

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 ($R_0 = 39.88$ offspring per individual), and finite rate of increase ($\lambda = 1.21/d$) 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 as 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

Bradysia difformis Frey was assigned to be a junior synonym of *Bradysia impatiens* Johannsen (Mohrig et al. 2013, Ye et al. 2017, Sueyoshi and Yoshimatse 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 Yoshimatse 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 injuring. 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

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 $(25 \pm 1)^\circ\text{C}$ 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): $R_0 = \sum l_x m_x$

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

Intrinsic rate of increase (day^{-1}): $r_m = \ln\left(\frac{R_0}{T}\right)$

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

Population doubling time (day): $Dt = \ln\left(\frac{2}{r_m}\right)$

where x represents the time interval in days, l_x represents the survival probability of female 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 ranged from shortest to longest with an order of $D1 < D7 < D3 < D5 < D8 < D2 < D4 < D6$. There was no significant difference ($P > 0.05$) between D1 and D7, and between D5 and D8, but significant differences were found between all other pairs of diets ($P < 0.05$).

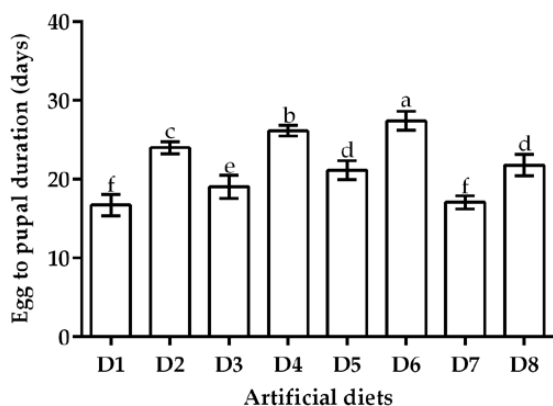


Fig. 1. Effect of artificial diet on the egg to pupal duration of *Bradysia impatiens*. Data represent the mean \pm SE. Means with different lowercase letters are significantly different according to Tukey's test ($P < 0.05$).

Survival of *B. impatiens*

Bradysia impatiens reared on the eight diets successfully completed to adult stage from egg stage; however, differences in survival were observed across the diets (Fig. 2). Greater survival for substage was appeared on D7 and D8. Egg survival rate was close to 100% on all diets except diet D5 (80%) (Fig. 2a). Larval survival rate of *B. impatiens* reared on D8 was the highest (98%), followed by that on D7, D3, and D2 (96, 88, and 86%, respectively) and lowest (70%) on D1 (Fig. 2b). The highest pupal survival rate was found on D8 (90%), which was significantly higher than that on D1 (54.4%) (Fig. 2c). The highest adult survival rate was observed with D8 (88%), followed by D7 (83%) (Fig. 2d).

Adult Longevity and Oviposition 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 offspring/individual) and λ (1.21/d) were also significantly greater on D7 than those obtained on other diets (Fig. 4b and c). Furthermore, *B. impatiens* had the shortest T (19.49 d) and Dt (3.67 d) on D7 (Fig. 4d and e). When reared on D6, the minimum values of r_m and λ were recorded ($r_m = 0.09/\text{d}$ and $\lambda = 1.10/\text{d}$), and the longest T and Dt were observed ($T = 29.16$ d and $Dt = 7.08$ d). The λ 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 Yoshimatse 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. *Bradysia 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,

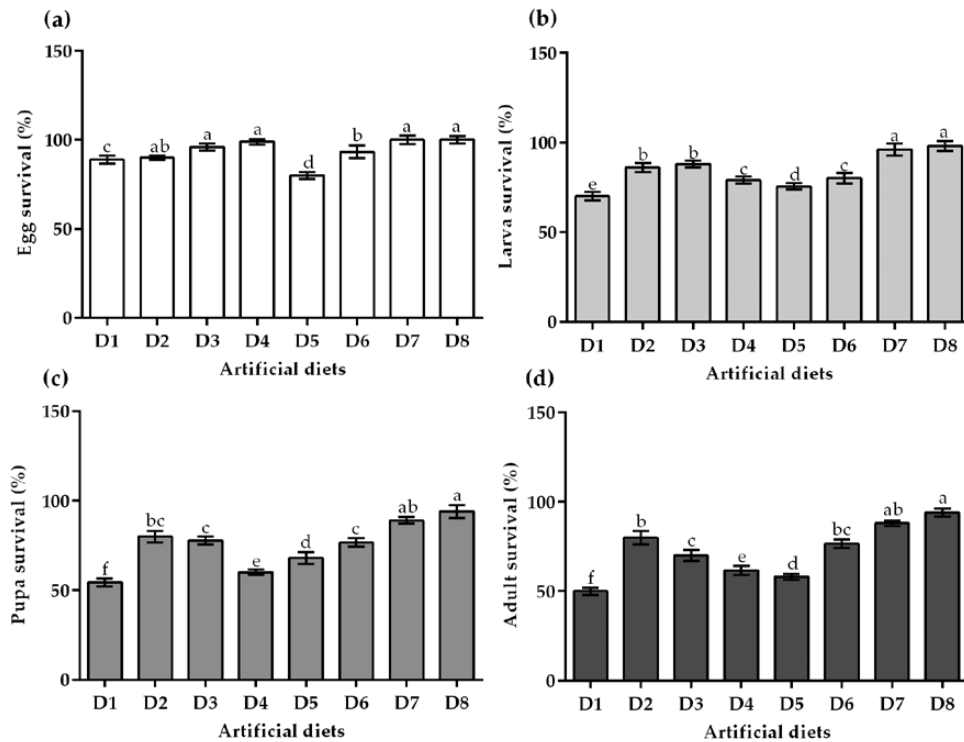


Fig. 2. Effect of artificial diet on the egg (a), larva (b), pupa (b), and adult (d) survival of *Bradysia impatiens*. Data represent the mean \pm SE. Means with different lowercase letters are significantly different according to Tukey's test ($P < 0.05$).

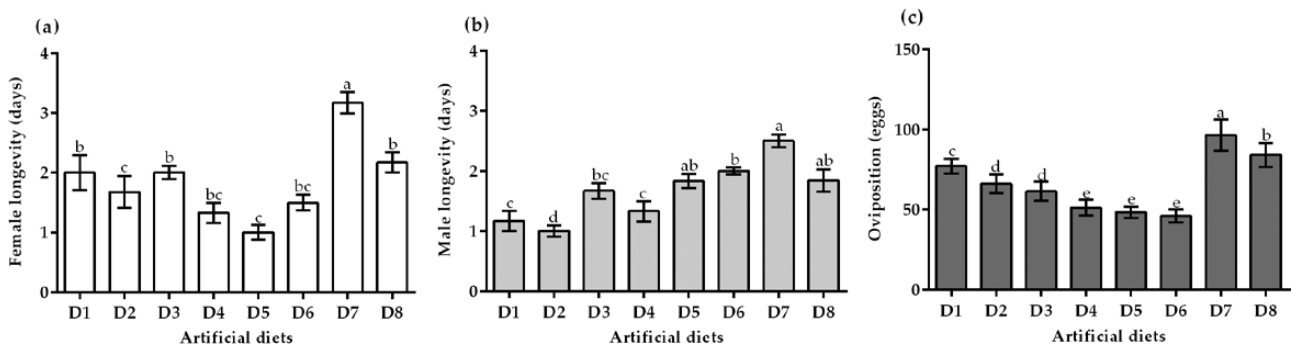


Fig. 3. Effect of artificial diet on the female longevity (a), male longevity (b), and oviposition (c) of *Bradysia impatiens*. Data represent the mean \pm SE. Means with different lowercase letters are significantly different according to Tukey's test ($P < 0.05$).

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 and oyster mushroom. It is reported that vitamin C is vital for molting, fertility, and maintenance of the normal growth and development of most insects (Nation 2001). Noticeably, Zhang et al. (2016) and Luo (2018), with the absence of vitamin C, also

reported similar observation. It appears that the origin of the *B. impatiens* larvae might have contributed to the artificial diets impact on its growth and development, regardless the presence of the vitamin C. This is probably due to the presence of chemical components in 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

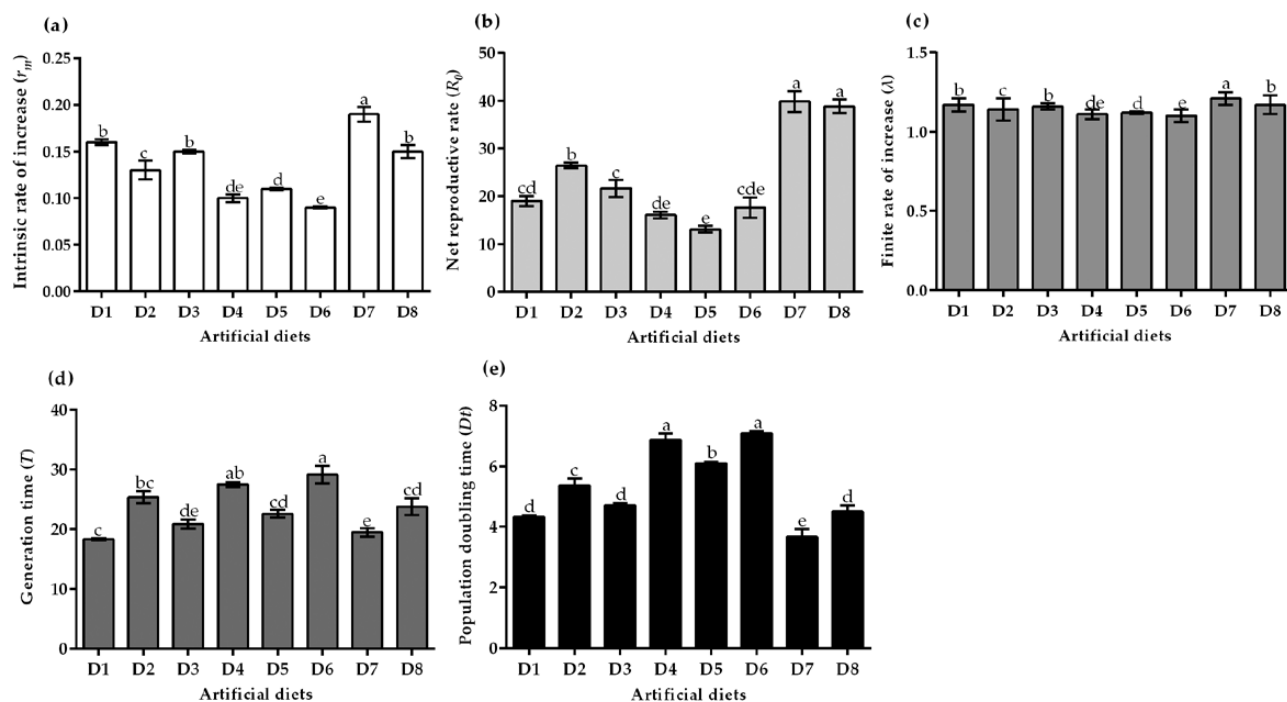


Fig. 4. Effect of artificial diet on the life table parameters of *Bradysia impatiens*. (a) r_m , intrinsic rate of increase (per day); (b) R_0 , net reproductive rate (offspring/individual); (c) λ , finite rate of increase (per day); (d) T , generation time (day); and (e) Dt , population doubling time (day). Data represent the mean \pm 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 characteristics (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. swainsoni* 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 , and λ were higher when *B. impatiens* was fed on D7 with the shorter T and Dt . 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 D8 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 ubiquitous molecule in animal and plant, particularly in fruits and vegetables, which reaches a concentration of over 20 mg 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 zea* (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, *Orgyia 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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Author Contributions

YG, CL: conceptualization, validation, and methodology; PQ: software; YG: formal analysis, writing—original draft preparation; YG, SG, KZ, and QZ: investigation, resources; CL: data curation, visualization, supervision, project administration, and funding acquisition; PQ, JC: writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

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