

**CONSEQUENCES OF COFFEE VARIETIES ON LIFE- HISTORY TRAITS OF
TOXOPTERA AURANTII (Hemiptera: Aphididae)**

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ABSTRACT

Coffee yields are primarily insects-limited. This study was conducted to determine the susceptibility of four common varieties which are under expansion in Rwanda. The influence of four coffee varieties BM71, BM139, Jackson2/1257 and Pop3303/21 on life history parameters of *Toxoptera aurantii* was studied in a controlled environment. The colony of *T. aurantii* was reared on varieties of coffee in the laboratory at $22 \pm 1^\circ\text{C}$ and photoperiod 16-8h (L:D). The survivorship, the number of offspring in the F1 generation ($\sum l_x m_x$), the intrinsic rate of natural increase (r_m), the finite rate of increase (λ) and the mean generation time (T) on each coffee variety were compared with host plant susceptibility. Results showed that there was a significant difference among coffee varieties for F1 generation ($\sum l_x m_x$). The population reared on BM139 had the highest intrinsic rate of natural increase ($r_m = 0.354$ individuals per day) and population doubling time (t_2) was 2.00 days while the population reared on BM71 had intrinsic rate of natural increase (r_m) was 0.237 and predicted time to double the population (t_2) was 2.92 days. Variety BM71 was less susceptible to *T. aurantii* while Jackson2/1257 was the most susceptible variety. Other varieties were moderately susceptible. Variety BM71 could prove useful in the development of an integrated pest management program for *T. aurantii*.

Keywords: *Toxoptera aurantii*, variety, life history, plant resistance, life table

1. INTRODUCTION

Coffea arabica L., is the backbone of the economy of developing countries (Nair, 2010). Two species of the genus *Coffea* are grown in East Africa, *C. arabica* and *C. robusta* (Bunn, Läderach, Rivera, *et al.*, 2015). *C. arabica* is preferred to other coffee species because it is taken as superior quality for its taste and organic nature and it offers superior cup quality and aroma (FUAD, 2010; Gemechu *et al.*, 2016). *C. robusta* is in decline due to its harsh flavour, its high susceptibility to pests, poor production and its susceptibility to low temperatures (Chu, 2012; Bunn *et al.*, 2015). Coffee production is constrained by complex factors, including losses due to

the damage by pests and diseases. The aphid *Toxoptera aurantii* has been reported as a pest of coffee for a long time (Le Pelley *et al.*, 1968; Barrera, 2008). The coffee aphid is one of the most important sucking pests on coffee, with a worldwide distribution (Lamb *et al.*, 1974; Wintgens *et al.*, 2004). In case of high infestation, *T. aurantii* causes significant damage, resulting in drying of the young plants (Waller *et al.*, 2007). In addition, the honeydew excreted by the aphids promotes the growth of saprophytic black fungi on the leaves and interferes with plant physiology such as photosynthesis (Barrera, 2008). The aphid is also a vector of the Coffee ring spot virus (Waller *et al.*, 2007). The fruiting and flowering stages of coffee are most sensitive to this pest and it is during this period that serious losses are recorded on mature coffee. *T. aurantii* is dark brown or black, its antennae have white and black bands; the most specific characteristics are stimulatory organ on the abdomen, black pterostigma and a single branch in the media of the forewing (Vacante *et al.*, 2012). It is reported to be the vector of different viruses for instance *Coffea ring spot virus* and *Citrus triteza virus* on citrus plants (Alford, 2014). The understanding of its biology in particular its life history on different varieties of coffee cultivated in Rwanda is very important for developing reliable management strategies. The present *T. aurantii* management strategy in Rwanda relies on the use of insecticide. The aphid is however developing resistance to pesticides (Whalon *et al.*, 2008). Using a chemical control strategy reduces the abundance of natural enemies and other beneficial insects, for example pollinators, and increases pesticide residues in the environment (Hazarika *et al.*, 2009). The use of resistant or less susceptible varieties to insect pest reduces the load of pesticides usage over the crop and is an efficient and environmentally friendly strategy to prevent infestation. In addition to this, resistant coffee varieties on which the shows delayed growth and development times may increase exposure time to natural enemies, resulting indirectly in a biological control strategy. Plant resistance is usually measured by visual screening of the plant in natural environment. This type of plant screening is however, more demanding in agriculture inputs like fertilizers, pesticides, capital, land, technology, labor and time. Simple, rapid and cheap methods have been used in the laboratory to screen plants for their resistance to insects. For instance, life table parameters, including the number of offspring in the F₁ generation ($\sum l_x m_x$), the survivorship, the intrinsic rate of natural increase (r_m), the finite rate of increase (λ) and the mean generation time (T) have been used to evaluate the susceptibility of the plant to the host in the laboratory. Using a life table helps to identify the current growth of the insect population and to predict the future population growth (Carey, 1993). The r_m is used to evaluate the susceptibility, where low values indicate that the variety is less susceptible, but in some cases the value of r_m may become high in less susceptible varieties (Kennedy *et al.*, 1995). Intrinsic rate of natural increase (r_m) is not enough to make a decision. Values of intrinsic rate of natural increase are complemented by the predicted number of offspring in F₁ generation to make the final decision. The present research was carried out to evaluate the life history parameters of *T. aurantii* on four varieties of *C. arabica* cultivated in Rwanda.

2. MATERIAL AND METHODS

Host varieties. On 19th November 2013, seeds of four commercial varieties of coffee (*C. arabica*) BM71, BM139, Jackson2/1257 and POP3303/21 were collected from Rwanda Agricultural Research Institute (2^o 06' S 29^o 48' E) in Southern Province; P.O. Box 621 Kigali. On 7th December 2013, Seeds were sown in forest soil in green house of Earth and Life Institute, ELI/catholic University of Louvain, Carnoy 4-5, 1348 Louvain –la- Neuve/Belgium. The first germination (BBCH-code: 00-09) appeared on January 12th, 2014. Generally, the seed germination took six weeks. On 5th February 2014, seedlings (BBCH-code: 10-19) were transplanted to pots (25-30cm) in a greenhouse maintained at a constant temperature of 25°C. Plants were kept separately, watered with rain water and checked every day. Neither synthetic fertilizers nor pesticide were applied to either seeds or seedlings. The coffee varieties were fed to aphids when they are starting to produce vegetative propagation materials (BBCH-code: 40-49).

Insect rearing. On 10th October 2015, laboratory colonies of *T. aurantii* were established with field-collected aphids from a tea plant *Camellia sinensis* L. in Rwanda (OCIR-THE, National Agriculture Export Board, Kitabi tea factory, Southern Province, 104 Kigali-Rwanda). Stock colonies were maintained on potted seedlings (25-30) cm tall of harrar variety of Coffee (the variety which was not tested in this experiment). The potted seedlings were caged to ensure that aphid could not escape from the plant. The research was carried out in an insect-rearing room at 22 ± 1°C, at ambient humidity (50% RH) and photoperiod 16: 8h (L: D). After a 2-month rearing period, colonies were ready to be used for the experiment.

Longevity and survivorship. On 15th October 2015, a cohort of 10 wingless adult aged of 8 days was transferred to the seedling (Harrar variety) and was allowed to reproduce for 24 h. On 16th October 2015, 10 apterous females, had been gently removed using a camel's hair paintbrush and their newborn larvae (aged of 1 day) were kept 10 new-born nymph per cage with 5 replicates and placed in an environmental room under stable optimum temperatures at 22±1°C, at ambient humidity of 50% RH and photoperiod 16: 8h (L:D). Every day; the survival and the development stage was observed to determine the nymph stage and its duration (Appendix 6). Aphids were checked daily until the last aphid died. Fresh coffee seedlings were provided once per week.

Fecundity. After the nymphs reached adulthood, four wingless adult females of *T. aurantii* were placed on the coffee seedlings. There were ten replicates. In total 40 individuals per variety were followed. Adult mortality and fecundity were recorded daily. Offspring were counted and removed from each seedling until the last female died. The whole experiment was conducted in the laboratory from December 15th, 2015 and ended on 16th February 2016.

Statistical analysis. From the daily observations, different biological parameters (appendix, 2, 3, 4 and 5), e.g. net reproductive rate (R_0), 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 cumulated net fecundity of *T. aurantii* on the four coffee varieties. Life history traits were described using equations (Carey, 1993) summarized in table 1. Significant differences among treatment means were determined by using Newman-Keuls multiple comparisons at a probability level of 5% ($P < 0.05$).

Table 1: Terminology and formulae used to calculate demographic parameters

No	Formula/Equation	Demographic parameter
1.	$1 = \sum_{x=0}^{\infty} e^{-rm} l_x m_x$	r_m , the equation must be solved by iteration
2.	$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$	R_0 , net reproductive rate (number of females/newborn females)
3.	$t_2 = \frac{\ln 2}{r_m}$	The time required by the population to double.
4.	$T = \frac{\ln R_0}{r_m}$	Generation time
5.	$\lambda = e^{rm}$	Finite rate of natural increase

3. RESULTS

The survival analyses showed that there was no significant difference between the survival curves of *T. aurantii* over time on the tested commercial coffee varieties. Both, the Log-rank (Mantel- cox) test ($\chi^2 = 6.4$, $df = 3$, $P = 0.09$) and Log-rank test for trend revealed that survival curves are not significantly different (Figure .1). The figure 1 shows that, there was a high mortality between the 15th day and 25th day.

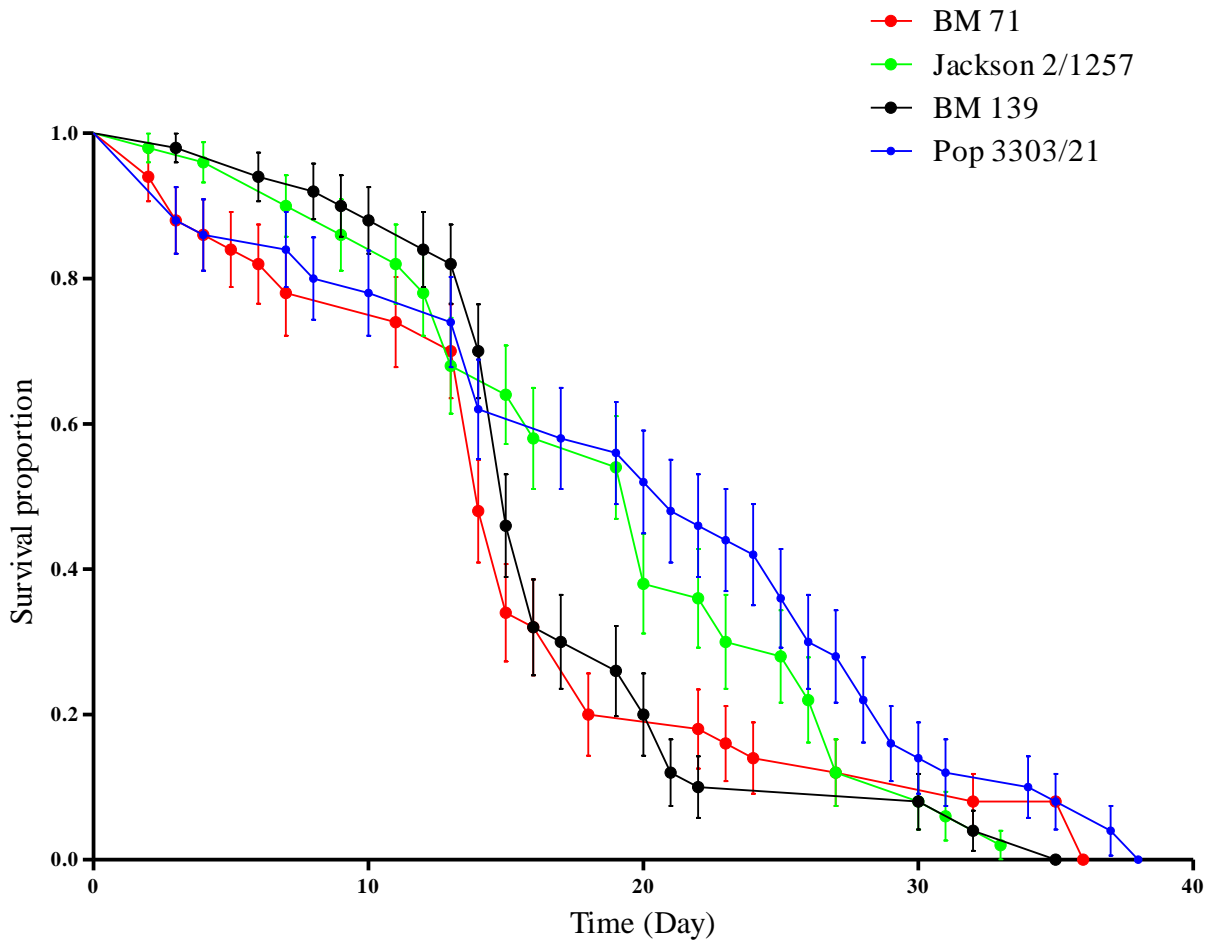


Figure 1. Percent survival of *T. aurantii* on the four coffee varieties (Mean+ SE); ($\chi^2= 6.4$, df = 3, P= 0.09).

The life expectancy at birth was 13.5 ± 0.5 days on BM71 against $18, 3 \pm 0.8$ days on Pop3303/21 (Table .2).

Table 2. Survivorship and life expectancy at birth of *T. aurantii* on coffee varieties

Survivorship and life stages	BM71	BM139	Jackson 2/1257	Pop3303/3
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Survivorship ¹	86.1 ± 0.5	96.1 ± 0.6	95.5 ± 0.5	89,0 ± 0.5
Development time (days)	9.67	6.6	7.6	9,0
First day of larviposition	8.0	9.0	10.0 ± 0.0	11,0
% of larviposition at day one	2.5 ± 0.0	22.5 ± 0.1	12.5 ± 0.1	7,3 ± 0.2
Life expectation (days)	13.5 ± 0.5	15.2 ± 0.6	17.9 ± 0.8	18.3 ± 0.8

The analysis of net cumulative fecundity (Figure 2) of *T. aurantii* on four different varieties of coffee revealed significant differences among them ($F_{3, 22} = 60.51; P < 0.001$). The value of cumulative fecundity was lowest on the variety BM71 and highest on Jackson 2/1257 while values of cumulative fecundity on other varieties were in between (Table 3). The feeding suitability was different among coffee varieties.

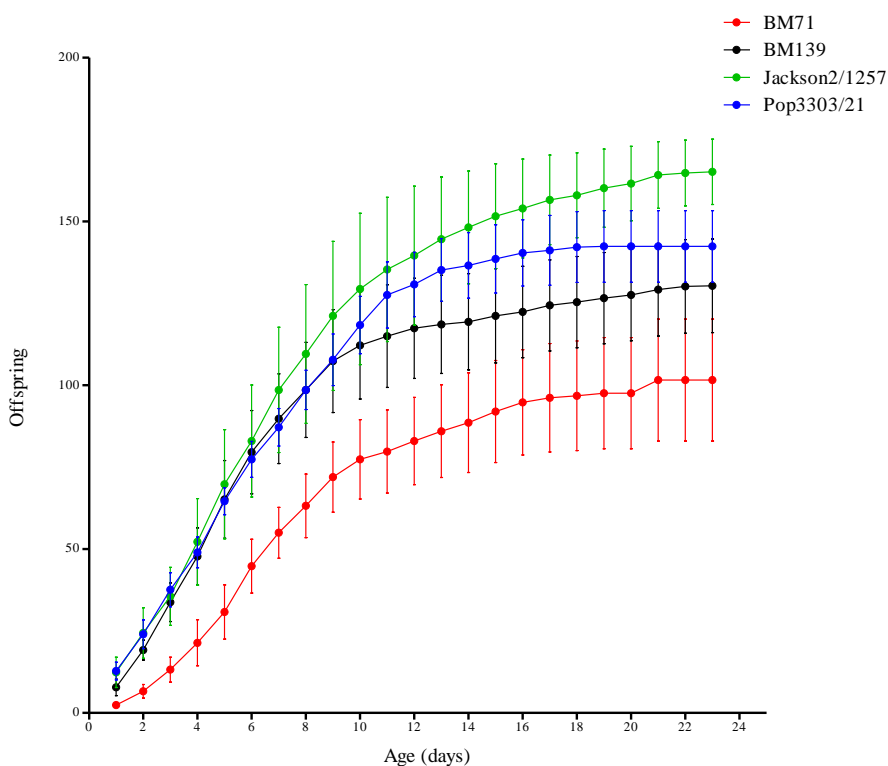


Figure 2. Cumulated net fecundity (Mean number ± Standard Error (SE)).

¹ The survival rate of immature stages (before they reach the adulthood stage)

The life and fecundity tables allow us to compare demographic parameters including the intrinsic rate of natural increase (r_m), net reproductive rate (R_o), finite rate of natural increase (λ), doubling time (t_2) and generation time (T) of the tested coffee varieties. Intrinsic rate of natural increase did not vary significantly (Table 3) between varieties ($F_{3,16} = 2.82, P = 0.346$). The intrinsic rate of natural increase value on BM139 ($r_m = 0.345$ individuals per day) indicates that under optimum conditions, the growth rate per day of population of aphid reared on BM139 was 0.345 individuals per capita. The intrinsic rate of natural increase of *T. aurantii* reared on BM71 had the lowest value ($r_m = 0.237$ individuals per day) and those on MB139 had the highest value ($r_m = 0.345$) while Jackson2/1257 and Pop3303/21 had intermediate values (Table 3). This is illustrated by the finite rate of increase (λ) which shows the factor by which a give population may be expected to multiply every day.

Table 3. Life table statistics for *T. aurantii* on different varieties of coffee

Life history traits	BM71	BM139	Jackson2/1257	Pop3303/3
R_o	6.30 ± 0.1	8.10 ± 0.1	10.13 ± 0.1	8.86 ± 0.1
$\sum l_x m_x$	170.1 ± 10.3	254.14 ± 11.8	322.3 ± 15.3	270.04 ± 13.1
r_m	0.237 ± 0.0	0.345 ± 0.0	0.325 ± 0.0	0.321 ± 0.0
λ	1.27 ± 0.1	1.41 ± 0.7	1.38 ± 0.6	1.38 ± 0.6
T	8.38 ± 0.4	6.63 ± 0.4	7.29 ± 0.5	6.72 ± 0.5
t_2	2.92 ± 0.2	2.00 ± 0.2	2.12 ± 0.1	2.15 ± 0.1

R_o : Net reproductive rate; $\sum l_x m_x$: Cumulated net fecundity; λ Finite rate of increase
 r_m : Intrinsic rate of natural increase; T: Generation time; t_2 : Doubling time .

The finite rate of increase was quite similar on Jackson2/1257 and Pop3303/21 ($\lambda = 1.38$) but had low value on BM71 ($\lambda = 1.27$). The values of R_o varied among varieties ($F_{3,16} = 5.33, P < 0.0097$ and were higher on Jackson 2/1257 ($R_o = 10.13$) and on Pop3303/21 ($R_o = 8.86$). The net reproductive rate of *T. aurantii* reared on BM71 and BM139 was lower than on the other two varieties (Table 3). The time needed for doubling (t_2) the population is 2.92 days on BM71 against 2.00 days on BM139. There was a small variation of T among varieties ($F_{3,16} = 3.1450, P = 0.054$). The time required for a population of *T. aurantii* to increase by a factor equal to R_o had the highest values on BM71 (8.38 days) and were the lowest on BM139 (6.63 days). Other coffee varieties Jackson2/1257 and Pop3303/21 had intermediate values of generation time (T).

4. DISCUSSION

Evaluating the susceptibility of a plant to an arthropod pest using life history traits and population growth parameters is a well-recognized technique (Golizadeh & Abedi, 2016; Golizadeh *et al.*, 2016). The lifecycle of *T. aurantii* was studied on four coffee varieties BM71, BM139, Jackson2/1257 and Pop3303/21 all of which it was to survive and reproduce. . The variety BM71 was less suitable to black citrus aphids while Jackson2/1257 was most suitable. The other tested coffee varieties were of intermediate suitability under laboratory conditions. The development time from first instar to adult ranged from 8 to 13 days for *T. aurantii* on coffee under optimum conditions. The shortest developmental time was on BM139 (8days) while the longest developmental period was on Pop 3303/21(13days). The duration of each instar varied between varieties but the difference was not significant; being shortest on BM139, BM71 and Jackson 2/1257 and largest on the variety Pop3303/21. The life expectancy at birth is 13.2 days on BM71. This indicated that, BM71 is the least susceptible variety. It is negatively affected the growth, the survivorship and the development of *T. aurantii*.

Intrinsic rate of natural increase (r_m) is a vital parameter to evaluate the growth of a population because even a small difference in the value of the intrinsic rate of natural increase (r_m) could make a big difference in the abundance of herbivore populations (Lawton *et al.*, 1979). The value of r_m indicates if a population increases geometrically ($r_m > 0$), remains constant in size ($r_m = 0$) or tends to extinction ($r_m < 0$) (Gotelli, 2008). The r_m of *T. aurantii* reared on four coffee varieties showed that the population increased exponentially. The lowest value of $r_m = 0.237$ individuals per day was observed on BM71 while the highest ($r_m = 0.325$ individual of females per female per day) was found on Jackson2/1257. However, intrinsic rate of natural increase is effective when the population has attained the stable age structure (Van Impe *et al.*, 1993). In fact, it is not only the r_m value which determines the susceptibility of varieties but also the measurement of predicted number of F1 generation and other demographic parameters must be taken into consideration before definitive conclusion can be drawn concerning the variety susceptibility.

Net reproductive rate (R_o) is the average number of female offspring that would be born to a birth cohort of females during their lifetime (Carey, 1993). Based on the R_o values, we noticed that there is a significant difference between coffee varieties ($F_{3, 22} = 60.51$; $P < 0.001$). The variety BM71 was not suitable ($R_o = 6.3$ female individuals/one newborn female) for the reproduction of *T. aurantii* while Jackson2/1257 was suitable with a high value of net reproductive ($R_o = 10.13$ female individuals/one newborn female).

The other demographic statistic used was, mean generation time T which according to (Carey, 1993) is defined in two ways: The first definition is the mean age of reproduction, which characterizes T as the mean interval separating the births of one generation from those of the next. The second definition of T is the time required for a population to increase by a factor equal to the net productive R_o . Results were interpreted taking into consideration the second definition. The variety BM71 which is less susceptible to *T. aurantii* requires 8.38 days to increase its population by the factor $R_o = 6.3$ females per one newborn female while the variety

Jackson2/1257 requires 7.29 days to increase its population by the factor $R_0 = 10.13$ females per one newborn female.

Black citrus aphids performed well on Jackson2/1257 and worst on BM71. This lower susceptibility of BM71 may be explained by poor fecundity and growth times observed on this variety which according to (La Rossa et al., 2013) also might be explained by the content of plant defense chemicals. According to (Awmack et al., 2002), the nutritional quality of the host affects the fecundity of insects at both individual and population scale. The variety BM71 may have both poor nutritional values and high content of antibiosis. Then this variety could prove useful in development of integrated pest management for *T. aurantii* in coffee plantations. It would be useful to investigate exactly which plant defense chemicals are involved in these varieties to get more information on their role in determining plant resistance in coffee.

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