

Effect of nitrogen fertilization on life table parameters and population growth of *Brevicoryne brassicae*

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Abstract

To investigate the effect of nitrogen fertilization on fitness of cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera Aphididae), oilseed rape (RGS003 cultivar) was treated with different nitrogen rates. Nitrogen fertilization treatments were 0, 50, 100, and 150% of recommended rates. The results showed that increase in nitrogen fertilization resulted in an increase in the soluble nitrogen concentration in plants. The aphid feeding on plants receiving higher nitrogen fertilizer (150%) had shorter total pre-reproduction period, longer reproductive period, as well as greater fecundity, but nitrogen fertilization had no effect on adult longevity. Life table parameters of aphid on various treatments were calculated, jackknife method was also used to determine the uncertainty of parameters. The intrinsic rate of increase in treatments of 0, 50, 100, 150% were determined as 0.28, 0.27, 0.31, and 0.35 (female/female/day), respectively. The results revealed that developed aphid on treated plants that received 150% nitrogen had higher reproduction ability compared with those developed on other treatments. The greenhouse study on aphid population growth on various treatments indicated that 21 day after infestation, highest number of aphid and lowest number of winged were found on treated cabbage with 150% of recommended nitrogen fertilization. This means that higher nitrogen fertilization improves the suitability of oilseed rape for cabbage aphid whose performances are then increased.

Key words: *Brevicoryne brassicae*, nitrogen fertilization, population abundance, intrinsic rate.

Introduction

Recently, cultivation of oilseed rape (*Brassica napus* L.) as a source of vegetable oil received a great attention in Iran (Baibordi and Malakooti, 2004). Insect pests are known as a major problem for oilseed rape crops. Cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera Aphididae) has been considered as an important pest in Iran and other parts of the world which causing considerable crop loss (Hughes, 1963; Khanjani, 2005). *B. brassicae* is a specialist on the Brassicaceae and prefers feed on younger plant tissues (Kennedy, 1958). Aphid feeding on young leaf results in weakening the plants and decreasing the amount and quality of seeds (direct damage). On the other hand, it produces large quantity of honeydew that in turn results in the growth of black mold on the leaves that decrease photosynthesis (indirect damage) (Costello and Altieri 1995). The population density on plant is so high that control is inevitable, which mainly is based on insecticides.

One of the most significant factors influencing the population dynamics of insect pest is quality of their food. Amount of plant nitrogen is a factor, which could show the quality of host plant for herbivorous insect (Mattson, 1980). Douglas (1993) stated that nitrogen level in the diet could change the performance of phytophagous insects. Often nitrogenous compounds are scarce in plant tissue, particularly in phloem sap (Mattson, 1980). Therefore, sucking insect such as aphids show strong response to change in nitrogen level in host plants (Van Emden, 1966) and the cabbage aphid is not exception from this rule. Evans (1938) stated that reproduction rate of the cabbage aphid is positively correlated with the nitrogen content of the plant. Van Emden (1966) reported

that fecundity and reproductive rate of cabbage aphid, on Brussels sprouts increase with increasing nitrogen fertilization. Van Emden and Bashford (1971) reported that the complex of asparagines and glutamine have positive correlation with relative growth rates (RGR) of cabbage aphid. Cole (1997) pointed out that, there is a relationship between amino acids in phloem saps of wild *Brassica* and intrinsic rate of increase in cabbage aphid.

Rape plants need lots of nitrogen to grow. Increasing nitrogen fertilization to produce more yields could result in higher aphids' damage (Koritsas and Garsed, 1985). The purpose of this study was to investigate the effect of various rates of nitrogen fertilization on cabbage aphid (biology, population growth index, and life table) on RGS003 cultivar.

Materials and methods

Insect source

The main culture of aphid was reared on hybrid oilseed rape (RGS003) that was sown in pots and maintained in greenhouse. Plants were infected by collected aphids from fields. The plants were sown and replaced weekly.

Nitrogen treatment

Soil test was carried out to determine the level of nitrogen in it. The soil without any nitrogen fertilization was used as check. The recommended level of nitrogen fertilization for oilseed rape plants in Iran is 150 kg/ha (Baibordi and Malakooti, 2004). To produce different levels of nitrogen fertilization, 50, 100, and 150 percent of recommended nitrogen were added to pots in two steps. 35 hybrid seeds (RGS003) were sown in each

plastic pot that contains 4.5 kg of soil (peat mass soil, sand and sterilized field soil). Based on weight of soil per hectare around the root of oilseed, and recommended weight of nitrogen fertilizer per hectare, at planting time $N_1 = 0$, $N_2 = 124.26$, $N_3 = 249.10$, $N_4 = 373.35$ (mg) of calcium nitrite per kg of soil were dissolved in 100 ml of distilled water and added to the soil of each pot. After emergence, weak seedlings were removed. The second fertilization was done in the 19th day of planting as $N_1 = 0$, $N_2 = 248.52$, $N_3 = 497.9$, $N_4 = 746.52$ (mg). Pots were controlled and checked daily and irrigated three times per week.

First experiment: measuring life history parameters

11 days after the second fertilization, young expanded oilseed rape leaves from each plant were removed for aphid feeding. Petioles were individually placed into a glass containing water. Glass and leaf were put in a cylindrical container (18 cm height, 8 cm diameter) to avoid aphid migration to other leaf. Cylindrical container had a hole on wall for ventilation. To have a cohort of the first nymphal stage with the same age (< 24 h), one young female aphid was put on each leaf in container. Rearing containers were placed in an environmental chamber (25 ± 1 °C, $70 \pm 10\%$ RH, and 14:10 L:D photoperiod). Four hours later, leaves were examined and adult female and produced nymphs except one of nymph were removed. From this moment, rearing containers were observed daily until all aphid were died. To provide fresh food, leaves were replaced with new young leaves (3 times per week). To examine the effect of fertilization on life table parameters of aphids, at least 40 replicates were used for each treatment.

Statistical analysis

Reproductive period of adult aphid, total pre-reproduction period (from birth to producing the first nymph), longevity period (from birth to death) and fecundity were calculated in four treatments and the collected data were subjected to statistical analysis.

Age specific fertility life table was constructed by age (x), age specific survival rate (l_x) and average aphids progeny in x age class (m_x). The most important parameter of fertility life table is the intrinsic rate of increase (r_m), which was estimated by using the following equation (Birch 1948):

$$\text{(equation 1)} \quad \sum L_x m_x e^{-r_m \text{ pivotal } x} = 1$$

Other parameters of fertility life table including: net reproduction rate ($R_0 = \sum L_x m_x$), generation time ($DT = \ln 2 / r_m$), population doubling time ($T = \ln R_0 / r_m$) as well as finite rate of increase ($\lambda = e^{r_m}$) were calculated; where in these equations L_x and *pivotal* x are $(l_x + l_{x+1}) / 2$ and $[x + (x + 1) / 2]$, respectively.

The statistical differences in r_m -values (and other life table parameters) were tested using jackknife pseudo-values. According to Meyer *et al.*, (1986), in the first step, r_m was calculated for the data using equation 1 (r_{all}). Then, one of the aphids was omitted and an r_m (\hat{r}_j) was calculated for the remaining aphids ($n - 1$). According to Meyer *et al.*, (1986), the jackknife pseudo-value (\tilde{r}_j) was computed for this subset of the original data

according to the equation $\tilde{r}_j = nr_{all} - (n - 1) \hat{r}_j$. This process was repeated for all possible omission of one aphid from the original data set. Finally n numbers of calculated \tilde{r}_j were used to calculate the mean (r_j) and SE for them by using equation 2 and 3.

$$\text{(equation 2)} \quad r_j = \frac{1}{n} \sum \tilde{r}_i$$

$$\text{(equation 3)} \quad \hat{SE}(r_j) = \sqrt{s_i^2 / n}$$

where, s_j^2 is the variance of the n jackknife pseudo-values ($\tilde{r}_1, \tilde{r}_2, \dots, \tilde{r}_n$). This process could be used to produce pseudo-values for other parameters (Maia *et al.*, 2000). To calculate different parameters, PersianRm software (Naveh *et al.*, 2004) was used. All statistical comparisons were done by using Proc GLM in SAS software.

Second experiment: greenhouse experiment

To investigate the effect of different treatments on aphid density under greenhouse conditions (25.18 °C, 38.27% RH) 10 pots with 22 plants were selected randomly for each treatment. Five days after second fertilization when plants were at the stage of 4-6 leaves, the youngest expanded leaf of each plant was infested with 10 wingless aphid females. Pots were covered by a fine mesh net and 21 days later, the total number of aphid and number of winged were counted in each pot. Statistical comparisons were done by using Proc GLM in SAS software.

Total soluble nitrogen of plants

70 leaves with the same age per treatments were randomly selected for nitrogen determination. These samples maintained in an oven at 60 °C for 2 days, and then were pulverized and total nitrogen measured using Kjeldhal method (Gupta, 2004).

Results

Laboratory biology

Biological characteristic of cabbage aphid on different treatments and their statistical comparisons were presented in table 1. Statistical analysis revealed that the shortest pre-reproductive period was on plants treated with the highest (150%) fertilization level ($F = 16.5$, $df = 3,187$, $P < 0.05$) and the longest reproductive periods were observed on normal (100%) and highest (150%) fertilization level ($F = 3.2$, $df = 3,187$, $P < 0.05$). Nitrogen fertilization also had a significant effect on female fecundity ($F = 18.8$, $df = 3,187$, $P < 0.05$) and higher number of offspring were produced per female on plants treated with higher nitrogen level. However, nitrogen fertilization could not lead to a significant difference on aphid longevity ($F = 1.8$, $df = 3,187$, $P > 0.05$).

Age specific fertility life table parameters

Trends in survival rate (L_x) and age-specific fecundity (m_x) data from different treatments were presented in figure 1. The mortality of the last cabbage aphid on the

Table1. Pre-reproduction period, reproduction period, longevity, and fecundity of *B. brassicae* as a function of nitrogen fertilization on rape plants. (mean \pm SE).

| Parameters* | % of recommended level of nitrogen fertilization | | | |
|--|--|-------------------|--------------------|-------------------|
| | 0% | 50% | 100% | 150% |
| Total pre-reproduction period (day) | 7.6 \pm 0.13 ab | 7.9 \pm 0.12 a | 7.5 \pm 0.13 b | 6.7 \pm 0.13 c |
| Reproduction period (day) | 10.5 \pm 0.64 ab | 9.9 \pm 0.59 b | 12.04 \pm 0.61 a | 11.9 \pm 0.6 a |
| Fecundity (total number of offspring per female) | 42.6 \pm 3.14 b | 47.5 \pm 2.88 b | 63.04 \pm 2.17 a | 70.3 \pm 2.94 a |
| Longevity (day) | 18.9 \pm 0.77 a | 20.2 \pm 0.84 a | 21.5 \pm 0.79 a | 20.5 \pm 0.79 a |

*Rows with the same letter were not significantly different based on Duncan's multiple range tests at $\alpha = 0.05$.

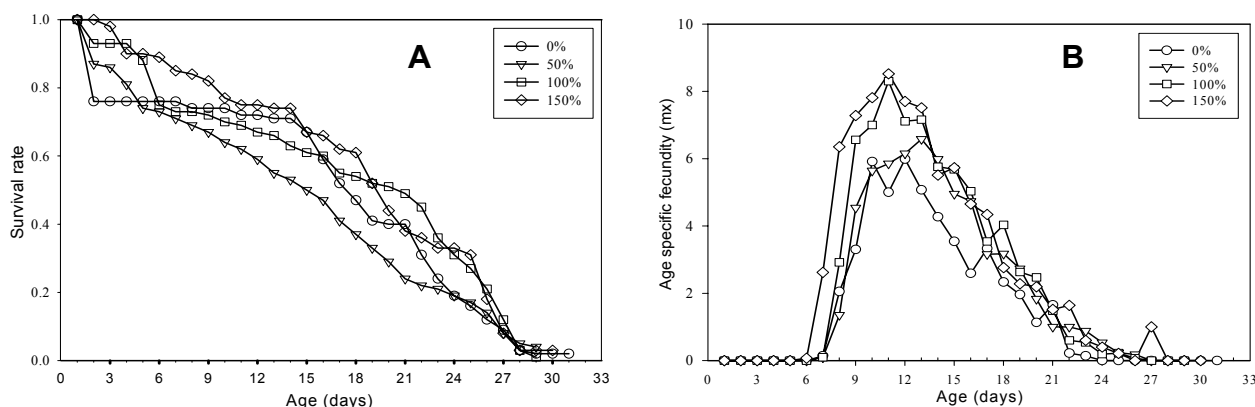


Figure1. Cabbage aphid survival rate (A) and age-specific fecundity (B) on treated plants with four nitrogen rates: 0, 50, 100, and 150% of the recommended level.

four treatments occurred on day 31, 29, 29, and 30 of the experiment. Age-specific fecundity (m_x) of cabbage aphid demonstrated that reproductive period in all four treatments lasts 17, 20, 20, 22 days, respectively.

Parameters of fertility life table of cabbage aphid on different treatments are presented in table 2. Statistical analysis demonstrated that none of fertility life table parameters had significance differences in the first two nitrogen treatments (0% and 50%). Aphids feeding on plants treated with higher fertilization (150%) had significantly shorter generation time (T) ($F = 11.6$, $df = 3,260$, $P < 0.05$), and the shortest population doubling time (DT) ($F = 14.3$, $df = 3,260$, $P < 0.05$). Aphids of this treatment had higher net reproductive rate (R_0) ($F = 10.1$, $df = 3,260$, $P < 0.05$) and were able to produce 55.4 female aphids in their lifespan. Statistical comparison also showed that finite rate of increase (λ) in treatment receiving 150% nitrogen was significantly

($F = 19$, $df = 3,260$, $P < 0.05$) higher than other treatments. Moreover, aphids feeding on this fertilization level were able to increase their population 1.432 times compared to preceding day. The intrinsic rate of natural increase (r_m) in treatments of 0, 50, 100, and 150% were determined as 0.283, 0.275, 0.312, 0.359 female/female/day, respectively.

As mentioned earlier, intrinsic rate of natural increase is the most importance among other life table parameters. Statistical analysis on jackknife's pseudo-values of r_m indicated that there was significant difference ($F = 18.5$, $df = 3,260$, $P < 0.05$) in treatments.

Host plant tissues analysis

Analysis of host plant tissues showed the nitrogen concentration, fresh and dried leaf weight of plants increased with increasing nitrogen fertilization (table 3). Plants fertilized at the highest nitrogen level of 150%

Table2. Life table parameters of *B. brassicae* on oilseed plants treated with various level of nitrogen fertilization (mean \pm SE)*.

| % of recommended level of fertilization | Parameters | | | | | |
|---|--------------------|--------------------|-------------------|--------------------|---------------------|--|
| | R_0 | T | DT | λ | r_m | |
| 0% | 31.05 \pm 2.89 c | 12.17 \pm 0.16 a | 2.45 \pm 0.07 a | 1.327 \pm 0.01 c | 0.283 \pm 0.008 c | |
| 50% | 30.09 \pm 3.20 c | 12.39 \pm 0.16 a | 2.52 \pm 0.08 a | 1.317 \pm 0.01 c | 0.275 \pm 0.009 c | |
| 100% | 44.43 \pm 4.07 b | 12.15 \pm 0.12 a | 2.2 \pm 0.05 b | 1.367 \pm 0.11 b | 0.312 \pm 0.008 b | |
| 150% | 55.41 \pm 4.65 a | 11.19 \pm 0.15 b | 1.93 \pm 0.04 c | 1.432 \pm 0.01 a | 0.359 \pm 0.008 a | |

*Mean \pm SE followed by the same letter within columns were not significantly different based on Duncan's multiple range tests at $\alpha = 0.05$.

R_0 : (net reproductive rate in number of offspring per female); T : (generation time in day); DT : (population double time in day); λ : (finite rate of increase); r_m : (intrinsic rate of natural increase in female/female/day).

Table 3. Influence of nitrogen fertilization on Fresh weight, Dry weight, Foliar N concentration (%) and N uptake.

| % of recommended level of fertilization | Fresh weigh (g) | Dry weigh (g) | Nitrogen concentration (%w/dry w) | Nitrogen uptake (g) |
|---|-----------------|---------------|-----------------------------------|---------------------|
| 0% | 340.38 | 48.39 | 2.18 | 1.05 |
| 50% | 369.45 | 52.13 | 2.63 | 1.37 |
| 100% | 438.95 | 55.63 | 2.68 | 1.49 |
| 150% | 569.73 | 56.24 | 4.14 | 2.32 |

contained the greatest nitrogen content in leaf tissue and by increasing fertilization level from 0 to 150% nitrogen uptake maximized, maximum uptake was 1.05 g for the lowest level and 2.32 g for the highest level.

Effect of fertilization on aphid population

Average aphids population on potted oilseed plants treated with different nitrogen level were presented in table 4. Statistical analysis showed that, number of aphids on treated oilseed plants significantly ($F = 38.6$, $df = 3,36$, $P < 0.05$) increased with increase in fertilization level. The greatest numbers of aphids were found on plants fertilized with 150% nitrogen. Winged aphids appeared at the end of the experiment. Raw data showed that winged density had reverse relationship with fertilization level. However, statistical analysis showed that there was no significant differences among treatments ($F = 0.5$, $df = 3,36$, $P > 0.05$) (table 4).

Discussion

Nitrogen content in host plant is known as a significant factor affecting developmental rate, aphid morph production (e.g. frequency of winged) (Dixon, 1987) and reproduction seasonal cycle (Dixon *et al.*, 1993). Our study confirmed that nitrogen fertilization level positively correlates with aphid performance. Increase in nitrogen fertilization level led to reduction in total pre-reproductive period, but it cannot significantly change longevity period. However, Moon *et al.*, (1995) reported that increase in nitrogen rates on wheat (in hydroponic culture) did not significantly change duration of pre-reproductive period, reproductive period, longevity, and reproduction rate of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov). In our experiment, maximum fecundity was achieved at the highest fertilization level. Van Emden (1966) showed that an increase in N or K fertilizer resulted in an increase in soluble nitrogen rates in Brussels sprouts var. Wroxton. They achieved maximum fecundity at the intermediate level of both nutrients (140 ppm N / 78ppm K) and reduction of fecundity considered to be affected by increase in nitrogen fertilization level or decrease in potassium fertilization level. Van Emden and Bashford (1969) reported similar results on other varieties of Brussels sprouts (Sutton early market). In other experiments that were done by these researchers, fecundity range of adult aphids were 15.4-41.4 (Van Emden, 1966) and 7.3-30.2 (Van Emden and Bashford, 1969) ((NH₄)₂SO₄ as fertilizer source), while in the present study fecundity range were 42.6-70.3 (calcium nitrate as fertilizer source). This indicates that

Table 4. Effect of nitrogen fertilization on wingless and winged aphid density* (mean ± SE).

| % of recommended level of fertilization | Aphid density | |
|---|-------------------|--------------|
| | wingless | winged |
| 0% | 1287.9 ± 69.43 d | 6.1 ± 1.57 a |
| 50% | 1896.1 ± 136.31 c | 4.6 ± 1.15 a |
| 100% | 2620.7 ± 152.79 b | 4.4 ± 1.61 a |
| 150% | 3168.1 ± 152.73 a | 3.9 ± 0.97 a |

*columns with the same letter were not significantly different based on Duncan's multiple range tests at $\alpha = 0.05$.

aphid development has influenced by host plant, nutrient status and fertilizer source (Chau *et al.*, 2005). Our results indicated that aphid survivorship on oilseed rape was not significantly correlated with nitrogen level in the leaves. Such results are in agreement with survivorship of *Aphis gossypii* Glover on chrysanthemum plants (Bethke *et al.*, 1998).

Variation in the intrinsic rate of population increase (r_m) could be attributed to three main factors: development rate, fecundity and longevity (Dixon, 1987) and it is often used to measure the influence of various environmental factors on population growth. Throughout the experiment, increase in nitrogen fertilization level resulted in increase in r_m . Wang *et al.*, (2006) reported that *Peregrinus maidis* (Ashmead) feeding on corn plant receiving the highest fertilization treatment of nitrogen, had greatest r_m .

Similarly, Nevo and Coll (2001) show that increase in nitrogen fertilization level in cotton resulted in an increase of r_m in *A. gossypii*. However, Jansson and Smilowitz (1986) observed that the highest level of nitrogen reduced the rate of population growth of *Myzus persicae* Sulzer on potatoes. Chau *et al.*, (2005) also showed that population growth rate of *A. gossypii* on chrysanthemum, increased with fertilization rates from 0 to 38 ppm of nitrogen and reached a plateau from 38 to 488 ppm. According to Cole (1997) the concentration of the important amino acids including tyrosine, alanine, leucine and glutamic acid, accounted for 43% of the variation in intrinsic rate of increase, they further stated that higher concentration of tyrosine and glutamic acid improved aphid performance.

An increase in aphid performance can also be the result of reduction in plant defense. The primary defense compound in oilseed rape plant is glucosinolates (Cole, 1997). Based on a view of earlier studies (Baibordi and Malakooti, 2004) and carbon nutrient balance hypothesis (reviewed by Price, 1997) the reduction of glucosi-

notolates content in plant could be occurred as a response to increase in nitrogen content, this in turn reduced plant defense. Therefore, like other aphids such as *A. gossypii* (Nevo and Coll, 2001), and *Hysterooneura setariae* (Thomas) (Jahn *et al.*, 2005), this phenomenon increased aphid performance. Probably, one of the reasons for non-significant change in life table parameters with increase in nitrogen fertilization from 0% to 50% is that these rates of nitrogen increase could not decrease plant defense or glucosinolates content significantly.

Nutritional quality of host plants should be considered in population growth modeling and designing management systems for insect pests. Using of such models as well as considering other agronomic and physiological characteristic could be effective factors in optimal use of fertilizer and it possibly reduces insecticide use, minimizing ground water contamination and reducing worker exposure to pesticide residues.

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