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Effect of Temperature on Life History of *Bemisia Tabaci* (Gennadius) (Homoptera: Aleyrodidae) on Cassava *Manihot Esculenta* Crantz

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Abstract

The aim of the present study was to evaluate the relationships between environmental temperatures and life-history traits of whitefly *Bemisia tabaci* on cassava in the Northern part of Vietnam. The influence of temperature on the biological characteristics of whitefly *B. tabaci* fed on cassava was evaluated at 20°C, 25°C, 27.5°C, and 30°C using individual insect rearing methodology. Results of the study showed that the development time from egg to adult of *B. tabaci* was influenced by temperatures. The data indicated that under different temperatures (20°C, 25°C, 27.5°C, and 30°C), the life cycle of *B. tabaci* were 41.46, 28.64, 24.29, and 20.25 days, respectively. The determination of lower developmental threshold and degree days for whitefly development were 11.2°C and 344.8 degree-days. There were 14.31 whitefly generations within 1 year on cassava suggesting this plant is a suitable host plant for the development and reproduction of *B. tabaci*. Female longevity ranged from 4.92 to 10.23 days. The fecundity ranged from 49.3 to 74.0 eggs/female. The mortality rate reached its highest rate of 36.27% at 30°C. Our results suggested that *B. tabaci* had high reproduction rates and demonstrated their positive fitness traits on cassava in a wide range of temperatures, being a potential important pest of cassava cultivars.

Keywords

Bemisia tabaci, cassava, temperature, life table, mortality rate

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Introduction

Whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is one of the most serious pests on many crops such as pulses, cotton, cucumber, tomatoes, eggplant, tobacco, and cassava in tropical and subtropical areas. The damage of crops caused by *B. tabaci* leads to reductions in yield and product quality. In addition, this pest can cause damages directly through plant sap-feeding and indirectly through the transmission of plant pathogenic viruses, primarily *begomoviruses* (Geminiviridae), *Crinivirus* (Closteroviridae), and

Carlavirus (Potyviridae), such as yellow leaf curl virus, bean yellow mosaic Virus**,** Sri Lankan Cassava Mosaic Virus (SLCMV) (Oliveira *et al*., 2001; Jones, 2003; Legg *et al*.,2014; Swati *et al.*, 2017; Khan & Wan, 2015; Wang *et al.*, 2018; Plant Protection Department of Vietnam, 2018). More than 900 host plants have been recorded for *B. tabaci* belongings to over 63 plant families (Perring, 2001; Buxton, 2005). Biological characteristics of *B. tabaci* have been studied for various host plants, different temperatures reported as cotton, tomato, tobacco, and soybean, etc. (Le Thi Lieu & Tran Dinh Chien, 2004; Touhidul & Ren, 2007; Tran Dinh Pha *et al.*, 2008; Dam Ngoc Han *et al*., 2012; Le Thi Tuyet Nhung, 2014; Khan & Wan, 2015). *B. tabaci* normally completes life development in 2 to 3 weeks in warm weather, but it takes approximately 2 months under cool conditions. The development time from egg to adult whitefly ranges from 15-70 days, dependending on the temperature and host plant (Swati *et al*., 2017). The longevity of *B. tabaci* adults ranges from 3.1 to 15 days at a temperature above 20°C, but the low temperature (10ºC) significantly increases the longevity to 30-60 days (Gerling *et al.*, 1986). The average number of eggs produced per female ranges from 50 to 400 (Swati *et al.*, 2017; Carabali *et al*., 2010).

In Vietnam, cassava is the third key food crop after rice and maize, with the cultivating area reaching 519,300ha with a yield of 10.1 million tons in 2019 (General Statistics Office of Vietnam, 2019). In 2018, it was reported that Sri Lankan Cassava Mosaic Virus (SLCMV) appeared on cassava in Vietnam due to whitefly *B. tabaci* ASIA1 transmission and raised concerns of widespread diseases (Uke *et al.*, 2018). The Plant Protection Department of Vietnam (2020) reported that a total of 42.000 hectares had been affected by SLCMV, which covered 17 provinces in the Southern and Central parts of Vietnam. Therefore, studies on cassava feeding whitefly and their life-history traits (i.e., development time, survival, and reproduction rate) in relation to environmental temperature are important to provide baseline information for the development of pest and disease control strategies on cassava production in Vietnam.

Materials and Methods

The culture of host plants

Cassava variety KM419 young plants were grown in small pots (10cm diameter and 8cm height) containing a mix of 1/3 prepared garden soil + 1/3 rice husk ash + 1/3 coconut coir and kept in a net cage (1m x 1m x 1m). Three-week-old plants were introduced to *B. tabaci* adults for their infestation in the stock culture.

The culture of *B. Tabaci*

Whitefly stock colony was collected from cassava fields at Hoa Binh province. Newly emerged adults of *B. tabaci* were kept on leaves of potted cassava plants, and the whole plants were placed in separated cages sized 40 x 40 x 80cm in the laboratory of the Department of Entomology, Faculty of Agronomy, Vietnam National University of Agriculture. The whitefly culture colony was then maintained under laboratory conditions with a temperature of 26-28ºC, relative humidity of 70-75%, and a 16:8h (L:D) photoperiod. The next generations of adults' *B. tabaci* colony were used as the test materials in this study.

The influence of temperature on the development, mortality, and fecundity of *B. tabaci*

Twenty pairs of early emerged adults of *B. tabaci* (20 pairs: male and female) were placed in each isolation plastic cages (45cm x Ø 20cm) containing a two-leaf cassava plant, and the top of the cage was covered with mesh for ventilation. Adult whiteflies were placed and left for 3 hours in the cages for laying eggs, then being removed. Deposited eggs were transferred into the climate chamber at 20, 25, 27.5, and 30 ± 0.5ºC, 70-75% RH, and a 16:8h (L:D) photoperiod. Eggs located on the cassava leaves were marked and kept in clip cages to prevent whiteflies from escaping. Daily observation was made under a binocular microscope to determine egg hatching, nymphal development, and adult eclosion. Individual nymphs were checked every 12h to determine the development time of each immature stage until adult whiteflies emerged, and the sex ratio was determined (Carabali *et al*., 2010).

Mortality rate

One-hundred freshly laid eggs were located and kept on the leaves of the cassava host. The plants were then transferred to the climate chamber. The number of hatching nymphs was counted to determine whitefly embryonic mortality. Mortality during the nymphal instars was calculated by counting the number of individuals developing to the next stage.

Adult longevity

Pairs of newly emerged *B. tabaci* adults were introduced in plastic cages, each of which contains a pot of two-leaf cassava plants. All cages were kept in climate chambers under 4 temperature conditions that were mentioned above for daily observations until all the adults died.

Fecundity

One newly emerged female and male were placed into the plastic cages attached to the cassava plant. The number of eggs deposited by a single female was counted daily until they all died. There were 11-14 replications for each temperature were observed.

Statistical analysis

One-way ANOVA was performed to examine the differences in the development times and if significant ($P \leq 0.0001$) differences were found, the Turkey post-hoc test was used to rank the group. To estimate the low developmental threshold temperature for each immature stage, they were regressed against temperatures using a least-square linear regression. Low developmental threshold temperatures were calculated as:

$$y = a + bx$$

where y = developmental rate, a = constant term, and b = regression coefficient.

The lower developmental threshold (T_o) is determined as:

$$T_o = -a/b$$

The sum of effective temperatures (K):

$$K = 1/b$$

The thermal units required for completion of development of each stage are determined according to the equation of thermal summation:

$$K = y\,(T - T_o)$$

where y = developmental rate, T = temperature in Celsius degree, T_o = lower threshold of development, and K = thermal constant (degree-days- DD) (Virachack *et al.*, 2018).

Results and Discussion

The influence of temperature on the development time of B. tabaci

The whitefly development time from eggs to adults decreased when the temperature increased (**Table 1**), whereby there was a significant difference between the egg and nymph development periods by the change of temperature from 20°C to 30°C ($F = 791.68$; $df = 3$; $P < 0.0001$). The longest developmental duration of *B. tabaci* recorded was 41.46 days at 20°C, while the shortest one lasted only 20.25 days at 30°C. At the temperature of 20ºC, 25ºC, 27.5ºC, and 30ºC, eggs lasted for 9.61, 6.16, 5.30, and 4.32 days, respectively. The results indicated that the development time of egg and fourth nymph (pupal) instar were longer than other stages, which is in accordance with the finding of other studies. Some authors have reported that mean development times of first to third nymphal *B. tabaci* were usually slightly shorter than for its remaining stages (Powell & Bellows, 1992; Sohani *et al.*, 2007). The pre-oviposition time of female *B. tabaci* was significantly different when rearing at different temperatures (2.85 days at 20°C, 2.09 days at 25°C, and 1.42 days at 30°C). The effects of temperature on development time of *B. tabaci* were demonstrated on other host plants. In a comparable study Sohani *et al.* (2007) obtained total developmetal time on cucumber at 20°C, 25°C, and 30°C were 34.84, 19.23, and 14.0 days, respectively. Powell & Bellows (1992) recorded development times of 38.20, 20.22, and 17.43 days on cucumber at 20°C, 25.5°C, 29°C, and 32°C, respectively. Other studies showed that on soybean at 17°C, 21°C, 25°C, 29°C, and 33°C the life cycle of whitefly were 62.3, 44.9, 27.7, 19.1, and 18.4 days, respectively (Dam Ngoc Han, 2013); at 24.4°C and 17°C on tomato, development times were 23.7 and 61.7 days, respectively (Le Thi Tuyet Nhung, 2014). Our study results confirm the consistent positive effect of temperatures on the development rate of whitefly.

Furthermore, Carabali *et al.* (2010) looked at rearing whitefly biotype B on different cassava varieties in Colombia, in which *B. tabaci* needed at least 33.3 days to complete its life cycle at 25ºC, 70% RH. The present result reported that the total life cycle of *B. tabaci* was only 28.64 days, thus further experiments are needed to explain the effects of cassava host plants on local whitefly populations.

Determining threshold temperatures and degree-days of *B. tabaci* on cassava will allow people to predict the number of generations in a year. The whitefly lower threshold temperature (T_o) varied from 10 to 12.0°C among developmental stages and degree-day (K) ranged from 59.2 to 80.6°C (**Table 2**). The influence of temperature on egg development was indicated by a linear regression equation (y = 0.0124x – 0.15) with a high correlation coefficient (r^2 = 0.99). This indicated that an increase in temperatures could lead to higher egg developmental rates, thus supporting the positive correlation between the variables. The lower developmental threshold (T_o) of *B. tabaci* eggs, therefore, was estimated to be 11.8°C, and the thermal constant for the development of eggs (K) was 80.6 day-degrees. Our results were higher than that of Sohani *et al.* (2007) who conducted research on whitefly on cucumber, whereby the lower developmental threshold (T_o) of *B. tabaci* eggs was 14.72°C, and the thermal constant was 64.44 day-degrees.

At the nymphal stage, the lower developmental threshold (T_o) of *B. tabaci* nymph instars was measured in the first, second, third, and fourth instar as 11.3, 11.4, 12.0, and 10.0°C, respectively, and the thermal constant (K) was 67.6, 59.5, 59.2, and 79.4 day-degrees, respectively. These results support the findings of Sohani *et al.* (2007) with whitefly on cucumber. The lower developmental threshold (T_o) of the first, second, third, and fourth nymphal instar were 11.3, 11.4, 12, and 10ºC, respectively. The thermal constant (K) was 67.6, 59.5, 59.2, and 79.4 day-degrees.

Our result also demonstrated that the egg to adult threshold temperature of *B. tabaci* was 11.2°C, which is consistent with the findings of Gerling *et al.* (1986) and Bosco & Caciagli (1998). However, the values for temperature threshold calculated here is slightly lower than those of Powell & Bellows (1992) (14.65°C on cotton and 16.71°C on cucumber), Sohani *et al.* (2007) (13.07°C on cucumber), and Le Thi Tuyet Nhung (2014) (12.37°C on tomato and 12.25°C on kohlrabi).

Table 1. Development times of *B. tabaci* on cassava at difference temperatures

Stages	Mean development time (days) (± SE)			
	20°C (n = 45)	25°C (n = 46)	27.5°C (n = 39)	30°C (n = 34)
Egg	$9.61^a \pm 0.11$	$6.16^b \pm 0.13$	$5.30^c \pm 0.09$	$4.32^d \pm 0.10$
1st nymphal instar	$7.55^a \pm 0.11$	$5.32^b \pm 0.08$	$4.00^c \pm 0.11$	$3.64^d \pm 0.12$
2nd nymphal instar	$6.85^a \pm 0.13$	$4.00^b \pm 0.10$	$3.63^c \pm 0.10$	$3.21^d \pm 0.08$
3rd nymphal instar	$6.94^a \pm 0.07$	$4.65^b \pm 0.08$	$4.23^c \pm 0.08$	$3.07^d \pm 0.05$
4th nymphal instar	$7.76^a \pm 0.10$	$5.89^b \pm 0.11$	$4.33^c \pm 0.10$	$4.00^d \pm 0.09$
Pre-Ovipostion	$2.85^a \pm 0.19$	$2.09^b \pm 0.09$	$1.86^{bc} \pm 0.10$	$1.42^d \pm 0.15$
Life cycle	$41.46^a \pm 0.33$	$28.64^b \pm 0.28$	$24.29^c \pm 0.37$	$20.25^d \pm 0.31$

Note: Humidity: 70- 75% RH. Means within a row followed by the difference letter are significantly different at P< 0.0001. n: Number observed.

Table 2. Developmental rates, threshold temperatures, and the thermal constants of *B. tabaci* on cassava

Stages	Rate regressed on temperature (°C) (X)				Y= -a+bx	R^2	T_o	K (DD)
	20°C	25°C	27.5	30°C				
Egg	0.104	0.162	0.189	0.231	y = 0.0124x - 0.15	0.99	11.8	80.6
1st nymphal instar	0.132	0.188	0.250	0.275	y= 0.0148x- 0.17	0.97	11.3	67.6
2nd nymphal instar	0.146	0.227	0.275	0.313	y = 0.0168x - 0.19	0.99	11.4	59.5
3rd nymphal instar	0.144	0.215	0.236	0.326	y = 0.0169x - 0.20	0.93	12.0	59.2
4th nymphal instar	0.129	0.179	0.231	0.249	y = 0.0126x - 0.13	0.97	10.0	79.4
Life cycle	0.026	0.038	0.047	0.055	y = 0.0029x - 0.03	0.99	11.2	344.8

The average number of degree-days needed to complete the egg to adult development was 344.8 degree-days , in agreement with the finding in other studies conducted at the same temperatures on bean (324 degree-days) and on cotton (316 degree-days) using simple regressions (Bosco & Caciagli, 1998; Zalom *et al*., 1985). On the other hand, our calculated number of degree-days is higher than the 229.52 degree-days and 195.0 degree-days on cucumber (Sohani *et al.,* 2007; Powell & Bellows, 1992) and 285.11 degree-days on tomato (Le Thi Tuyet Nhung, 2014). According to this result, the possible number of generations of *B. tabaci* in Hanoi, Vietnam is 14.31 generations per year on cassava plant, with the total thermal units required per year for *B. tabaci* is 4.935 degree-days. Similar studies also reported that 14.51 generations of *B. tabaci* can occur within 1 year on tomato (Le Thi Tuyet Nhung, 2014) or 11-15 generations can occur within 1 year depending on the climate conditions **(**Raveesh & Charu, 2018).

Mortality rates of *B. tabaci* adults

Mortality rates of *B. tabaci* on cassava under four different temperature conditions are shown in **Table 3**. The mortality rates of *B. tabaci* egg, nymph stages were affected greatly by temperature. Mortality during the embryonic stage, first, second, third, and fourth nymphal instars at 30°C were 18.0%, 6.10%, 3.90%, 4.05%, and 4.23%, respectively. Pre-adult mortality was the lowest at 19.26% at 25°C, whereas it was 22.72% at 27.5°C and 36.27% at 30ºC. At 20ºC, the mortality rate of *B. tabaci* was also high, at 25.99%.

We found a higher mortality of eggs than nymphs. In general, the most sensitive nymphal stage was the first nymphal instar. Sohani *et al.* (2007) found that on cucumber, the total mortality of *B. tabaci* was the highest, at 20°C (45.8%); at 25°C and 30°C, the mortality rates were 20.9% and 17.3%, respectively. Our result is in accordance with the previous study of Le Thi Tuyet Nhung (2014), whereby the total pre-adult mortality of *B. tabaci* on kohlrabi at 17°C (24.18%) was higher than that at 24.4°C (16.41%). In addition, Khan & Wan (2015) recorded the total mortality from whitefly eggs to adults, showing that the mortality was 34.4% on tomato and 28.2% on cotton. The mortality rate of the immature stages on cassava *M. carthaginensis* and *M. esculenta* in Colombia was 40.0% and 72.5%, respectively (Carabali *et al.,* 2010). Results from our study indicate that similar to most ectothermic organisms, the *B. tabaci* was sensitive to changes in temperatures. In other words, changes in temperatures had an influence on its development times and survival.

Longevity of *B. tabaci* adults

At all studied levels of temperature, the mean adult longevity of *B. tabaci* showed its negative correlation to environmental temperature (**Figure 1**). The longevity of females and males decreased as the temperature increased. The data indicated that temperature was a highly significant factor affecting the longevity of both females (F = 35.48; df = 3; P< 0.0001) and males (F = 80.22; df = 3; P< 0.0001). The maximum longevities observed for an individual whitefly were 10.23 and 9.29 days for females and males at 20°C. Mean adult longevity

Table 3. Pre-adult mortality of *B. tabaci* reared on cassava at different temperatures

Stages	The mortality rates (%) at different temperatures (°C)			
	20°C	25°C	27.5°C	30°C
Egg	14.00 (14)	9.00 (9)	11.00 (9)	18.00 (18)
1st nymphal instar	4.65 (4)	3.30 (3)	3.37 (3)	6.10 (5)
2nd nymphal instar	2.44 (2)	2.27 (2)	3.49 (3)	3.90 (3)
3rd nymphal instar	1.00 (3)	1.16 (1)	1.20 (1)	4.05 (3)
4th nymphal instar	3.90 (3)	3.53 (3)	3.66 (3)	4.23(3)
Total	25.99 (26)	19.26 (18)	22.72 (19)	36.27 (32)

Note: Sample size (n) in parenthesis is the number dying in each stage.

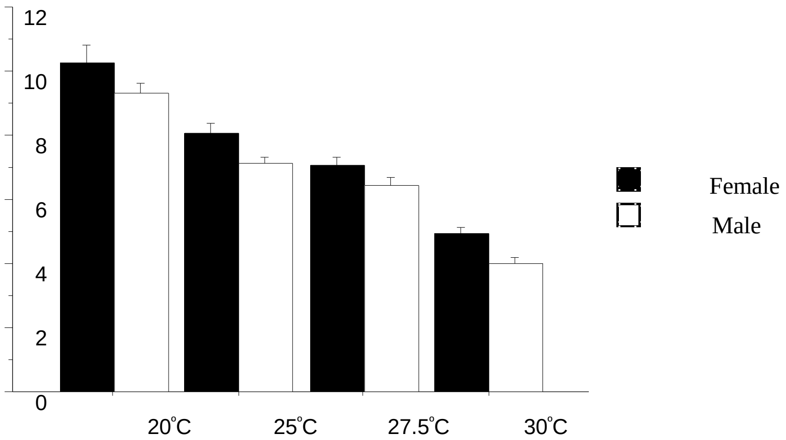

Figure 1. Longevity of *B. tabaci* adults at different temperatures

of females and males were significantly lower at 27.5°C with 7.07, 6.47 days in comparison with those at 25°C with 8.09, 7.11 days, respectively. The shortest longevity of *B. tabaci* was observed at 30°C and 4.92 days (female) and 4.0 days (male). Our present study collected data were slightly lower than those reported by previous publications at the same temperatures. The research of Sohani *et al.* (2007) showed that whitefly on cucumber had longer longevity than those indicated in this study, whereby the females lived 34.14, 26.75, and 16.88 days and male lived 29.50; 17.50 and 11.23 days at the temperature of 20°C, 25°C, and 30°C, respectively. Fekrat & Shishehbo (2004) found that on aubergine, at 20°C, 25°C, and 30°C, females lived for 18.14, 13.14, and 8 days, respectively; while males survived for 12.71, 9.78, and 5.92 days, respectively. However, Carabali *et al.* (2010) found at 25°C on wild and commercial cassava (*M. carthaginensis* and *M. esculenta*), the longevity of *B. tabaci* were only 5.1 and 3.1 days, which were shorter than our present result. The females lived significantly longer than males (F = 9.58; df = 1; P = 0.007). Similar results also obtained in the present study were seen in Fekrat & Shishehbor (2004), Sohani *et al. (*2007), and Khan & Wan (2015).

Fecundity of B. tabaci

Generally, *B. tabaci* fecundity is highly variable and dependent on temperature, host-plant species, and cultivar (Khan & Wan, 2015). *B. tabaci* fecundity varied among temperature when they were reared in cassava (**Table 4**). There were statistically significant effects of temperature on mean total fecundity (F = 27.07; df = 3; P< 0.0001) and mean daily fecundity (F

Table 4. Total fecundity, daily oviposition, and sex ratio of *B. tabaci* on cassava at different temperatures

Traits	Temperature			
	20°C	25°C	27.5°C	30°C
Mean total fecundity (eggs/female)	$59.77^a \pm 2.06$	$65.82^b \pm 2.28$	$74.00^c \pm 2.22$	$49.3^d \pm 1.28$
Mean daily fecundity (eggs/day/female)	$6.01^a \pm 0.32$	$8.30^b \pm 0.42$	$10.59^c \pm 0.42$	$10.27^{bc} \pm 0.54$
Sex ratio (female/male)	1.01/1	0.97/1	1.13/1	1.26/1

Note: Means within a row followed by the difference letter are significantly different at P< 0.0001.

= 22.44; df = 3; P< 0.0001). The highest average number of eggs per female was 74.0 at 27.5°C and the lowest one of 49.3 eggs/female was at 30°C. Meanwhile, the mean daily number of eggs per female at 27.5°C and 30°C were 10.59 and 10.27, respectively.

In our study, whitefly reared on cassava showed their lower capacity of egg deposition in comparison with previous studies. The mean total eggs deposited per female recorded in four temperature experiments treatments (20ºC, 25ºC, 27.5ºC, and 30ºC) were 59.77, 65.82, 74.0, and 49.3, respectively. Fekrat & Shishehbor (2004) reported that on aubergine *B. tabaci* laid, a total of 78.6, 71.3, and 51.8 eggs at 20°C, 25°C, and 30°C, respectively. Sohani *et al.* (2007) reported that *B. tabaci* deposited total number of eggs same above temperature conditions on cucumber were 150.29, 263.75, and 204.71 eggs/female, respectively. Carabali *et al.* (2010) explained how the unlocal type B strain of *B. tabaci* survived and laid 8.2 eggs/female when reared on *M. esculenta* in Columbia, thus, our present result supports the ability of adaptation and/or differentiation of whitefly population on cassava.

Daily fecundity of whitefly *B. tabaci* increased as temperature increased. At 20°C, 25°C, 27.5°C, and 30°C, the average numbers of eggs laid per female per day were 6.01, 8.30, 10.59, and 10.27, respectively. Daily oviposition of *B. tabaci* observed in the present study was higher than the value cited by Fekrat & Shishehbo (2004), where the daily ovipositions of *B. tabaci* on aubergine were 3.9, 5.0, and 5.8 eggs/day/female at the temperature of 20ºC, 25ºC, and 30ºC, respectively. Khan & Wan (2015) reported that the mean daily number of laid eggs ranged from 4-5 on tomato and 5-6 on cotton at 25°C. Sohani *et al.* (2007) found that at 20°C, 25°C, and 30°C on cucumber *B. tabaci* laid 4.24, 9.92, and 12.75 eggs/day/female. The most favorable temperature for the development and reproduction of *B. tabaci* on cassava was 25-27.5°C

Sex ratio varied in *B. tabaci* from 1/1 (female/male) at 20°C, 25°C, and 27.5°C and 1/1.26 at 30°C. This result is consistent with the results of previous research of Sohani *et al.* (2007), whereby the sex ratio from 1/1 (male/female) at 20°C to 1/1.49 at 30°C. Khan & Wan (2015) reported at 25°C, *B. tabaci* sex ratios were 52:48 and 55:45 female to male ratio on tomato and cotton hosts, respectively.

Conclusions

The present study is one of the few studies on biological characteristics of *B. tabaci* on cassava in Vietnam. The ideal temperature for whitefly survival on cassava was between 25 and 27.5°C, which explains why they can build up population and play a key role on the transmission of SLCMV in the Southern and Central parts of Vietnam. Based on our results, the whitefly lower developmental threshold was 11.2°C and it was estimated that 14.31 generations could occur within 1 year on cassava, suggesting that *B. tabaci* is a potential threat in developing whitefly population and spreading SLCMV on cassava in the Northern part of Vietnam. In addition, global warming could promote *B. tabaci* invasion by increasing their survival, which increases negative impacts to the new agroecosystem.

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