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Monitoring *Aethina tumida* (Coleoptera: Nitidulidae) With Baited Bottom Board Traps: Occurrence and Seasonal Abundance in Honey Bee Colonies in Kenya

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ABSTRACT The population dynamics of the honey bee pest *Aethina tumida* Murray (small hive beetle) have been studied in the United States with flight and Langstroth hive bottom board traps baited with pollen dough inoculated with a yeast *Kodamaea ohmeri* associated with the beetle. However, little is known about the population dynamics of the beetle in its native host range. Similarly baited Langstroth hive bottom board traps were used to monitor the occurrence and seasonal abundance of the beetle in honey bee colonies at two beekeeping locations in Kenya. Trap captures indicated that the beetle was present in honey bee colonies in low numbers all year round, but it was most abundant during the rainy season, with over 80% trapped during this period. The survival of larvae was tested in field releases under dry and wet soil conditions, and predators of larvae were identified. The actvity and survival of the beetle were strongly influenced by a combination of abiotic and biotic factors. Larval survival was higher during wet (28%) than dry (1.1%) conditions, with pupation occurring mostly at 0–15 cm and 11–20 cm, respectively, beneath the surface soil during these periods. The ant *Pheidole megacephala* was identified as a key predator of larvae at this site, and more active during the dry than wet seasons. These observations imply that intensive trapping during the rainy season could reduce the population of beetles infesting hives in subsequent seasons especially in places where the beetle is a serious pest.

KEY WORDS *Aethina tumida*, *Kodamaea ohmeri*, *Pheidole megacephala*, honey bee, Kenya

Studies of the population dynamics of organisms provide information about their occurrence, seasonal abundance, population structure, adaptive behavior associated with seasonal changes, and biotic and abiotic factors that influence population dynamics (Nicholson 1985, Emden and Williams 1974, Wallner 1987). Such information can be exploited for use in population prediction models and for developing effective pest management programs (Watt 1962, Liebhold and Tobin 2008, Waters and Stark 1979). The population dynamics of several insects have been studied in Africa (Sétamou et al. 2000, Gnonlonfin et al. 2008, Ndenga et al. 2006, Moritz 2002), but few studies have focused on pests associated with honey bees, despite the immense economic value of honey bees in food production and biodiversity conservation.

The small hive beetle *Aethina tumida* Murray, a pest of honey bee colonies, interacts with its host differently depending upon the honey bee subspecies. The interaction can range from a mere nuisance in colonies of African honey bees (Lundie 1940) to a serious pest when it infests European honey bee colonies (Sanford 1998, Hood 2004). Both larvae and adults of the beetle live in honey bee colonies, where they feed on pollen, brood, and honey, but the larvae inflict the most damage (Elzen et al. 1999). Adults of the beetle can be monitored using traps baited with pollen inoculated with a yeast *Kodamaea ohmeri* (NRRL Y-30722) associated with the beetle (Teal et al. 2006; Torto et al. 2007b, 2007c; Arbogast et al. 2009a, 2009b). This lure combined with a bottom board trap has been used successfully in the United States to monitor populations of adult beetles in European honey bee colonies maintained in Langstroth hives. In Kenya, unconfirmed reports from beekeepers managing honey bee colonies in Langstroth hives suggest that the small hive beetle can be a damaging pest of honey bee colonies at certain times of the year. These reports prompted us to investigate the occurrence and seasonal abundance of the beetle in honey bee colonies at selected beekeeping sites in Kenya using the bottom board trapping system. We also attempted to determine the abiotic and biotic factors that influence the seasonality of the beetle's activity, with a view to generating knowledge that can be used to educate beekeepers to make management decisions for the beetle.

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Materials and Methods

Field Sites. Two beekeeping sites in Kenya – the International Centre of Insect Physiology and Ecology (*icipe*) campus in Nairobi (01° 13′ 25.3″S, 36° 53′ 49.2″E), at ≈1,600 m above sea level and Ndalani in Matuu (01° 5′ 6.3″S, 37° 28′ 13.1″E), at ≈1,100 m above sea level – were used for the study. Both sites are located in semiarid areas, and are ≈70 km apart. The Ndalani site is a key beekeeping zone in Kenya. Honey bees at the two sites are kept in Langstroth hives. Four colonies were randomly selected at each site for the study.

Rearing of Insects. Adult small hive beetles were collected from honey bee hives at the *icipe* apiary and raised in the laboratory on moistened pollen dough (consisting of 4% pollen with sugar, soy, yeast, and water) at a temperature of 26 ± 2°C and relative humidity 50 ± 5% in round plastic bowls (10 cm in diameter × 14 cm deep) with perforated lids. Eggs laid in the food substrate were reared on the same diet until the larvae reached the wandering stage. One hundred and fifty larvae were removed from the rearing media and placed on moist filter paper in petri dishes 2 h before their use.

Odor-Baited Traps. Bottom board traps, as previously described (Torto et al. 2007b), were baited with inoculated pollen dough (50 g), which was prepared by mixing *K. ohmeri* with double-distilled water and commercial pollen dough (4% pollen with sugar, soy, yeast, and water; Global Patties, Airdrie, Alberta, Canada) at 1:100:1,000 by wt, and allowing the dough to ferment for 5 d (Torto et al. 2007a).

Experiment 1: Comparison of Yeast-Inoculated and Noninoculated Pollen Dough Traps. In previous studies, the efficacy of inoculated pollen dough as a bait for luring small hive beetles from honey bee colonies into bottom board traps was compared with nonbaited traps Torto et al. 2007b). In this experiment, we compared the efficacy of inoculated pollen dough with noninoculated pollen dough in luring the beetle into similar traps in similar hives (Langstroth) located at the *icipe* campus, Nairobi. Four honey bee colonies were selected at random at the site, and trapping was carried out for 10 wk (August–October 2007). The traps were checked, beetles were counted every week, and the lure was changed every 4 wk.

The total numbers captured with yeast-inoculated and noninoculated pollen dough were compared using a χ^2 one-sample test with a null hypothesis that equal numbers of beetles were trapped using either bait. The analysis was carried out at an α level of 0.05.

Experiment 2: Comparison of the Seasonality of Occurrence of the Beetle at Two Sites in Kenya. Based on the results of experiment 1, bottom board traps baited with inoculated pollen dough were used to compare captures of beetles from the colonies at the two sites for a full year. At the Nairobi site, the traps were set up from November 2007 to October 2008, and from February 2008 to January 2009 at the Matuu site. The traps were checked once per week, and the bait was changed monthly. Trap catch was expressed as the number of beetles captured per week. Weekly captures were pooled to calculate monthly means throughout the study period. Rainfall data for Kasarani-Nairobi and Ndalani-Matuu was obtained from the Kenya Meteorological Department. The influence of rainfall on mean monthly trap catch was examined by linear regression using SAS Proc REG (SAS Institute 2003).

Experiment 3: Identification of Soil Factors Influencing Beetle Survival. Based on the results of experiment 2, this experiment was carried out to identify potential soil factors that influence the survival of juvenile stages of the beetle before they emerge as adults. The experiment was carried out during April and May 2009 at the *icipe* Nairobi campus beeyard. April and May were the months of peak beetle abundance at both sites. The primary purpose of the experiment was to determine the relationship between soil moisture level and the depth at which beetles pupate. Attempts were also made to identify biotic factors in the soil that regulate beetle populations.

The soil at the experimental site, to a depth of 25 cm, was comprised of 10–12% silt, 27–37% clay, and 50–60% sand (Crop Nutrition Laboratory Services, Nairobi, Kenya). A transect, consisting of four quadrats spaced 20 m apart, was laid out on the soil surface. Each quadrat was delimited by a frame (30 cm square and 10 cm deep) constructed of 0.3-cm plywood and inserted to a depth of 5 cm into the soil, taking care to disturb the soil as little as possible. Wandering stage larvae were placed on the soil surface in each frame, during early evening (5:45–6:20 p.m.) to minimize desiccation. After 7–8 d, the soil in each frame was excavated in 5-cm increments to a depth of 25 cm to recover the released beetles, and the number of beetles recovered at each depth was expressed as a proportion of the total number recovered at all depths. The experiment was repeated three times (16–23 April, 28 April–5 May, 11–18 May 2009) with 98, 150, and 150 larvae per frame, respectively. For each repetition, the proportions in the four quadrats were averaged. Soil moisture content was measured with sensors placed ≈15 cm from each quadrat at depths of 10, 20, and 25 cm, and readings were taken from these sensors daily with a digital moisture meter (Watermark-Irrometer, Riverside, CA). The sensors and moisture meter measure soil water tension (the physical force actually holding water in the soil) expressed as kilopascals (kPa; 1 kPa = 1 centibar). As soil loses moisture, soil water tension rises, so moisture content and kPa are inversely related.

For each quadrat, the number of beetles recovered at each depth was expressed as a proportion of the total number recovered at all depths, and for each repetition of the experiment, the proportions for the four quadrats were averaged. The relationship between depth and mean proportion recovered was examined by regression analysis, and the number of beetles released was compared with the number recovered using a one-sample χ^2 test.

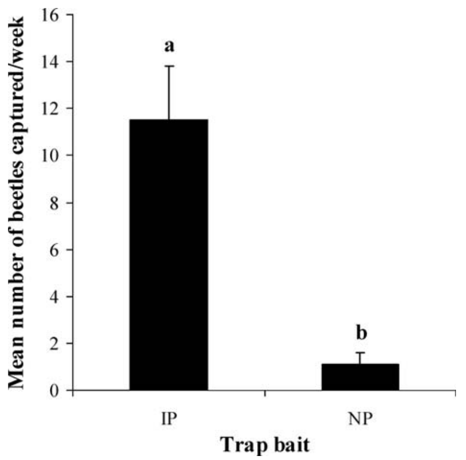

Fig. 1. Mean (+SE) number of beetles captured per week in traps baited with inoculated pollen (IP) and noninoculated pollen (NP) from August to October 2007. Bars with the same letter are not significantly different. Means separated by a one-sample χ^2 test (1 df). H_0: number of beetles captured/wk in both trap types are equal at $\alpha = 0.05$.

Results

Experiment 1: Comparison of Yeast-Inoculated and Noninoculated Pollen Dough Traps. A total of 126 beetles was captured in the traps during the trapping period, with ≈92% captured in the yeast-inoculated pollen dough traps. The mean number of beetles captured per week in the trap baited with yeast-inoculated pollen dough was significantly higher than that for traps baited with noninoculated pollen dough ($\chi^2 = 8.58$, df = 1, $P = 0.003$) (Fig. 1).

Experiment 2: Comparison of the Seasonality of Occurrence of the Beetle at Two Different Sites. The pattern of trap catch at the two sites was similar (Figs. 2a and 3a), with the number of beetles captured increasing with increasing rainfall. At both sites, there were two peak periods in the year when trap captures of the beetle were high. These peaks coinicided with the periods of the highest rainfall at the sites: March–May and September–November for Nairobi, and March–May and November–January for Ndalani in Matuu. The relationship with rainfall for the two sites is best described in Figs. 2b and 3b. Linear regression analysis of trap catches and rainfall with the origin as the intercept were significant at both beekeeping sites (Nairobi-*icipe:* $F = 22.82$; df = 1, 11; $P = 0.0006$, adjusted $R^2 = 0.6451$; Matuu-Ndalani: $F = 13.68$; df = 1, 11; $P = 0.0035$, adjusted $R^2 = 0.5138$), where R^2 is the coefficient of determination. The intercept was set at the origin ($x = 0$, $y = 0$) because no beetles were expected to be trapped during periods with no rainfall.

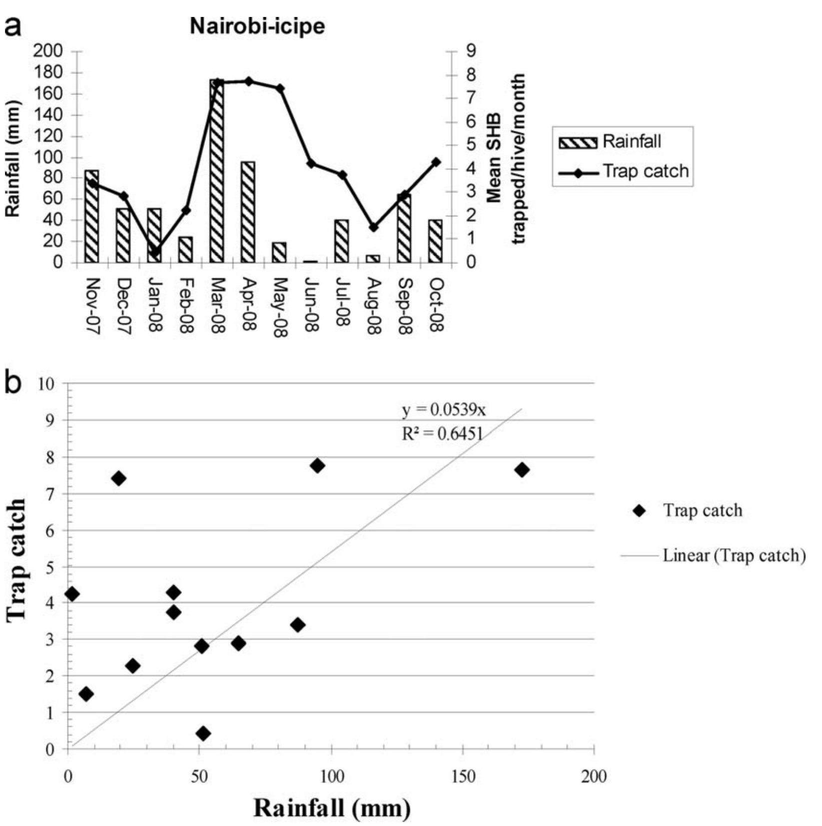

Fig. 2. (a) Mean number of adult beetles trapped/hive/mo for a full year from four honey bee colonies located at the *icipe* campus-Nairobi in Kenya, and (b) relationship between captures of adult beetles and rainfall from this site.

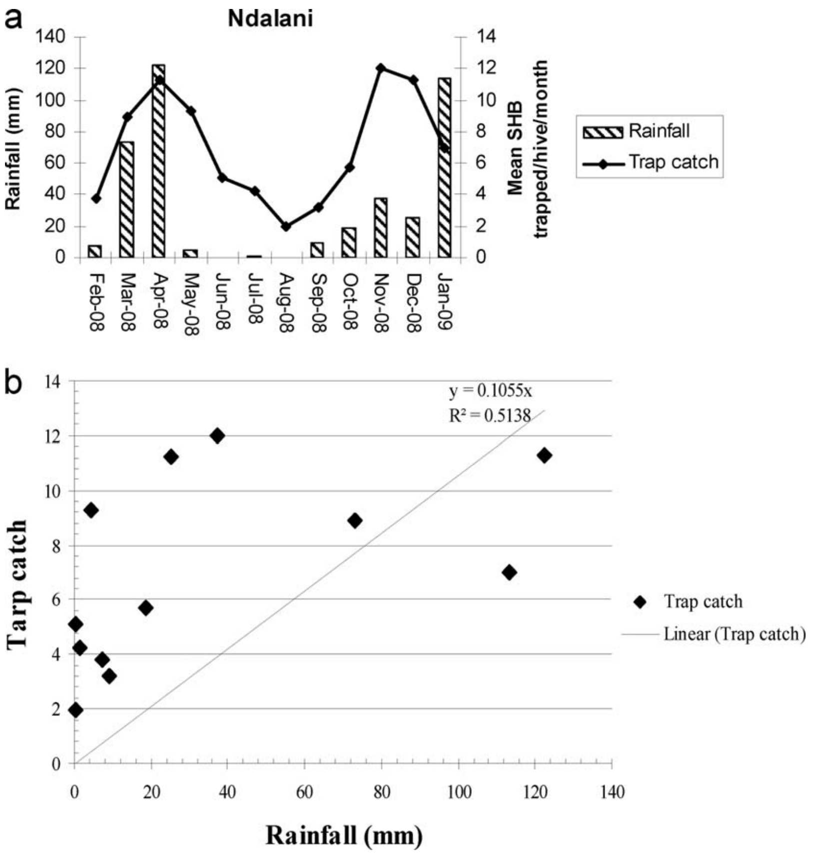

Fig. 3. (a) Mean number of beetles trapped/hive/mo for a full year from four honey bee colonies located at the Matuu site, Ndalani in Kenya, and (b) relationship between captures of adult beetles and rainfall from this site.

Experiment 3: Identification of Potential Soil Factors Influencing Beetle Survival. Of the 392 larvae released in the first experimental run, none were recovered. Of the 600 larvae released in each of the second and third runs, only 52 (8.7%) were recovered in the second run and 317 (52.8%) in the third. Of the beetles recovered, slightly more than half (58.6%) were prepupae, and the remainder were pupae. The mean proportion of beetles (pupae and prepupae) recovered from the soil declined exponentially with depth (Fig. 4).

Soil moisture readings at the various soil depths and daily records of the amount of rainfall at the study site (source: Kenya Meteorological Services) are summarized in Fig. 5. The period 16–23 April was the driest, whereas 28 April–5 May was the wettest. The top 20-cm depth was dry during the beginning of the experiment until 21 April, after which it stayed wet, whereas soil at the 25-cm depth gradually lost its moisture because of the low amount of rainfall during this period, which was not enough to soak through the soil to this depth.

The generalist ant species *Pheidole megacephala* (Hymenoptera: Formicidae) was observed preying on larvae of the beetle within 10 min after they were released. The specimens were identified by Stefan Cover (Harvard University, Museum of Natural History, Cambridge, MA), where a voucher specimen has been deposited.

Discussion

The results showed that traps baited with *Kodamaea*-inoculated pollen dough were more effective in trapping small hive beetles in honey bee colonies

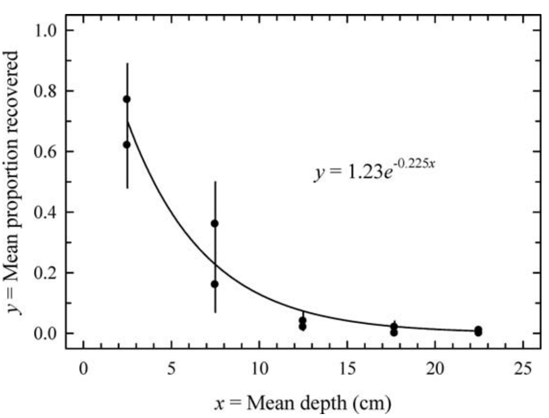

Fig. 4. Relationship between depth in the soil and proportion of released larvae recovered after 7–8 d (as prepupae and pupae). Error bars indicate ± SE, R^2 = 0.948, adjusted R^2 = 0.942. Analysis of variance: $F_{1,8}$ = 146.4, $P < 0.0001$.

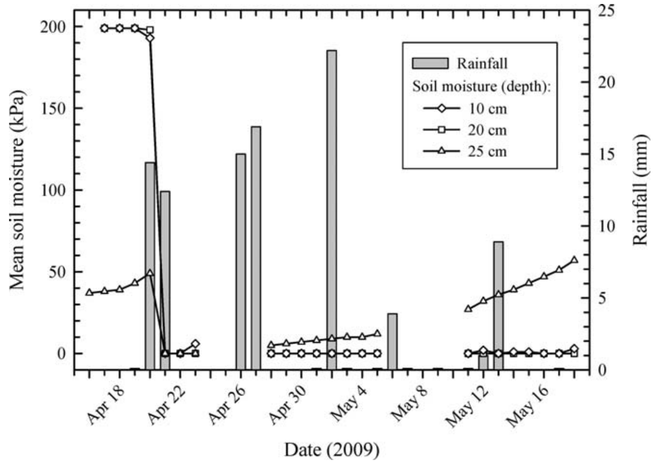

Fig. 5. Variation in soil moisture and rainfall during the study period. Rainfall records are given for each day. Trace amounts of rainfall (<0.1 mm) are plotted as 0.1 mm to indicate dates of occurrence. Soil moisture content is given only for days in which an experiment was in progress.

than were traps baited with pollen dough alone, which is in agreement with earlier laboratory and field studies with the European honey bee in the United States (Torto et al. 2007b). The effectiveness of the lure in attracting beetles and the importance of the yeast as a component of the lure were confirmed. As previously shown, the yeast ferments pollen, releasing volatiles that include components of the honey bee alarm pheromone that are important olfactory cues in attracting the beetles to bee colonies (Torto et al. 2007c).

The results also show that trap captures in general were low all year round, indicating a low infestation of honey bee colonies at the two sites. However, the seasonal patterns of occurrence at the two study sites were similar and followed the seasonal pattern of rainfall. This suggests that the amount of rainfall influences abundance and may serve as a useful tool in predicting seasonal beetle activity and infestation of managed honey bee colonies. Rainfall plays an important role in the population dynamics of many insects (Birch 1957, Wallner 1987). Given that the beetle completes its life cycle in moist soil, it is not surprising that trap captures of the beetle were high in the yeast-inoculated pollen traps during the rainy season. This suggests that more beetles completed their development in the soil during these months. The results of the larval burrowing assay are in agreement with this assessment because larvae were found to burrow deeper into the soil for pupation during the drier periods. In addition to moisture content, the nature and type of soil may also determine the depth to which larvae burrow for pupation (Ellis et al. 2004).

Whereas the results suggest that the larvae may possess an innate ability to detect the most favorable moisture level for development and survival, the results also suggest that burrowing deeper into the soil during the dry season to pupate may confer an added advantage. It appears that this behavior enables larvae to avoid close contact with the generalist ant predator *P. megacephala,* which was found preying on released larvae close to the surface of the soil. Interestingly, this ant is considered a potential biological control agent for several economically important insect pests in Africa (Carroll and Janzen 1973, Way and Khoo 1992, Dejean et al. 2007), but it shows a greater preference for feeding on decomposing plant and animal tissues (Tinzaara et al. 2005).

In summary, this study has shown that adults of *A. tumida* are present in honey bee colonies all year round at the experimental sites used for the study in Kenya. Trap captures were low, suggesting that absconding of bees at these sites may be the result of factors other than small hive beetle infestation alone. Traps baited with *Kodamaea*-inoculated pollen dough were more effective in trapping the beetle. Both abiotic and biotic factors play a significant role in limiting populations of the beetle.

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