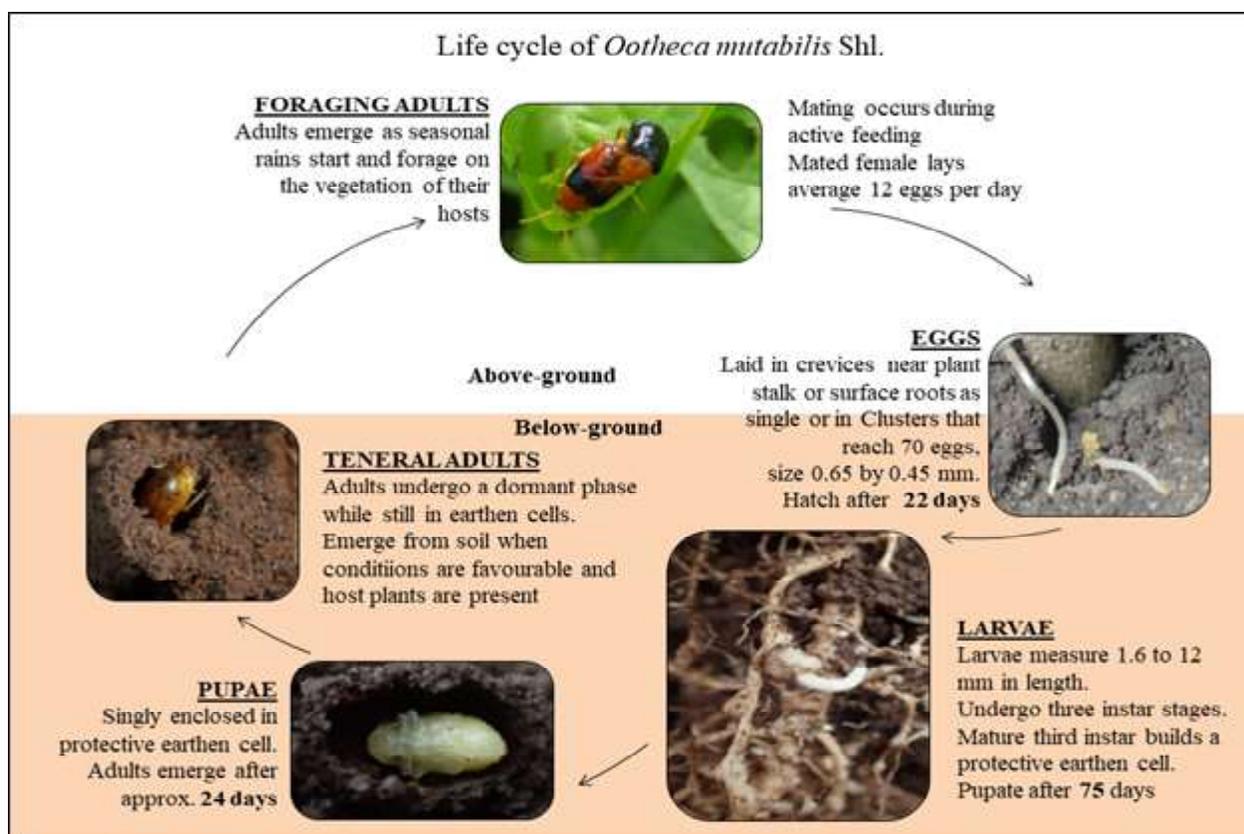


Figure 5. An info graphic for the depicted summary of life cycle of *Ootheca mutabilis* as observed under screenhouse conditions.

Table 3. Duration of development of life stages of *Ootheca mutabilis* under screenhouse conditions

Development phase*	Mean (days) ± SEM	Range (days)
Egg to first instar	21.6 ± 1.03	19–25
First to second instar	22.2 ± 2.15	14–28
First to third instar	40.6 ± 2.52	21–63
First instar to pre-pupa	64.8 ± 2.56	56–77
First instar to pupa	75.0 ± 2.00	70–84
First instar to adult	98.5 ± 2.26	84–112

*All development durations for larvae, pupae and adults start at larval neonate as reference stage

Days lived by adults

Overall, the mean number of days lived by the *O. mutabilis* adults recovered from screenhouse experiments was 27.3 days. Generally, females (33.7 days) lived significantly ($t_{(d.f = 14)} = -3.57, p < 0.05$) longer than their male (17.5 days) counterparts. The range of days lived by male and female beetles were 10 to 21 and 11 to 47 days, respectively. The females from the screenhouse experiment did not live to lay any eggs, even after being reared in the same way as adults collected from the field.

Discussion

Egg clustering and oviposition site preference

This study has provided findings on the *ex-situ* developmental biology of *O. mutabilis*, with emphasis on the oviposition and morphological changes; and development duration on common bean. Females preferred laying eggs in clusters as a strategy to promote survival. Beetles lay eggs in larger clusters whenever predator threats are anticipated (Paul *et al.*, 2015). In addition, eggs laid and glued together in clusters reduce the chance of individuals being searched and eaten by predators; in what is called the “*random search theory*”, emphasised by Agarwala and Dixon (1993). Solitary predators can only eat a few, thus the greatest proportion of eggs would survive the attack (Paul *et al.*, 2015). However, the actual threat towards predation was not assessed in this study; therefore, further studies are required. Furthermore, females laid eggs nearest to the plant or covered spaces and crevices, as another strategy to promote survival. Oviposition sites covered by debris and crevices protect eggs from natural enemies, such as predators and parasitoids; and harsh environmental conditions such solar radiation and excessive heat that would otherwise desiccate them or lower fitness of mother and offspring (Fatouros *et al.*, 2020). Laying eggs near the plant stalk and roots also ensures that emerged neonates quickly find food.

Egg morphology and role of oviposition site

The shape and size of the eggs of *O. mutabilis* is similar to what was earlier reported appearing in elliptical and translucent yellow (Kortenhaus and Wagner, 2010). The eggs were barely a millimetre in size, thus are indistinguishable from soil particles without magnification. This, together with hidden oviposition sites, help the eggs to avoid the searching predators and parasitoids (Fatouros *et al.*, 2020). However, the impact of this cryptic nature in protecting *O. mutabilis* eggs needs further investigations in real life natural enemy simulations.

Development duration of life stages

The eggs hatched after 22 days, a duration that is quite longer than that on cowpea as reported by Ochieng (1978). Longer duration can be an advantage during management because it provides ample time to prepare for control of larvae, after the first sight of ovipositing adults in the field. Although this study focused on common bean, there are no known studies to suggest the direct effects of host on rate of egg hatching for herbivorous insects. Instead, the rate of egg development has a positive relationship with temperature; whereby an increase in temperature influences the enzyme kinetics leading to shorter development time and thus higher rate of development (Régnière *et al.*, 2012). However, the rate of increase is not infinite beyond maximum threshold temperatures that impend the survival of the insect's life stage (Khan *et al.*, 2009).

Larval development lasted 75 days, whereby the third instar's duration was the longest compared with duration of other instars (Table 3), making them the most damaging (Fig. 6). This stage was characterised with vigorous feeding on the root system that caused yellowing of potted bean plants at the density of more than ten mature larvae per pot (Fig. 6). This observation is similar to that of Minja *et al.* (2003) who observed that the magnitude of damage on root system by *Ootheca* larvae increased with larval population density.



Figure 6. Beans affected by *Ootheca mutabilis* larvae: A) Plants with yellowed leaves grown with third instars predominant in the pots; and B) ground stem of the bean plant damaged (white arrow) by the beetle larvae (black arrows).

The voracious feeding of third instars also allows for accumulation of enough food reserves to sustain the pre-pupal and pupal stages. The third instar of *O. mutabilis* increased in size up to five times that of first instar. Larvae feeding and growth should highlight the need to pay attention to their damage during control, though hitherto ignored.

The total development time of *O. mutabilis* from egg to adult lasted 120 days, which was longer than the growth duration of common bean for the bush type at (65 to 75 days) (MAAIF, 2019). This suggests that a single life cycle of *O. mutabilis* cannot be completed in the presence of the same bean crop. The evidence from this study also indicates the possibility of only one generation of *O. mutabilis*

for an entire common bean growth cycle in the field. The crop will have been harvested; hence leaving the juvenile life stages (larvae and pupae) in the soil. This suggests that non-host crops such as maize (*Zea mays* L.) should be rotated with common bean in order to break the biological cycle of the beetles (Ampofo and Massomo, 1998). *Oothea mutabilis* is also known to feed on other crops in the Leguminosae family such as cowpea (*Vigna unguiculata* L.) and soybean (*Glycine max* L.) (Lutaakome et al., 2022; Olaboro et al., 2024); thus, these crops should not be rotated with common bean.

The adults recovered and reared in the greenhouse barely fed and females did not lay eggs during the time they leaved. This may also explain the reason for diapause observed in *Oothea* species, where the teneral beetles remained in the soil until the next host crop emerges (Ampofo et al., 2005). The development duration also contrasts with that reported by Ochieng (1978), where *O. mutabilis* completed development 20 days earlier on cowpea in Nigeria. The discrepancies may be due to differences in the hosts, pest biotype and environmental conditions in the respective studies. Different hosts tend to provide different qualities of nutrients; which affect the insect's growth and development (Gou et al., 2020); and this was the possible cause of the discrepancies.

The adults from the greenhouse-reared colony did not lay eggs; compared with those collected from the field that laid eggs. The newly emerged *Oothea* adults, undergo obligatory state of diapause characterised with immature ovaries (Ochieng, 1978; Ampofo et al., 2005). At this stage, the morphologically mature adult beetles undergo a state of physiological dormancy in the soil; while waiting for the next seasonal rains (Lutaakome et al., 2022). However, the initial signalling stage, and cues for breaking of dormancy are still not known. Diapause is common to other species from the *Oothea* genus, such as *O. bennigseni*, where adults emerge naturally from soil at the onset of rains during March and April, coinciding well with breaking of dormancy (Ampofo et al., 2005). Since the adults in this study were directly recovered from soil, this calls for further investigations into the life history and other life table parameters to further understand how the beetles overcome diapause.

Conclusion

This study has revealed that females of *O. mutabilis* beetles lay clustered eggs hidden in crevices near plant roots, which ensures that emerged larvae quickly find food; thus, enhancing survival. The development biology of the beetle occurs entirely in the soil near the host's root zone. The larvae's feeding actions lead to yellowing of the leaves of the plants. Also, since older larvae remain in the soil at the end of growing cycle of common bean, these act as a source of infestation for the next host crop that is planted in the same or nearby field. The information on development biology and morphological characteristics of life stages can be relied on during scouting for *Oothea* spp. to identify infestation in the field and designing appropriate control.

Recommendation

Recommended management practices may include tillage practices that expose the larvae, pupae and teneral adults to natural factors such as sun rays and natural enemies. Other practices such as crop rotation with non-host plants can disrupt the development and survival of the beetles hence controlling the population. Farmers should avoid rotating common bean with similar hosts such as those from the Leguminosae family. Since this study could not conclude on the longevity and reproductive potential of *O. mutabilis* needed for population predictions, further investigations are needed to know number of eggs are laid per female and for what duration.

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References

- Abate, T., and Ampofo, J. K. O., 1996. Insect pests of beans in Africa: Ecology and management. *Annual Review of Entomology* 41(1):45–73. <https://doi.org/10.1146/annurev.en.41.010196.000401>
- Agarwala, B. K. and Dixon, A. F. G., 1993. Why do Ladybirds lay eggs in clusters? *Functional Ecology*, 7(5):541–548. <https://doi.org/https://www.jstor.org/stable/2390130>
- Ampofo, J. K. O., Hallenwenger, U., Massomo, S. M. and Ulicky, E., 2005. Participatory IPM development and extension: the case of bean foliage beetles in Hai, Northern Tanzania. PABRA Millennium Workshop, pp. 87–95.
- Ampofo, J. K. O. and Massomo, S. M., 1998. Host effects on bean foliage beetle (Coleoptera: Chrysomelidae) emergence pattern in northern Tanzania. In: SADC Regional Programme on Beans in Southern Africa pp. 109–110. CIA. <https://naldc.nal.usda.gov/nal/dc/download.xhtml?id=IND23288569andcontent=PDF>
- Ampofo, J. K. O., Mziray, H. and Minja, E., 2001. Make available more options for managing soil productivity and bean pests pp. 256–257. Centro Internacional de Agricultura Tropical. Cali, Colombia. https://doi.org/http://ciat-library.ciat.cgiar.org/articulos_ciat/bean_entomology
- Arionget, F. 2012. Observation of some aspects of the biology of bean leaf beetle (*Ootheca* spp.). College of Agricultural and Environmental Science, Makerere University. Special research project. pp. 28
- Beebe, S., Rao, I. M., Mukankusi, C. and Buruchara, R., 2012. Improving resource use efficiency and reducing risk of common bean production in Africa, Latin America and the Caribbean. In C. H. Hershey (Ed.), *Eco-Efficiency: from vision to reality*. pp. 1–18. Centro Internacional de Agricultura Tropical (CIAT). <http://hdl.handle.net/10568/54656>
- Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J. C., Muthoni, R. and Abang, M., 2011. Development and delivery of bean varieties in Africa: the Pan-Africa Bean Research Alliance (PABRA) model. *African Crop Science Journal* 19(4):227–245.
- Buruchara, R., Mukankusi, C. and Ampofo, J. K. O., 2010. Bean disease and pest: Handbooks for small-scale seed producers (R. Buruchara, C. Mukankusi, and K. Ampofo (eds.); 4th ed.). Centro Internacional de Agricultura Tropical (CIAT). Pp. 78.
- Costa, E. N., Ribeiro, Z. A., de Souza, B. H. S. and Boiça Júnior, A. L., 2014. Oviposition preference assessment of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) for different soybean genotypes. *International Journal of Pest Management* 60(1):52–58. <https://doi.org/10.1080/09670874.2014.900586>
- Fatouros, N. E., Cusumano, A., Bin, F., Polaszek, A. and Van Lenteren, J. C., 2020. How to escape from insect egg parasitoids: A review of potential factors explaining parasitoid absence across the Insecta. *Proceedings of the Royal Society B: Biological Sciences*, 287(1931). <https://doi.org/10.1098/rspb.2020.0344>
- Floater, G. J., 1996. The Brooks-Dyar rule and morphometrics of the processionary caterpillar. *Australian Journal of Entomology*, 35, pp. 271–278. <https://doi.org/10.1111/j.1440-6055.1996.tb01402.x>
- Food and Agriculture Organization (FAO), 2022. FAOSTAT data. <https://www.fao.org/faostat/en/#data>. Last accessed on 20th March, 2023

- Gou, Y., Quandahor, P., Zhang, Y., Coulter, J. A. and Liu, C., 2020. Host plant nutrient contents influence nutrient contents in *Bradysia cellarum* and *Bradysia impatiens*. *PLoS ONE* 15(4):1–13. <https://doi.org/10.1371/journal.pone.0226471>
- Grobbelaar, E., 2008. On the identity of *Ootheca bennigseni* Weise, *O. mutabilis* (Schönherr) and *O. meridiana* sp. n. (Chrysomelidae: Galerucinae), bean and cowpea pests in the Afrotropical Region. *African Entomology* 16(1):7–22. <https://doi.org/10.4001/1021-3589-16.1.7>
- Halerimana, C., 2019. Distribution of bean leaf beetles and associated yield losses in Uganda. Department of crops and Horticultural production, Makerere University. Msc. thesis, pp. 76.
- Halerimana, C., Kyamanywa, S., Olaboro, S., Paparu, P., Nkalubo, S. T., Colvin, J., Cheke, R. A., Wagner, T., Seal, S. E., Kriticos, D. J., and Otim, M. H., 2021. Distribution and relative abundance of bean leaf beetles (*Ootheca* spp.) (Insecta: coleoptera: Chrysomelidae) in Uganda. *Insects* 12:1048. <https://doi.org/10.3390/insects12111048>
- Joda, A. O. J., 2019. Developmental biology of *Aspavia armigera* (Fabricius, 1775) (Hemiptera: Pentatomidae) on rice (*Oryza sativa* L.) (Poaceae) and three other hosts in Nigeria. *Polish Journal of Entomology* 88(4):349–361. <https://doi.org/10.2478/pjen-2019-0023>
- Khan, M., Gregg, P. and Mensah, R., 2009. Effect of temperature on the biology of *Creontiades dilutus* (Stål) (Heteroptera: Miridae). *Australian Journal of Entomology* 48:210–216. <https://doi.org/10.1111/j.1440-6055.2009.00705.x>
- Kilimo-Trust., 2012. Development of Inclusive Markets in Agriculture and Trade (DIMAT): The nature and markets of bean value chains in Uganda. pp. 56. http://www.undp.org/content/dam/uganda/docs/UNDPUG_PovRed_Value Chain Analysis Report Honey 2013 Report.pdf
- Kortenhaus, S. and Wagner, T., 2010. Revision of *Ootheca* Chevrolat, 1837 from tropical Africa—redescriptions, descriptions of new species and identification key (Coleoptera: Chrysomelidae, Galerucinae). *Zootaxa*, 2659, pp. 1–52.
- Larochelle, C., Katungi, E. and Cheng, Z., 2016. Household consumption and demand for bean in Uganda: Determinants and implications for nutrition security. In: proceedings of the 5th International Conference of the African Association of Agricultural Economists, September 23–26, 2016, Addis Ababa, Ethiopia.
- Lutaakome, M., Kyamanywa, S., Paparu, P., Olaboro, S., Halerimana, C., Nkalubo, S. T. and Otim, M. H., 2022. Host and seasonal effects on the abundance of bean leaf beetles (*Ootheca* spp.) (Coleoptera: Chrysomelidae) in Northern Uganda. *Insects*, 13(9). <https://doi.org/10.3390/insects13090848>
- Ministry of Agriculture Animal Industry and Fisheries (MAAIF)., 2019. Beans training manual for extension workers in Uganda (pp. 1–81). MAAIF, Entebbe, Uganda. <https://www.agriculture.go.ug/wp-content/uploads/2019/09/Beans-training-manual-for-extension-workers-in-Uganda.pdf>, accessed on 26th February 2025
- Minja, E., Ampofo, J. K. O. and Mziray, H., 2003. Verification of farmers' indigenous knowledge on the influence of *Ootheca* larval feeding on bean root development in Northern Tanzania. In: Integrated Pest and Disease Management in major Agroecosystems. Project PE-1. Annual Report 2003. CIAT. https://doi.org/http://ciat-library.ciat.cgiar.org/articulos_ciat/report2003/ipm_2003_05.pdf (first accessed: 30th January 2018)
- Ochieng, R. S., 1978. Studies on the bionomics of cowpea leaf beetle, *Ootheca mutabilis*. In: S. R. Singh, H. F. Van Emden, and T. A. Taylor (Eds.), *Pests of grain legumes: Ecology and control*. pp. 187–191. Academic press, London, United Kingdom.
- Olaboro, S., Kyamanywa, S., Lutaakome, M., Paparu, P., Halerimana, C., Nkalubo, S. T. and Otim, M. H., 2024. Feeding preferences of the bean leaf beetle (*Ootheca* spp.) (Coleoptera: Chrysomelidae): Insights for targeted pest control strategies in Uganda. *Insects* 15(7): 516. <https://doi.org/10.3390/insects15070516>

- Paul, S. C., Pell, J. K. and Blount, J. D., 2015. Reproduction in risky environments: the role of invasive egg predators in Ladybird laying strategies. *PLoS ONE* 10(10): 1–14. <https://doi.org/10.1371/journal.pone.0139404>
- Paul, U. V, Ampofo, J. K. O., Hilbeck, A. and Edwards, P., 2007. Evaluation of organic control methods of the bean beetle, *Oothea bennigseni*, in East Africa. *New Zealand Plant Protection* 60:189–198.
- Régnière, J., Powell, J., Bentz, B. and Nealis, V., 2012. Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modelling. *Journal of Insect Physiology* 58(5):634–647. <https://doi.org/10.1016/j.jinsphys.2012.01.010>
- Riedell, W. E., Osborne, S. L., Lundgren, J. G. and Pikul, J. L., 2011. Nitrogen fertilizer management effects on soybean nitrogen components and bean leaf beetle populations. *Agronomy Journal* 103(5):1432–1440. <https://doi.org/10.2134/agronj2011.0113>
- Rodríguez De Luque, J. J. and Creamer, B., 2015. Major constraints and trends for common bean production and commercialization; establishing priorities for future research. *Agronomía Colombiana*, 32(3), pp. 423–431. <https://doi.org/10.15446/agron.colom.b.v32.n3.46052>
- Siddiq, M. and Uebersax, M. A., 2013. Dry beans and production, processing and nutrition - An overview. In: M. Siddiq and M. A. Uebersax (Eds.), *Dry beans and production, Processing and Nutrition*. pp. 3–22. John Wiley and Sons, Inc. Ames, Iowa, USA.
- VSN International., 2011. *GenStat for Windows Release 14 Reference (12th edn.)*. Hemel Hempstead, UK. <http://www.genstat.co.uk/>
- Wortmann, C. S., Kirkby, R. A., Eledu, C. A. and Allen, D. J., 1998. Atlas of common bean (*Phaseolus vulgaris*) production in Africa. In: International centre for Tropical Agriculture. (CIAT publication No. 297) Media series 600.