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ORIGINAL RESEARCH ARTICLE

Responses of four coffee varieties to infestations of the aphid Toxoptera aurantii (Hemiptera: Aphididae)

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Abstract
Insect pests can significantly reduce coffee yields. Coffee trees are susceptible to phytophagous insects, including the black citrus aphid *Toxoptera aurantii*, (Hemiptera: Aphididae). This pest can substantially reduce yields, especially during severe and prolonged infestations. This study aimed to determine the susceptibility of coffee varieties BM71, BM139, Jackson 2/1257, and Pop 3303/21 which are currently being expanded in Rwanda. We analyzed the survivorship, the number of offspring in the F1 generation ($\sum l_x m_x$), intrinsic rate of natural increase (r_m), finite rate of growth (λ), and mean generation time (T) for each variety to assess its susceptibility. Laboratory experiments were followed by field trials in a completely randomized block design with 24 replicates under natural environmental conditions. The number of aphids on the soft upper leaves of each plant was assessed weekly. At the same time, natural enemies were collected and preserved in 70% alcohol for future identification, except for butterflies and entomopathogens. Results revealed significant differences among the coffee varieties. The variety BM71 can be the least susceptible, while Jackson 2/1257 can be the most susceptible, with the other varieties showing moderate susceptibility. The natural enemy communities in the field experiments can be dominated by ladybird beetles, with *Cheilomenes lunata* and *C. sulphurea* (Coleoptera: Coccinellidae) being the most prevalent species. We recommend further evaluation of the quality of BM71 and its indirect effects on beneficial insects to promote Integrated Pest Management strategies. Studies should also explore ways to enhance the conservation and effectiveness of natural enemies, especially ladybird beetles, to strengthen sustainable pest management practices in coffee farming systems in Rwanda.

Keywords Completely randomized block design · Life history traits · Natural enemies · Variety

Introduction

Coffea arabica L. (Gentianales: Rubiaceae) is a crucial economic commodity for many developing countries and can play a significant role in agricultural exports and foreign exchange earnings (Gizaw et al. 2022). In East Africa, coffee cultivation is essential for both local livelihoods and national economies (Wang et al. 2015). In Rwanda, for instance, coffee contributes significantly to the country's foreign exchange earnings (Ngarukiye 2023). This contribution is vital, as coffee is one of the most traded agricultural commodities, with Rwanda positioning itself as a key exporter, especially in the global market for high-quality arabica coffee. However, coffee farmers in Rwanda face numerous challenges that threaten the sustainability and profitability of coffee production. These challenges include

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pests and diseases that can significantly reduce yields, quality, and profitability for coffee growers.

In Rwanda, farmers primarily cultivate two species of the coffee genus: *C. arabica* and *C. robusta* (Chu 2012; Bunn et al. 2015). While both species are important for local economies, *C. arabica* is the dominant species grown due to its superior quality, taste, and aroma. *C. arabica* is renowned for its smooth flavour and organic nature, making it highly sought after in the global market for premium coffee. In contrast, C. robusta has declined in popularity, mainly because of its harsh flavour profile, high susceptibility to pests, and vulnerability to low temperatures, which limit its adaptability to certain growing environments (Chu 2012; Bunn et al. 2015). Consequently, *C. arabica* production remains the focus for many coffee farmers in the region.

However, coffee production faces several challenges, with one of the most significant and persistent issues being the damage caused by pests, particularly sucking insects like aphids. The coffee aphid, *Toxoptera aurantii* (Hemiptera: Aphididae) has been recognized as a longstanding threat to coffee crops (Le Pelley 1968; Barrera 2008). *T. aurantii* is a sucking insect that feeds on the sap of young coffee plants, causing direct damage to the plants and indirectly facilitating the growth of black fungi (fumagine) on the leaves due to the honeydew excreted by the aphids (Le Pelley 1968; Barrera 2008). This honeydew not only promotes fungal growth but also interferes with critical plant processes such as photosynthesis, thereby impacting the overall health and productivity of coffee plants. Additionally, *T. aurantii* is a vector for the Coffee Ring Spot Virus (Waller et al. 2007), a harmful disease in coffee production, further exacerbating the challenges faced by coffee farmers.

The damage caused by *T. aurantii* is particularly severe during the flowering and fruiting stages of coffee when the plants are most vulnerable to pest infestations. During this period, aphid populations can proliferate rapidly, leading to substantial losses in both yield and quality. If left unchecked, the damage can be extensive, resulting in the drying and death of young plants, and in severe cases, significant reductions in coffee production. The pest's ability to reproduce quickly and its widespread distribution across coffee-growing regions make *T. aurantii* a persistent threat to coffee cultivation (Lamb and others 1974; Wintgens and others 2004).

Traditionally, the most common method for controlling coffee pests, including *T. aurantii*, has been the use of synthetic pesticides. However, this approach has raised several environmental and ecological concerns. Over-reliance on chemical control has led to the development of pesticide resistance in pest populations, including aphids (Whalon et al. 2008). Additionally, pesticide use reduces the abundance of natural enemies and other beneficial insects, such as pollinators, which are essential for maintaining ecosystem services environment (Hazarika et al. 2009). In regions like Tanzania's Kilimanjaro, studies have shown that coffee trees treated with insecticides often have higher aphid populations compared to untreated trees, likely due to the negative effects of pesticides on natural predators (Lyimo et al. 2017). As a result, there is growing recognition that chemical control is not a sustainable solution, and alternative pest management strategies should be promoted.

In response to these challenges, researchers and farmers can explore alternative methods of pest control that reduce reliance on chemical pesticides and promote more sustainable agricultural practices. One such approach is the use of resistant coffee varieties, which can slow or delay the growth and development of aphids, thereby reducing pest populations and minimizing the need for chemical interventions (Golizadeh et al. 2016). By selecting coffee varieties that are naturally resistant or less susceptible to pests, farmers can reduce pesticide use, improve the resilience of their crops, and promote ecological sustainability. Resistant varieties also can offer the added benefit of enhancing biological control, as aphid populations may remain exposed to natural predators for longer periods, further suppressing pest numbers.

The identification and development of resistant coffee varieties have been a focus of research in many coffee-producing countries, including Rwanda. The country has actively promoted the use of improved coffee varieties as part of its strategy to increase both the quantity and quality of coffee production. Varieties such as BM71, BM139, Jackson 2/1257, and Pop 3303/21 have been introduced to smallholder farmers to improve production and reduce the impact of pests (Boudreaux and Ahluwalia 2008; Boudreaux 2010). These varieties have been recommended for their potential to withstand pest pressures while maintaining high-quality output, thereby contributing to the long-term sustainability of Rwanda's coffee sector.

The present research aims to contribute to this effort by evaluating the life history parameters of *T. aurantii* on four coffee varieties currently cultivated in Rwanda. Understanding the biology and life cycle of this pest on different coffee varieties will provide valuable insights into the development of more effective and targeted pest management strategies. The study uses laboratory-based life table parameters, such as fecundity, survivorship, and intrinsic rate of natural increase (r_m), to assess the susceptibility of different coffee varieties to aphid infestation. Life tables are widely used in pest management research as they help quantify the growth and reproductive potential of insect populations, allowing for more accurate predictions of pest dynamics and better-informed decisions regarding pest control (Carey 1993).

The laboratory experiments have been complemented by field experiments, as field conditions differ significantly from controlled laboratory settings. Our objective was to assess the susceptibility of these coffee varieties under natural environmental conditions to determine whether the laboratory results could be confirmed. By combining laboratory data with field experiment data, this research can contribute to the development of a comprehensive management strategy that minimizes the need for chemical pesticides and promotes sustainable coffee production in Rwanda.

Materials and methods

Step 1. Laboratory experiments

On November 19th, 2013, we collected seeds of four coffee varieties (*Coffea arabica*): BM71, BM139, Jackson 2/1257, and POP 3303/21, from the Rwanda Agriculture and Animal Resources Board (20°06' S, 29°48' E) in the Southern Province, P.O. Box 621, Kigali. On December 7th, 2013, the seeds were sown in potting soil in a greenhouse at the Earth and Life Institute (ELI) of Université Catholique de Louvain, at Carnoy 4–5, 1348 Louvain-la-Neuve, Belgium. The first germination (BBCH-code: 00–09) occurred on January 12th, 2014, with the entire germination process taking approximately six weeks. On February 5th, 2014, the seedlings (BBCH-code: 10–19) were transplanted into 25–30 cm pots and placed in a greenhouse with a constant temperature of 25 °C. The plants were watered with rainwater and monitored daily, and no synthetic fertilizers or pesticides were applied to the seeds or seedlings.

On October 10th, 2015, laboratory colonies of *T. aurantii* were established from field-collected aphids found on tea plants (Camellia sinensis L.) in Rwanda (National Agriculture Export Development Board, Kitabi Tea Factory, Southern Province, 104 Kigali, Rwanda). The stock colonies were maintained on potted seedlings (25–30 cm tall) of the Harrar variety of coffee (a variety we did not test in this experiment). The potted seedlings were caged to prevent the aphids from escaping. The research was conducted in an insect-rearing room at a temperature of 22 ± 1 °C, with ambient humidity (50% RH) and a photoperiod of 16:8 h (L: D). After two months of rearing, the colonies were ready for experimentation.

On October 15th, 2015, a cohort of ten wingless adult aphids, aged 8 days, was transferred to the Harrar variety seedlings and allowed to reproduce for 24 h. On October 16th, 2015, the 10 apterous females were carefully removed, and ten newly born nymphs (aged of 1 day) were placed on each variety with five replicates. The cages were then kept in a room under stable and optimal conditions (22 ± 1 °C, 50% RH (relative humidity), and a 16:8-hour photoperiod). Survival and development were observed daily to determine the nymphs' stage and duration. Aphids were regularly monitored and checked daily until the last aphid died. We provided fresh coffee seedlings once a week.

Once the nymphs reached adulthood, four wingless adult females of *T. aurantii* were placed on each coffee seedling, with ten replicates per variety. A total of 40 individuals per variety were monitored. Adult mortality and fecundity were recorded daily, with offspring being counted and removed from each seedling until the last female died. The entire experiment was conducted in the laboratory from December 15th, 2015, to February 16th, 2016.

Step 2. Fields experiments

The field experiments were established in Rwanda (2°19'60" S, 29°40'00" E), in Nyarugenge District, at an altitude of approximately 1600 m above sea level (Mwendo village). On July 18th, 2013, seeds from four commercial coffee varieties; BM71, BM139, Jackson 2/1257, and Pop 3303/21, were collected from the Rwanda Agriculture and Animal Resources Board (02°06'S, 029°48'E), Southern Province, P.O. Box 621, Kigali. These varieties were selected because they are the most widely cultivated in Rwanda's provinces. On August 13th, 2013, the seeds were separately sown in forest soil, and on December 20th, 2013, 400 seedlings were potted in polyethene bags. The plants were grown in a mixture of organic compost and soil in a 4:1 ratio, with daily watering using rainwater. No pesticides were applied to the seeds or seedlings. The plants were kept under natural environmental conditions and hardened off by July 30th, 2014, before being planted in the experimental plots.

After land preparation, the seedlings, aged 10 months, were planted in the experimental field on September 4th, 2014. The four commercial coffee varieties were placed under natural conditions, where natural pest contamination occurred. The plants were arranged in three complete randomized blocks of 42 m² each, with 20 m of uncovered ground between blocks. Each block consisted of 32 seedlings (eight per variety), separated by 2 m of mulched ground. A farm compost (5 kg), made from animal urine and decomposed grasses, was used to fertilize each plant at planting. No pesticides or mineral fertilizers were used during the experimental period.

Data collection

Two weeks after planting (September 17th, 2014), all visible black aphids (larvae and adults) and green scales on the soft leaves at the top of the plants were counted weekly until February 24th, 2015. The assessments were carried out

between 8:00 a.m. and 11:00 a.m., with blocks alternated each week during the sampling sessions.

At each sampling session, natural enemies were collected using the following methods: Coffee plants were shaken by hand twice, and the falling arthropods were collected in a white bag, counted, and preserved in alcohol (70% ethanol) for later laboratory identification. Predators were also caught manually, considering the entire plant. Flying insects, such as syrphids, were captured using a mosquito net attached to a stick. In October 2015, the morphological identification of natural enemies was conducted using a binocular, hand lens, and insect identification keys (Beavis and others 1988).

Data analysis

The data from laboratory experiments; from the daily observations, various biological parameters, such as net reproductive rate (Ro), intrinsic rate of natural increase (r_m), finite rate of birth (λ), generation time (T), and doubling time (t_2), were calculated. Survivorship curves were compared using survival analyses in GraphPad Prism 5. Analysis of variance was performed to compare the cumulated net fecundity of *T. aurantii* on the four coffee varieties. Life history traits were described using the equations (Carey 1993). Significant differences among treatment means were determined using Newman-Keuls multiple comparisons at a probability level of 5% ($P<0.05$).

For field experiments data, we tested the effect of treatments on the number of aphids counted every week on 3Blocks using a Generalized Linear Mixed Model with a linked function of Poisson distribution (Poisson-GLMM) but discarded the latter due to substantial overdispersion of aphid counts. Instead, we adopted the negative binomial (error distribution: negative binomial (NB-GLMM) with a log-link function for those comparisons. We included in the model varieties (treatments), dates of data collection, and years as fixed factors while we considered blocks as random factors, and the number of aphid populations counted in each plot as response variables. All GLMM analyses were performed using R version 4.2.3 Copyright (C) 2023, The R Foundation for Statistical Computing for Mac. If the treatment type was found to be statistically significant, post hoc analyses of the effects of treatments were then performed to know which treatment was different from others. In addition, the natural enemy's abundance was analyzed by analysis of variance (ANOVA), and the means were compared with Tukey's test at $P<0.05$. All the analyses were performed using Graph Pad Prism 5.1 software (Dennis Radushev, San Diego, USA).

Results

Laboratory results

The survival analysis indicated that there is no significant difference among the survival curves of *T. aurantii* overtime on the tested coffee varieties (Fig. 1.)

The feeding suitability varied among the coffee varieties. The analysis of the net cumulative fecundity of *T. aurantii* on four different coffee varieties revealed significant differences among them (F3, 22 = 60.51; $P<0.001$). Cumulative fecundity is low on the BM71 variety and high on Jackson 2/1257, with the other varieties showing intermediate values (Fig. 2).

The intrinsic rate of natural increase did not vary significantly between varieties (F3,16 = 2.82, $P=0.346$). The intrinsic rate of natural increase value for BM139 (r_m = 0.345 individuals per day) indicates that the population growth rate per day of aphids reared on BM139 was 0.345 individuals per capita. The intrinsic rate of natural increase for *T. aurantii* reared on BM71 had the lowest value (r_m = 0.237 individuals per day), while BM139 had the highest value (r_m = 0.345). Jackson2/1257 and Pop3303/21 had intermediate values (Table 1). This is further illustrated by the finite rate of increase (λ), which shows the factor by which a given population is expected to multiply each day.

The finite rate of increase was similar for Jackson2/1257 and Pop3303/21 ($\lambda=1.38$) but lower for BM71 ($\lambda=1.27$). The values of Ro varied among the varieties (F3,16 = 5.33, $P<0.0097$), with higher values for Jackson2/1257 (Ro = 10.13) and Pop3303/21 (Ro = 8.86). The net reproductive rate of *T. aurantii* reared on BM71 and BM139 was lower than on the other two varieties. The time required for the population to double (t_2) was 2.92 days on BM71 compared to 2.00 days on BM139. There was a small variation in generation time (T) among the varieties (F3,16 = 3.1450, $P=0.054$).

Field results

Significant differences were found in aphid infestations on coffee varieties ($\chi^2=9677.3$; df = 3; $P<0.001$). BM139 was the most infested while BM71 was the least infested variety. However, the varieties Jackson 2/1257 and Pop3303/21 were moderately infested (Fig. 3).

The aphid population varied highly significantly over time ($\chi^2=9946.7$; df = 23; $P<0.001$). Between November 2014 and January 2015, the aphid populations increased and started to decrease after January 2015. It decreased to zero after January 27, 2015, and remained relatively rare throughout the observation period.

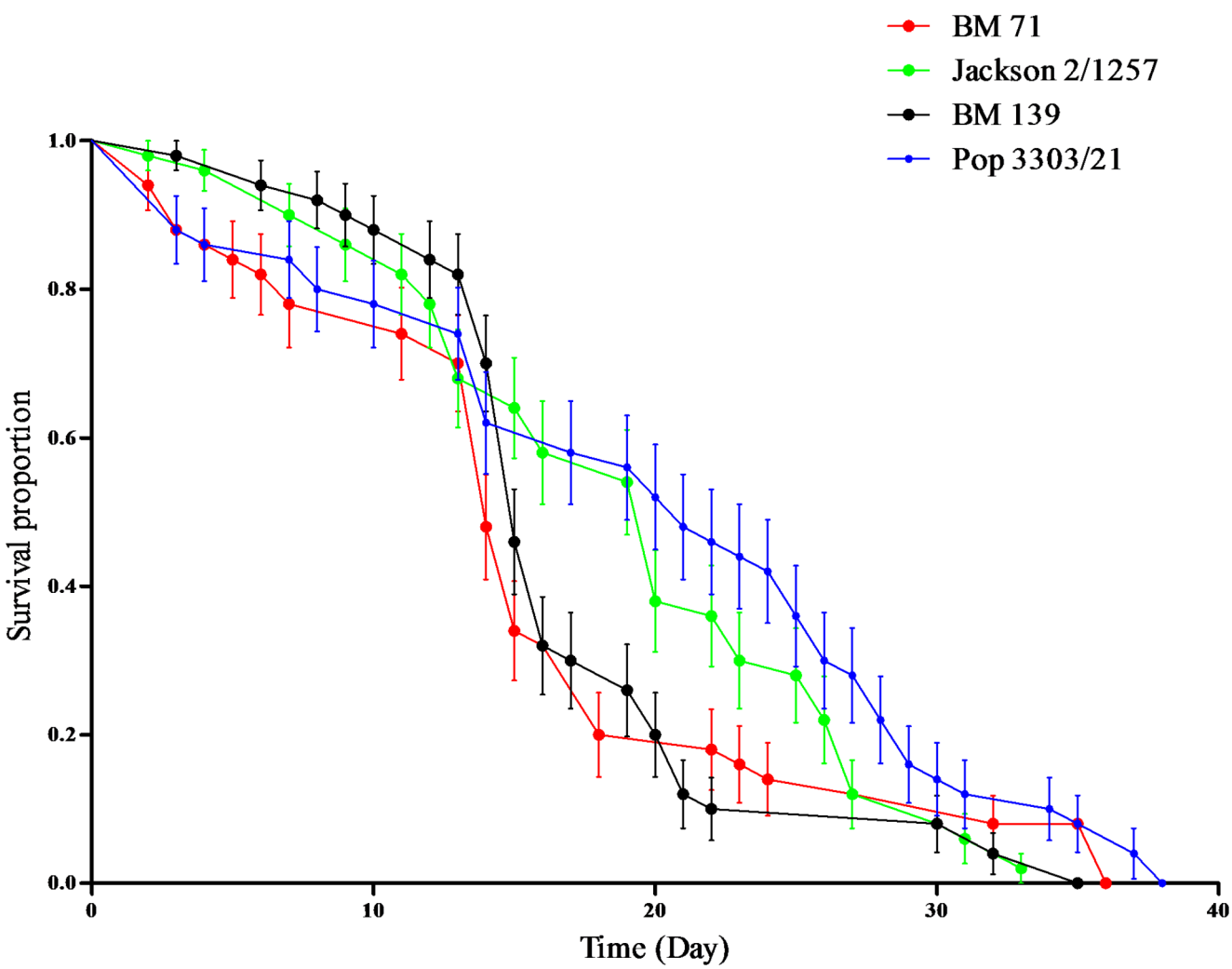


Fig. 1 Proportion survival of *T. aurantii* on the four coffee varieties (Mean + SE)

Different species of insects and fungi (Fig. 4) were collected during field experiments. Ladybird beetles were found feeding on black aphids.

Discussion

After performing ad-hoc test to separate means using Newman-Keuls multiple comparisons (Table 1); there is significant variability in the susceptibility of coffee to the black citrus aphids, *T. aurantii*. Laboratory and field experiments indicate that the variety BM71 can be less susceptible, while BM139 can be the most susceptible to *T. aurantii*. This variability in aphid infestation among coffee varieties may be from differences in chemical composition. For instance, tea plants can emit over 80 volatile compounds (e.g., (E)-2-hexenal), which can attract black aphids. Similar molecules can exist in commercial coffee varieties at varying concentrations and can influence their attractiveness to aphids. Analyzing the chemical content of these varieties and evaluating their impact on the feeding preferences of *T. aurantii* is crucial. Additionally, elevated nitrogen and potassium levels in nutritional solutions have been linked to increased nymph and adult insect populations in coffee plants over time. Therefore, assessing the quantities of nitrogen and potassium in BM71 is important.

The presence of natural enemies, such as ladybird beetles and the moth *Eublemma scitula*, plays a crucial role in reducing aphid and green-scale infestations over time (Murphy 1991). The larvae of *E. scitula* cover themselves with the dead bodies of scales for self-protection against ants and other predators. These predatory butterflies have also been observed feeding on olive scales in Tunisia (Mansour et al. 2011), citrus pests in the Mediterranean region (Vacante and Gerson 2012), and green scales in coffee plantations in Kenya (Murphy 1991).

Other predators were identified in our coffee experiments and are likely to feed on black citrus aphids; among these,

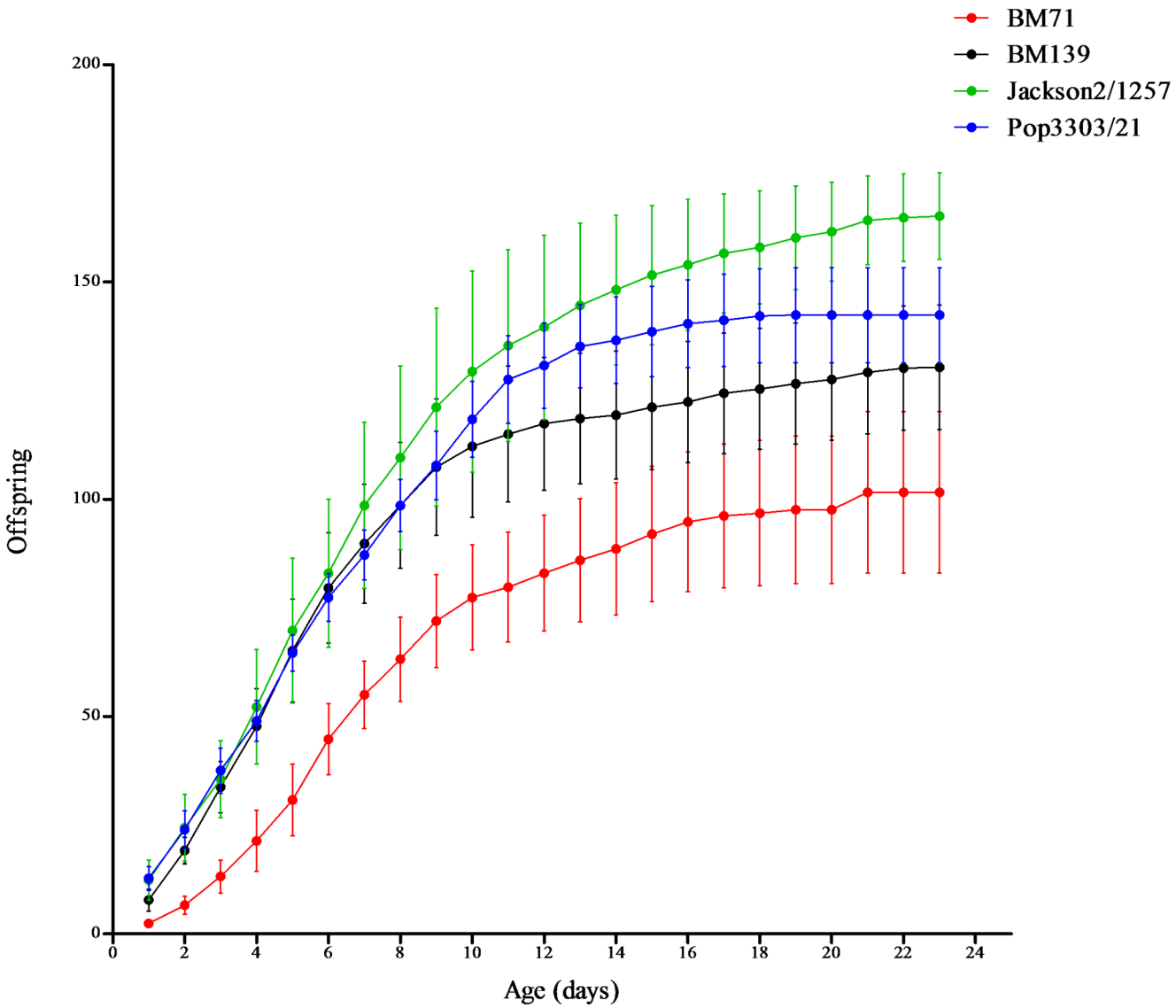


Fig. 2 Cumulated net fecundity (Mean number ± Standard Error (SE)

Table 1 Mean separation of four coffee varieties based on Newman-Keul's multiple comparison. comparison. marked with Ns indicate no statistically significant difference, suggesting similar responses between those varieties. The significance of differences between pairs of varieties is indicated by asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$)

Newman-Keuls's Multiple Comparison	Summary	95% CI of diff
BM71 vs. BM139	*	-295.0 to -11.65
BM71 vs. Jackson2/1257	***	-376.6 to -93.24
BM71 vs. Pop3303/21	**	-324.9 to -41.54
BM139 vs. Jackson2/1257	ns	-223.3 to 60.10
BM139 vs. Pop3303/21	ns	-171.6 to 111.8
Jackson2/1257 vs. Pop3303/21	ns	-89.98 to 193.4

the species *Cheilomenes lunata* was the most predominant predator observed. These findings are consistent with previous research, which highlights that *C. lunata* is widely reported across (Murphy 1991; Magagula et al. 2001; Saethre et al. 2011; Bayissa et al. 2016).

In conclusion, this study highlights significant variability in the susceptibility of coffee varieties to *T. aurantii*, with BM71 showing resistance and BM139 being more susceptible. The role of natural enemies, particularly *C. lunata* and *E. scitula*, can be pivotal in reducing pest populations, supporting the integration of biological controls with less susceptible varieties for sustainable pest management. Future research should focus on chemical content of coffee varieties, the impact of soil nutrients, and enhancing biological control strategies while validating these findings through long-term field trials in diverse agroecosystems.

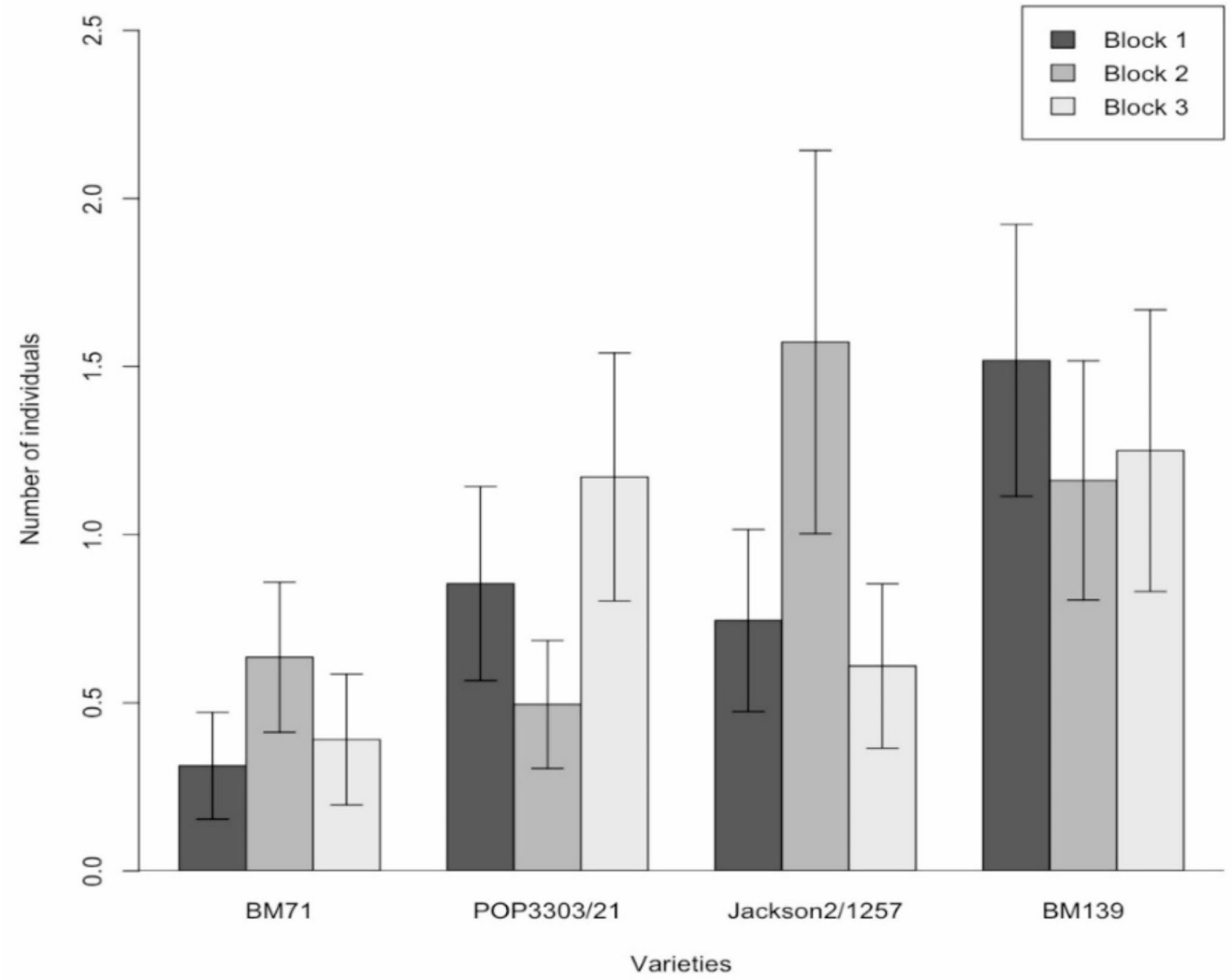


Fig. 3 Variation of aphid populations (Mean ± SE) on the four coffee varieties

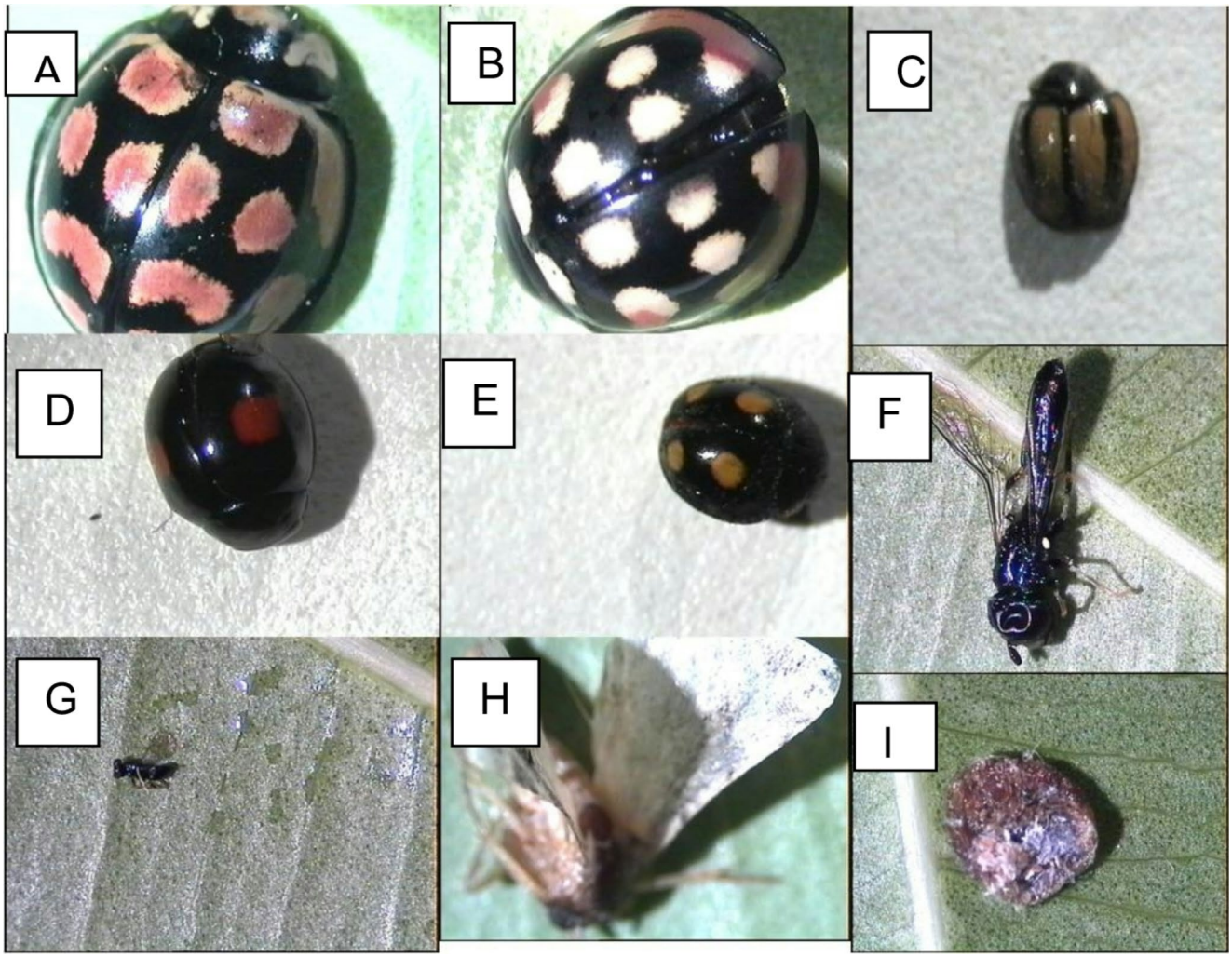


Fig. 4 Natural enemies and entomophotorales: **A**: *Cheilomenes lunata* Fabricius (Coleoptera: Coccinellidae), **B**: *Cheilomenes aurora* Gerstäcker (Coleoptera: Coccinellidae), **C**: *Chilocorus distigma* (Coleoptera: Coccinellidae), **D**: *Chilocorus nigripes* Mader (Coleoptera: Coccinellidae), **E**: *Hyperapsis* sp (Coleoptera: Coccinellidae), **F** & **G**: Syrphidae, **H**: *Eublemma scitula* (Lepidoptera: Erebidae), **I**: Entomophthorales (*Paecilomyces fumosoroseus* (Hypocreales: Cordycipitaceae)

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Author contributions HA Carried out the experimental designs, GL analyzed the data, MM confirmed the identity of *T. aurantii*, while RNDT and HT supervised the work.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest related to this article. This research was conducted independently and without funding or support from any organization or party with a direct interest in the results.

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