Artificial Diet Influences Population Growth of the Root Maggot *Bradysia impatiens* (Diptera: Sciaridae)

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Abstract

In order to investigate the effects of artificial diets on the population growth of root maggot *Bradysia impatiens*, its population growth parameters were assayed on eight artificial diets (Diet 1, D2, D3, D4, D5, D6, D7, and D8). Results showed that developmental duration from egg to pupa was successfully completed on all eight artificial diets. However, the egg to pupal duration was shortest, while the survival rate of four insect stages was lowest when *B. impatiens* was reared on D1. When *B. impatiens* was reared on D7 and D8, the survival rate, female longevity, and female oviposition were higher than those reared on other diets. When *B. impatiens* was reared on D7, the intrinsic rate of increase (*r_m* = 0.19/d), net reproductive rate (*R_n* = 39.88 offspring per individual), and finite rate of increase (*λ* = 1.21/d) were higher for its population growth with shorter generation time (*T* = 19.49 d) and doubling time (*Dt* = 3.67 d). The findings indicate that the D7 artificial diet is more appropriate for the biological parameters of *B. impatiens* and can be used an indoor breeding food for population expansion as well as further research. We propose that vitamin C supplement added to the D7 is critical for the improvement of the *B. impatiens* growth.

Key words: *Bradysia impatiens*, artificial diet, life table, population parameters

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*Bradysia diffinis* Frey was assigned to be a junior synonym of *Bradysia impatiens* Johannsen (Mohrig et al. 2013, Ye et al. 2017, Sueyoshi and Yoshimatsu 2019), which is an emerging pest in agricultural and forestry worldwide in Asia, Europe, North America, South America, and Africa with an extensive range of hosts (Hurley et al. 2010). In China, it was recorded on edible fungi (Zhang et al. 2008, Shen et al. 2018) and important vegetables, such as chive (*Allium tuberosum* Rottl. ex Spreng), lily (*Lilium brownii* var. viridulum Baker), spring onion (*Allium fistulosum* L.), and broad bean (*Vicia faba* L.) (Gou et al. 2015a, Zhang et al. 2016). It is reported that the larvae of *B. impatiens* (also known as *B. agrestis* Sasakawa, 1978) injured cucumber (*Cucumis sativus* L.) in Japan, and caused heavily losses (Sueyoshi and Yoshimatsu 2019). *Bradysia impatiens* has the characteristics of short developmental duration (about 21 d from egg to adult in growth chambers at 25°C when fed on Chinese chive) (Liu et al. 2015a) and high fecundity (Shen et al. 2018). Additionally, *B. impatiens* larvae attacked the subterranean part of the plant or fed on the mycelium of oyster mushroom (*Pleurotus ostreatus*; Zhang et al. 2008, 2014; Han et al. 2015; Wu et al. 2016), making it difficult to detect early injury. Therefore, its control and prevention is extremely onerous. *Bradysia impatiens* can spread pathogens either directly or indirectly (Santos et al. 2012), such as *Verticillium albo-atrum* Reinke and Berthold in alfalfa (*Medicago sativa* L.), *Botrytis cinerea* Persoon ex Fries and *Colletotrichum fragariae* Brooks in strawberry (*Fragaria ananassa* Duch), and *Pythium aphanidermatum* (Edson) Fitzp in cucumber (*Cucumis sativus* L.) (Braun et al. 2010, 2012). For pest prevention and control, it is critical to understand the biological characteristics of an insect pest, and a sufficient supply of the target insect is the basis for research (Chen et al. 2016).

At present, the main feeding materials for *B. impatiens* to provide research material on its biology are culture medium, humus, and natural foods. Previous study reported that Potato Dextrose Agar medium supplemented with strains of tea oyster mushroom can be used for rearing *B. impatiens* (Zhang et al. 2008). Zhang et al. (2014) raised *B. impatiens* with putrefied broad bean and studied the emergency rhythm, mating behavior as well as sex pheromone. Gou et al. (2015a) studied the effects of different host plants on the biological characteristics of *B. impatiens* and revealed that Chinese chive is beneficial to its growth and reproduction. Liu et al. (2015b) studied the effects of different temperatures on the growth and reproduction of *B. impatiens* feeding on Chinese chive rhizomes, and found that 25°C is the optimum temperature for its survival. Zhang et al. (2016) raised *B. impatiens* on Chinese chive, broad bean, lettuce, and other natural foods, and found that chive and broad bean...
resulted in the greatest population growth. Other foods have also been used to raise *B. impatiens* indoor, such as bean dregs and sterilized humus with soybean (*Glycine max* (Linn.) Merx.) meal (Cheng et al. 2018, Shen et al. 2019). Wang et al. (2014) reported that insect feeding activities and their food intake are affected by internal and external factors, such as environmental conditions and food sources.

Artificial diets can make insects grow orderly and have consistent physiology, and solve the shortage of seasonal food and the difficulty of large-scale indoor breeding (Jain and George 2004, Cai et al. 2016, Pinto et al. 2019), which are the bottlenecks in *B. impatiens* research. In addition, using artificial diets to rear insects could promote better knowledge about biology, behavior, and nutritional requirements of insects, and such information is the basis for further management (Pinto et al. 2019). However, studies involving in artificial diets affected the population parameters of *B. impatiens* are scarce.

Insects depend on particular diets to obtain the required combination of essential nutrient for survival, growth, and fecundity (Behmer 2009). Proteins and carbohydrates are reported as the two most important macronutrient necessary for insect’s development, growth, and fecundity. This is due to their ability to provide the essential amino acids and energy, respectively, that influence insect’s development (Karasov and del Re 2007, Simpson and Raubenheimer 2012). The influence of artificial diet chemical compounds on the growth and development differ among insects (Duffey and Stout 1996). The variation of ingredients used in artificial diets for insect culturing determines its effects on the insect and this is correlated to plant defense. For instance, high-energy protein casein, proteins and carbohydrates are reported as the two most important macronutrient necessary for insect’s development, growth, and fecundity. Thus, to obtain the required combination of essential nutrient for survival, growth, and fecundity (Behmer 2009).

Vitamin C (VC), also known as l-ascorbic acid, ascorbic acid, or ascorbate, served as a phagostimulant or growth promoter for herbivorous insect, and its deprivation in diets resulted in impaired development of insects (Crickermore et al. 1998). In addition, VC is vital for both plants and animals, owning to its antioxidant activity and its roles as a regulator of gene expression and cell signaling (Goggin et al. 2010). The effects of a basal diet with enhanced levels of VC on the growth of the wasp *Apriona swainsoni* (Coleoptera: Chrysomeloida) manifested mainly in increasing the probability of egg hatching and adult emergence (Liu et al. 2018).

In this study, we investigated the developmental duration, survival rate, adult longevity, and oviposition ability of *B. impatiens* with eight artificial diets. We also assayed population growth parameters, including intrinsic rate of increase (*r*), net reproductive rate (*R*), finite rate of increase (*λ*), generation time (*T*), and doubling time (*Dt*). The aim of this study was to obtain optimal artificial diets for indoor rearing of *B. impatiens*, so as to provide basic materials for its biological characteristics research and further integrated control. Beyond that, we propose that VC supplement is critical for the improvement of the *B. impatiens* growth.

### Materials and Methods

#### Artificial Diets

Fresh Chinese chive and cucumber vine without insecticides were collected from the experimental field of Gansu Agricultural University in Lanzhou, Gansu Province, China (30°57′20″N, 103°41′54″E). Oyster mushroom roots were gathered from the mushroom house of the Botanical Garden in Lanzhou. These plants were dried in an oven at 90°C and ground into powder. Agar, yeast, sorbic acid, benzoic acid, and VC were supplied by Sangon Biotech Co. (Shanghai, China). The composition of the diets is described in Table 1.

The diet constituents were weighed as shown in Table 1 and added in boiling distilled water as follows. First, Chinese chive and oyster mushroom powders (or cucumber powder) and yeast were added and stirred for 2 min. Second, agar was added and stirred for 2 min. Finally, sorbic acid, benzoic acid and VC were added and stirred for 1 min. The diets were then placed into eight glass Petri dishes (15 cm) and stored at 4°C after cooling.

#### Insects

Larvae of *B. impatiens* were originally obtained from chive fields in Gangu County (105°7′2″N, 34°45′22″E), Gansu Province, China. They were kept in Petri dishes (9 cm) with wet filter papers (Gou et al. 2019) and fed on eight artificial diets separately for three generations in growth chambers with a photoperiod of 16:8 (L:D) h and light intensity of 588 Lux at (25 ± 1)°C and 65–70% relative humidity. Egg specimens laid within 12 h, where from populations continuously raised with each diet for three generations, were randomly selected from the Petri dishes containing each of artificial diet for life cycle study.

#### Population Growth Parameters Measurement

For each artificial diet, 30 newly laid eggs were counted under stereomicroscopy and placed into Petri dishes (9 cm) containing wet filter papers and artificial diets. There were eight diets, which constituted eight treatments. Each treatment was replicated four times and a total of 120 eggs (30 × 4) was used for each diet. The eggs were placed around the diets. The hatched larvae from these eggs were reared on the same diet sequentially. Freshly emerged adults, male and female were transferred to individual transparent plastic containers (15 × 9 cm) moistening with filter paper and paired with a 1:1 = male:female ratio (Gou et al. 2020a). If the number of males emerged was less than the females on a given day in any treatment, then males from an extra set of insects reared in parallel at the respective artificial diet were used. Fresh diets were supplied 1–2 d as food for larvae (eggs, pupae and adults do not need feeding). Appropriate water were replenished as needed in Petri dishes and transparent plastic containers. For each replicated treatment, we

| Constituent | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 |
|-------------|----|----|----|----|----|----|----|----|
| Chinese chive (g) | 20 | 20 | 15 | 15 | 5 | 5 | 20 | 20 |
| Oyster mushroom (g) | 20 | 0 | 5 | 0 | 15 | 0 | 20 | 0 |
| Cucumber (g) | 0 | 20 | 0 | 5 | 0 | 15 | 0 | 20 |
| VC (g) | 1 | 1 | 1 | 1 | 5 | 5 | 2 | 2 |
| Yeast (g) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Sorbic acid (g) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Benzoic acid (g) | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Distilled water (ml) | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
recorded daily whether the individual was alive or dead, the survival time, the developmental stage, and the total number of eggs per female. The study was conducted in the growth chambers at (25 ± 1)°C with a photoperiod 16:8 (L:D) h, 588 Lux light intensity, and 65–70% relative humidity.

Calculation of Population Growth Parameters
Population growth parameters of *B. impatiens* reared with each of the eight artificial diets were estimated using the following equations (Birch 1948, Ma and Liu 2016, Zhang et al. 2020):

Net reproductive rate (offspring/individual): \( R_0 = \sum I_x m_x \)

Mean generation time (day): \( T = \frac{\sum x m_x}{\sum m_x} \)

Intrinsic rate of increase (day\(^{-1}\)): \( r_m = \ln \left( \frac{\lambda}{T} \right) \)

Finite rate of increase (day\(^{-1}\)): \( \lambda = e^{r_m} \)

Population doubling time (day): \( D_T = \ln \left( \frac{1}{r_m} \right) \)

where \( x \) represents the time interval in days, \( I_x \) represents the survival probability of females during the period of, and \( m_x \) represents the average numbers of oviposition during the period of \( x \).

Statistical Analysis
All statistical data analyses were carried out with SPSS statistics software (Version 19.0 for Windows, SPSS, Chicago, IL). Data were subject to one-way analysis of variance and the means were compared using Tukey’s test (\( P < 0.05 \)). No data required transformation to meet the requirements for analysis of variance.

Results

Egg to Pupal Duration of *B. impatiens*
Developmental duration of *B. impatiens* from egg to pupa varied significantly among artificial diets (Fig. 1). The longest was on D6 (27.41 d) and shortest was on D1 (16.74 d). Egg to pupal duration varied significantly among artificial diets (Fig. 1). The longest was on D6 (29.16 d) and shortest was on D1 (19.49 d). Female longevity on D7 was prolonged by 1.17, 1.49, 1.84, 1.16, 2.17, 1.67 and 1 d compared with those on D1, D2, D4, D3, D5, D6, and D8, respectively. Male longevity was longest on D7 (2.5 d) and shortest on D2 (1 d) (Fig. 3b). Female and male longevity was distinctly longer on D7 compared with the other diets. Oviposition was much higher when *B. impatiens* was reared on D7 (96.47 grains) than those on the other diets (Fig. 3c), followed by D8 (84.19 grains), D1 (77.15 grains), D2 (66.16 grains), D3 (61.44 grains), D4 (51.33 grains), and D5 (48.41 grains). Oviposition was the lowest (46.03 grains) when *B. impatiens* was reared on D6.

Survival of *B. impatiens*
Artificial diet affected the longevity of *B. impatiens* adults. Female longevity varied from longest to shortest with an order of D7 > D8 > D3 > D1 > D2 > D4 > D6 > D5, with longest (3.17 d) on D7 and shortest (1 d) on D5 (Fig. 3a). Female longevity on D7 was prolonged by 1.17, 1.49, 1.84, 1.16, 2.17, 1.67 and 1 d compared with those on D1, D2, D4, D3, D5, D6, and D8, respectively. Male longevity was longest on D7 (2.5 d) and shortest on D2 (1 d) (Fig. 3b). Female and male longevity was distinctly longer on D7 compared with the other diets. Oviposition was much higher when *B. impatiens* was reared on D7 (96.47 grains) than those on the other diets (Fig. 3c), followed by D8 (84.19 grains), D1 (77.15 grains), D2 (66.16 grains), D3 (61.44 grains), D4 (51.33 grains), and D5 (48.41 grains). Oviposition was the lowest (46.03 grains) when *B. impatiens* was reared on D6.

Population Growth Parameters of *B. impatiens*
The effects of the artificial diets on the life table parameters of *B. impatiens* are presented in Fig. 4 and Supp Table A1 (online). The highest \( r_m (0.19/d) \) occurred when *B. impatiens* was reared on D7 and was significantly higher than that on other seven diets (Fig. 4a). The \( R_0 (39.88 \text{ offspring/individual}) \) and \( \lambda (1.91/d) \) were also significantly greater on D7 than those obtained on other diets (Fig. 4b and c). Furthermore, *B. impatiens* had the shortest \( T (1.94 \text{ d}) \) and \( D_T (3.67 \text{ d}) \) on D7 (Fig. 4d and e). When reared on D6, the minimum values of \( r_m \) and \( \lambda \) were recorded (\( r_m = 0.09/d \) and \( \lambda = 1.10/d \)), and the longest \( T \) and \( D_T \) were observed (\( T = 29.16 \text{ d} \) and \( D_T = 7.08 \text{ d} \)). The \( \lambda \) was not significantly affected by the artificial diets (Fig. 4c).

Discussion
The larvae of *B. impatiens* inhabit various groups of plant and fungi, for instance, Chinese chive, oyster mushroom and cucumber (Zhang et al. 2008, Liu et al. 2015b, Sueyoshi and Yoshimatsu 2019, Gou et al. 2020b). The current experiments were designed to assess how the three main host plants and VC affect *B. impatiens* population growth by replacing Chinese chive and oyster mushroom with cucumber, or by reducing the amount of Chinese chive, oyster mushroom, and cucumber, or by increasing VC supplement. *Bradyssia impatiens* originated from the same population and the feeding conditions were consistent, which ensured the same starting point among different treatments.

Our findings revealed that developmental duration from egg to pupa was shorter on D1 (16.74 d) and D7 (17.07 d), the survival rate of each insect stage was higher with D8 with a mean of 86.6%, and the oviposition was greater with D7 (96.47 grains). These results are in agreement with the observations of Zhang et al. (2016) and Luo (2018), who obtained a shorter development, higher survival rate,
and greater oviposition, when rearing *B. impatiens* on the Chinese chive and oyster mushroom.

Previous study indicates that insects generally need a better source of vitamins in minor quantities, due to their inability to synthesize vitamins (Dadd 1973). Vitamins are reported to play a major role as cofactor of the enzyme catalyzing metabolic pathways, as well as cofactor for several enzymatic stages in the fatty acid synthesis, and a component of the enzyme pyruvate carboxylase (Tuz and Hagedorn 1992). This suggest that the presence of the vitamin C probably acted as a cofactor for the synthesis of other significant nutrient components, which contributed to the shorter development, higher survival rate, and greater oviposition of the *B. impatiens* when reared on the Chinese chive, which plays equivalent role in this insect body.

It is investigated that insects generally obtain their ascorbic acid from their natural diet; however, for the best growth and development, they need the optimum requirement in their diet (Chapman 1998). The required amount of ascorbic acid in the insect diet reflects its potential in fertility, growth, and development (Genc 2004). This shows that *B. impatiens* probably obtains vitamin C from their natural diet, though their growth and development are inhibited due to inadequate amount of vitamin C in their natural diet.

Life table is an important method to evaluate the dynamics of insect populations, especially intrinsic rate of increase \(r_m\) and net reproductive rate \(R_0\) are used as important indicators to measure.

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**Fig. 2.** Effect of artificial diet on the egg (a), larva (b), pupa (c), and adult (d) survival of *Bradysia impatiens*. Data represent the mean ± SE. Means with different lowercase letters are significantly different according to Tukey’s test \((P < 0.05)\).

**Fig. 3.** Effect of artificial diet on the female longevity (a), male longevity (b), and oviposition (c) of *Bradysia impatiens*. Data represent the mean ± SE. Means with different lowercase letters are significantly different according to Tukey’s test \((P < 0.05)\).
insect population change trend (Gou et al. 2015b). The $r_m$ is not only reflects the ability of population growth but is also regarded as an ideal parameter for the comparison of population biological characterists (Ma et al. 2016, Zhang et al. 2020). For example, the $r_m$ was shown to be closely related to the developmental duration, survival rate, and fecundity of the wasp A. sawanoi population (Liu et al. 2018). The length of developmental duration is directly related to the population growth rate and the number of generations (Liu et al. 2018). Furthermore, a shorter developmental duration and a stronger reproduction reflect the adaptability of insects to specific hosts (Moreau et al. 2006, Sun et al. 2017). In our study, $r_m$, $R_0$, $\lambda$, and $T_d$ were higher when B. impatiens was fed on D7 with the shorter $T_d$ and $R_0$. Furthermore, the egg to pupal duration observed in this study was shorter, and oviposition were higher with D7. Consequently, the results of our experiment suggest that the D7 could be the best artificial diet among eight diets tested for the population growth of B. impatiens in a favorable condition. This was probably because the D7 contains compounds that meet the nutritional requirement of B. impatiens. Diet 1, D2, D7, and D8 are mainly composed of sufficient amount of Chinese chive, oyster mushroom, and cucumber. Moreover, the D7 and D8 diets contain twice as much VC as the other diets. The D7 contains less oyster mushroom than the D7 diet and is the second suitable diet (Fig. 4a–c) next to the D7 diet, indicating that the artificial diet based on Chinese chive and oyster mushroom with ratio of 1:1 and ample VC could promote the growth, development, and reproduction of B. impatiens.

Vitamin C is a apparently a ubiquitous molecule in animal and plant, particularly in fruits and vegetables, which reaches a concentration of over 20 mm in chloroplasts and occurs in all cell compartments (Nishikimi and Yagi 1996, Smirnoff and Wheeler 2000). Vitamin C is essential for herbivorous insect, not only due to its effect on the population growth, but also for its antioxidant activity, and roles as a regulator of gene expression and cell signaling, as well as an enzyme cofactor (Smirnoff and Wheeler 2000). Zhuo et al. (1981) and Zeng (2018) found that in the absence of VC, cotton bollworm Helicoverpa armigera (Lepidoptera: Noctuidae) could not complete its life cycle, and the survival reduced, the growth and development delayed, and the pupa weight declined. Lin and Liu (1996) also found, by increasing the VC contents in the artificial diet, that the pupa weight and survival rate of spotted borer Proceras venosatum (Lepidoptera: Pyralidae) were increased, whereas the development was shortened. It was also reported that VC promoted the growth and development of tobacco budworm Heliothis virescens (Lepidoptera: Noctuidae) (Coudron et al. 2009), migratory locust Locusta migratoria (Orthoptera: Acrididae) and desert locust Schistocerca gregaria (Orthoptera: Acrididae) (Goggin et al. 2010), and silkworm Bombyx mori (Lepidoptera: Bombycidae) (Kanafi et al. 2007). The best characterized roles of VC are to protect critical tissues of corn earworm Helicoverpa zeae (Lepidoptera: Noctuidae) larvae from reactive oxygen species (ROS) and to protect the midgut epithelium of tent caterpillar Malacosoma disstria (Lepidoptera: Lasiocampidae) and whitemarked tussock moth Orgya leucostigma (Lepidoptera: Lymantriidae), from oxidant plant allelochemicals, such as tannins and phenolics (Aabid 2016).

In short, we conclude that the D7 artificial diet is more appropriate for the population growth of B. impatiens as indicated by improved biological parameters and that VC supplement is critical for the improvement of the population growth.

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