Interactions of undescribed *Drilus* beetle larvae with pestiferous *Limicolaria flammae* (Gastropoda: Achatinidae): are there prospects for biocontrol?

Abstract

The garden snail, *Limicolaria flammae* is a seasonal pest of several horticultural crops in most of the agroecological areas in Nigeria. The level of damage to crops is especially enormous in the South because of its ability to feed on different crops and the timing of abundance, the early rain season, which usually coincide with onset of rain-fed crop production thereby expose seedlings and grown-up crop plants to serious damage. In this study, we recorded occurrence data of *Limicolaria flammae* in okra, carrot, sweet potato, lettuce and maize within a single multi-crop organic farm in order to understand its food preference. Activities of the predatory beetle larvae, *Drilus* sp was observed within the environment. The appearance of the larvae was described photographically and its interaction with *L. flammae* was studied to generate some data on the proportions of snail mortality caused by *Drilus* sp and other mortality factors that naturally regulate the population dynamics of *L. flammae* within the environment. There was no statistically significant difference in the average number of snails recorded in the different cropping fields [F (4, 15) =1.369, P=0.291].. Activities of *Drilus* larvae against *L.* flammae was higher in okra and carrot plots and snail mortality due to the larvae attack were significantly higher compared to what was recorded in other crop plots [F (4, 30) =3.998, P=0.01].The *Drilus* larvae predate on *L. flammae* by appertural entry, biting through the soft tissues around the mantle to gain entrance and moult into instar larvae within the snail shell in 15-20 days, leaving its exuviae behind in the shell in all the specimens observed (n=40). The *Drilus* species being reported in this study is morphologically distinct from other described species in available literature. The study suggested that the *Drilus* species is less known and there are potentials that the species could be useful in inundative biocontrol approaches for the management of pestiferous gastropod populations.

Key words: inundative biocontrol, predatory larvae, mortality, gastropod, populations

INTRODUCTION

The garden snail, *L. flammae* occurr in uncultivated plots, farms, home gardens, lawns and road sides as herbivores, especially during wet seasons in Nigeria. Although there is no authoritative data on the distribution and abundance of *L. flammae* in different agroecological areas in Nigeria, there are reports on the presence of the species and their potentials to cause economic damage on important plants (Oke and Chokor, 2009). *Limicolaria flammae* attain pest status when the environment is modified for agriculture (Awodiran and Ogunjobi, 2016) and they appear to be polyphagous, showing no feeding discrimination for specific types of crops. There is evidence that the spread of *Limicolaria* is potentially damaging to horticulture and crop production (Slug and Pradesh, 2017; Justin et al., 2019). Incidentally, early annual rainfall in Southern part of Nigeria, usually between February and March and commencement of rain-fed cropping activities often coincide with the period of abundance of *Limicolaria* species (Tyouwua, 2017), hence there is invasion and in synergy, these conditions are suspected to fascinate availability of food and increase in their populations.

Feeding activities of land snails in general can be responsible for huge loss on horticultural and ornamental crops in different agroecological regions in Nigeria. Apart from direct feeding on foliage, presence of faecal contaminants and slime trails on leafy vegetables reduce market value. Despite the awareness of the potential economic loss incurable from *L. flammae* invasion of cultivated lands, studies on sustainable prevention and control have not received sufficient attention. There is lack of information on the ecology and interaction of *L. flammae* with its natural enemies that sustain some sorts of ecological balance, which could be exploited in developing sustainable biocontrol component of an integrated management approach against the pestiferous gastropod community. Natural enemies of *L. flammae* are known to include mammals, birds and arthropods (Baker, 2004). Collection of wild snails by humans for food and many cultural practices in cropping systems, such as bush burning and deep tilling, exert significant pressure on population dynamics and abundance of land snails and eliminate their tendencies to assume the status of a serious pest. Above all, predation by the larvae of *Drilus* beetle, with over 130 species currently documented around the world (Kundrata and Bocak, 2017), has been identified as obligate or facultative predator of snails. *Drilus* larvae attack on snails leave specific marks of their activities in the shell, for example, in form of larvae exuviae within the shell or characteristic entry or exit hole (Němec and Horsák, 2019). Recently, the biology of two species of *Drilus* beetles native to Netherlands and their pattern of predation on black snails was described (Baalbergen et al., 2016) Several other reports from different parts of the world indicate that *Drilus* species are diverse and very many species of the predatory beetle are yet to be described (Sormova et al, 2018).

In a hectare multi-crop farmland in Southern Nigeria, significant damage to vegetative parts of maize, sweet potatoes, okra, lettuce and carrot was caused by feeding activities of *L. flammae* and within the area, predatory activities of undescribed species of *Drilus* larvae community was observed. In this report, we provide first hand observations on propensity of *L. flammae* to attack specific crops with regards to aspects of food preference behaviours and described the mode of interaction of the *Drilus* species with the land snails. Quantitative data on intensity of predation by the species of the *Drilus* larvae beetle within the single organic multi-crop environment was generated and the potentials of *Drilus* beetle larvae as a biocontrol agent of pestiferous gastropods was discussed.

Materials and methods

Snail distribution and cause of mortality

Samples were collected between August and September 2019 during the rainy season and the study site was selected based on the observed foraging activities of *L. flammae* and interactions between *Drilus* larvae and *L. flammae* observed in previous years. The rainfall pattern in this region is bimodal, with the first rain around March/April and peaks in July followed by a short break in August. The second rain peak is usually in October and with mean annual rainfall of 2528 mm, mean temperature of 26.9°C and relative humidity of 70–80%. The cultivated area was approximately 0.5 acre coverage for each of the following crops; okra, maize, carrot, lettuce and sweet potatoes.

In order to explore the population of snails in relation to crop types, as indicator of food preference behaviour and the predatory activities of *Drilus* beetle larvae in the different crop plots, five positions measuring 9 x 9 m^2 each were pegged and marked using line rope within each 0.5 acreage. The surveyed area within each plot represented approximately 76% of the cultivated area. Spotlight collection of live snails and dead snails or shells was done. Thereafter, the dead snails were cracked and opened up to observe for presence or absence of exuviae as evidence of *Drilus* beetle larvae attack or mortality due to other factors. Photographs of representative samples of snail shells containing exuviae of *Drilus* larvae was recorded.

Interaction of *Drilus* beetle with *Limicolaria flammae*

In order to prepare photographic evidence of the mode of interaction of Drilus beetle larvae with the snails, randomly selected snails and Drilus larvae (Snail: Drilus, 10:5) were kept in transparent plastic box (L x B x H=30cm x 25cm x15cm) and visually observed. The mode of attack and entry of the predatory larvae into the snail was photographed. The time taken by the larvae to emerge from the snail's shell counting from the time it has completely entered the shell was recorded for 40 specimens.

Statistical analysis

Data on the number of snails collected from the five cropping fields and the number of snails killed due to *Drilus* larvae attack and other causes were tested for compliance with parametric statistical procedure and thereafter subjected to Analysis of Variance Test (ANOVA). Where there were significant differences, a post-hoc statistical test was conducted to separate the means using Tukey's honestly Significant Difference (HSD) test.

Results

Visual appearance of *Drilus* larvae

The larvae is heavily sclerotized with bright orange and black colour mix and hairy. The head and the first segment next to the head is bright orange in colour, the 2nd and the 3rd segments are equally orange but each has two black patches. The first three legs of the larvae are bright orange in colour. The next five segments (4th-8th) and the legs are black. The rear segments, 9th-11th and the attached legs are deep orange and contrasting to the fore segments (Figure 1).

Mode of attack

The predatory larvae essentially attacked actively moving snails by crawling on the shell from behind and biting through the soft tissues close to the mantle of the snail with its highly sclerotized and powerful mandibles multiple times, causing retraction of the snail into the shell while the attack continued (Figure 2). Leakage of body fluid of the snails under attack occurred within 4-6 hours. In the first 6-8 hours of attack, some parts of the larvae remained outside the shell but as the feeding on the soft tissues of the snail continued, the larvae eventually completely entered into the shell. The *Drilus* larvae remained in the shell for 15-20 days, moult within the shell and emerge

Figure 1. Photograph of the predatory *Drilus* beetle larvae

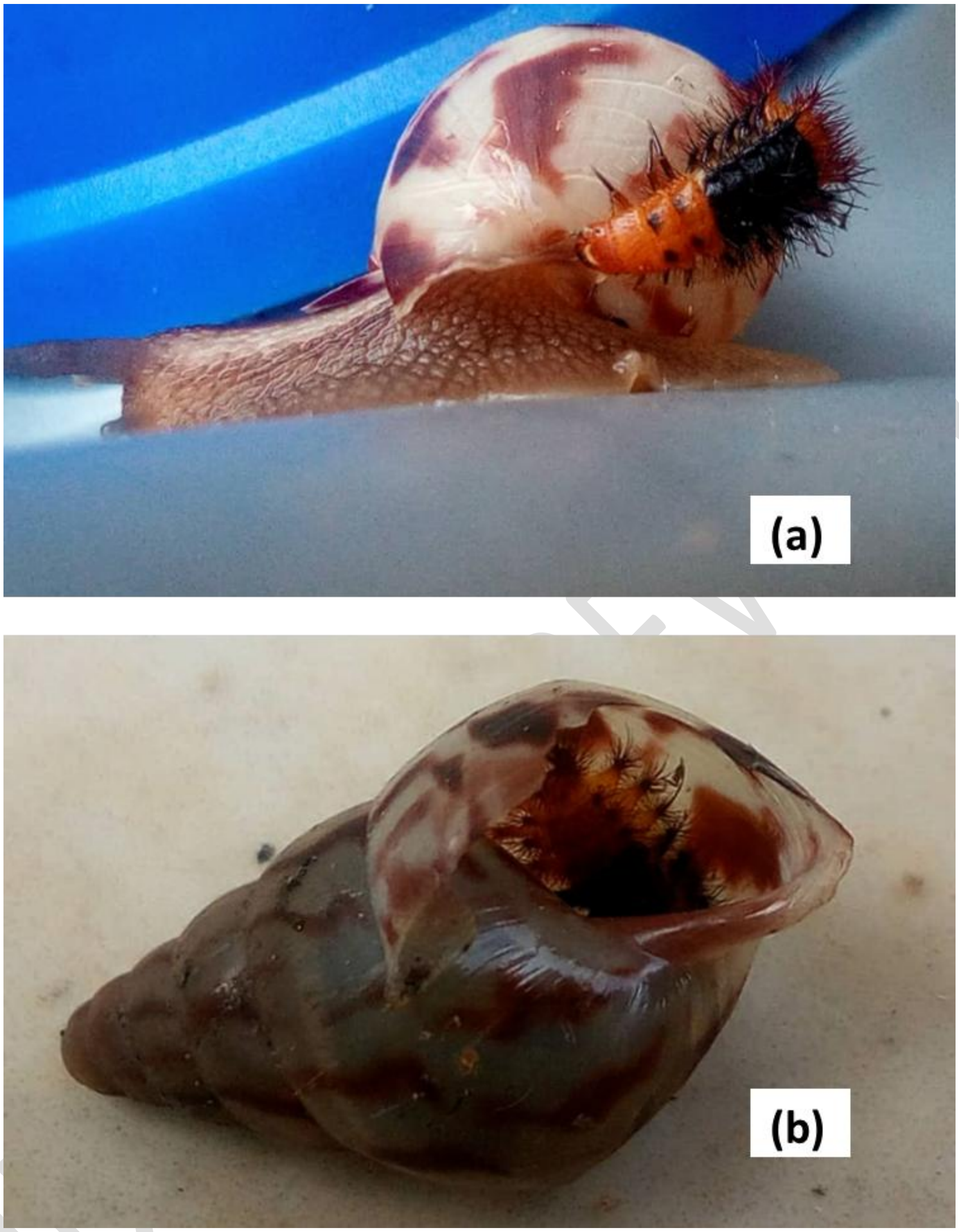

Figure 2. Larva of *Drilus* sp preying on *Limicolaria flammea* (a) the predatory larvae crawled on the shell and has bitten the snail multiple times (b) the snail retracted into its shell and the attack continued until the larvae gained entrance into the internal organs of the snail.

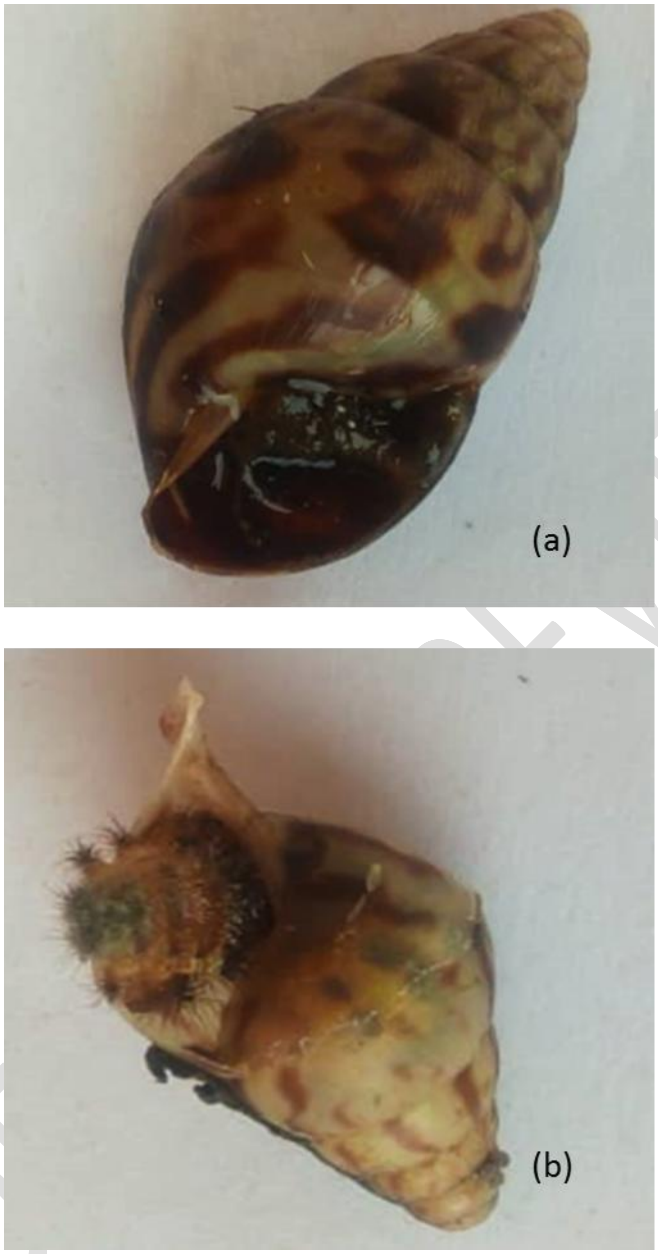

Figure 3. *Limicolaria flammea* killed by larvae of *Drilus* sp (a) dead snail with rotting tissues (b) shell of the dead snail cracked to show presence of exuviae

to attack another snail within 24 hours and most of the attacks occurred at night. The exuviae of the larvae was present in the shell of all the snails killed by *Drilus* larva (Figure 3).In all the cases observed in this study (n=40), the mode of entry into the snail by the predatory larvae was appertural and moulting occurred within the shell before the emergence of the instar larvae. Each larva attacked and killed up to three snails within a period of eight weeks.

Snail count and predation by *Drilus* larvae

Table 1. shows the averages of the number of live snails recovered from okra, lettuce, carrot, potatoes and maize plots were not significantly different statistically [$F(4,15)=1.369$, $P=0.291$]. There were significant differences in the number of dead snails and the empty shells recovered from the different crop fields [$F(4, 30)=3.998$, $P=0.01$]. In the okra, lettuce and carrot fields, signiccantly higher number of dead snails and empty shells were recovered compared to potato and maize plots. There were significant variabilities in the number of dead snails in relation to *Drilus* attack and other mortality factors within crop types [$F(4, 30)=3.97$, $P=0.011$]. Evidence of snail mortality due to *Drilus* larvae attack in okra field was significantly the highest, followed by the value recorded in the field planted with carrot but comparable in maize, potato and lettuce cultivated areas. The number of dead snails and empty shells without *Drilus* larvae exuviae in the different crop fields were comparable. In the okra, lettuce, carrot, potato and maize fields, *Drilus* exuviae was found in 69%, 18%, 50%, 50% and 40% of dead snails or empty shells respectively.

Discussion

The *Drilus* species observed in this study has unique colour patterns and colour intensity that separates it from other species earlier described in many parts of the world where there is.

Table 1. Estimate of snail count and proportions killed by larvae of *Drilus* sp and other mortality factors in different crop fields

	Surveyed Crop fields				
Parameters	Okra	Lettuce	Carrot	Potatoes	Maize
Live snails	40 ± 4a	37 ± 4a	32 ± 4a	35 ± 8a	32 ± 7a
Dead snails/shells	13 ± 2a	11 ± 3a	10 ± 2a	4 ± 2b	5 ± 2b
Mortality by *Drilus* beetle attack	9 ± 1a	2 ± 1b	5 ± 2a,b	2 ± 1b	2 ± 1b
Mortality by other causes	4 ± 1a	9 ± 2a	5 ± 1a	2 ± 1a	3 ± 1a
Mortality by Drilus (%)	69	18	50	50	40
Mortality by other causes (%)	31	82	50	50	60

occurrence of *Drilus* species. Baalbergen *et al.* (2016) proposed that colour differentiation is the most obvious character that can be used to separate instar larvae of *Drilus* species. Several species of *Drilus* have been described and many more are being discovered. As far as we know, this Nigerian specimen being reported is clearly separated from all previously reported species in terms of external morphology. However, the behaviour of the species as an obligate predator of snails and its mode of interaction with *L. flammae* was similar to that described earlier by Baalbergen *et al*. (2016). The mode of entry of this species is appertural, biting the soft tissue around the mantle to create space for entry. Several species of *Drilus* are known to bore entry holes on snail shell, but that did not occur between the *Drilus* species and *L. flammae*.

The biology and the life history of the species was not observed beyond its external appearance and the time taken by the instar larvae to moult and emerge from the shell of the prey, which was 15-20 days for about forty specimens evaluated. In other studies, some species of *Drilus* emerge from the host shell in about 30 days (Baalbergen et al., 2016). It can be suggested that this observed difference in part of the life stage may be characteristic or may be related to effects of temperature and relative humidity. There is currently no study to suggest the effect of temperature on life cycle of *Drilus* species but aspects temperature and humidity relations as it modulates life cycle of arthropod species are well known. Thus far, it can be suggested that the current Nigerian specimen being reported is an undescribed *Drilus* species.

The snail, *L. flammae* had no preference for any of the crops, considering the results of the snail count that showed no significant difference in their average number in the different crop fields. Reports on food preference behaviours in snails showed that highly nutritive and juicy plants such as cabbage, lettuce, watercress, potato skins are the most preferred and snail population density on a particular crop can be considered as an indicator of preference (Santos et al., 2018). It was observed that all the sampled crops in the current report were significantly attacked.

Snail mortality due to *Drilus* attack could be separated from other mortality factors by the presence of exuviae left behind by the instar larvae in the shell (Němec and Horsák 2019). The number of recovered snail shells with evidence of *Drilus* larvae exuviae was significantly higher in okra and carrot fields but comparable in maize, potatoes and lettuce plots. The ability of a predator to locate its host may vary in different crops as plant morphology may influence the ability of a predator to locate its prey

. In entomological pest-host plant and natural enemies complex, studies have shown that some plants attract natural enemies of pests differentially (Xiu et al., 2019; Rashedi et al., 2019) and the possibility of similar interactions in gastropod pest, host and natural enemies complex cannot be ruled out. These may be responsible for the variabilities in the predatory capabilities of the *Drilus* larvae against *L. flammae* in the different crops. There is no data to compare variabilities in predatory activities, host location capabilities and aggregation patterns of *Drilus* larvae on snails in different crops. In the okra field, the proportion of snail shells containing *Drilus* larvae exuviae was 69% against 31% by other mortality factors. In all the crop fields examined, *Drilus* larvae was responsible for a proportion of the dead snails recovered. Other mortality factors in snail populations include birds, some other insects from the Order Diptera and rodents: although these agents were present in the study area, their activities were not identified in the study.

The larvae of *Drillus* beetle, an obligate predator of snails, was capable of feeding on many snails within its larval stage. Thus, considering the functional role of the predatory larvae on natural population regulation in snail communities, there are potentials in adopting the species in inundative biocontrol of pestiferous gastropods. More so, the female insect in other described species are known to be highly prolific, capable of laying up to 250 eggs that hatch into the larvae within 30 days and seek to attack its first victim within 24 hours after eclosion (Baalbergen et al., 2018). The hyper-metamorphosis character of *Drilus* species (Faucheux, 2014; Baalbergen et al., 2016) that allows prolonged larval form for up to two years is a beneficial quality which could likely enhance its biocontrol capabilities.

In line with good ethics in Integrated Pest Management strategies, bioprospecting for new species is key and it is clearly necessary to document this Nigerian *Drilus* sp and its activities on the pestiferous *L. flammae* populations. However, detailed studies are required in the aspects of taxonomic classification, bioecology and the life history of the species.

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