

Effect of Nitrogen Fertilization on *Tetranychus urticae* Koch (Acari: Tetranychidae) Populations on Common Bean Cultivars

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Abstract: The impact of nitrogen fertilization on bean plants (*Phaseolus vulgaris* L.) and *T. urticae* population dynamics were investigated in the field at Khomein, Markazi Province, Iran, during 2008-2010. In this study, six bean cultivars (Chiti beans: Khomein and Ks21189, Red beans: Akhtar and Ks31169 and White beans: Pak and G11867), with five nitrogen levels (0, 11.5, 23, 46 and 69 kg ha⁻¹ N as Urea 46% N), were evaluated in a factorial experiment based on randomized complete blocks design with four replications. Results of analysis of variance for numbers of adult or immature stages of *T. urticae* were showed that significant variations were observed both among nitrogen treatments and among six bean cultivars, in each year. So, combined analysis for three were showed that significant variations were observed in year (Y), cultivar (C), nitrogen (N) and interaction between cultivar and nitrogen (C×N). The results showed that 69 kg ha⁻¹ N, was the most effective on numbers of adult or immature stages of *T. urticae*. This study indicated that increasing nitrogen level in the range of 0 to 69 kg ha⁻¹ resulted in enhanced mite population on bean leaves in field.

Key words: *Phaseolus vulgaris* % *Tetranychus urticae* % Nitrogen levels % Population dynamics

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops in terms of both economy and nutrition and widely grown in different regions of the world and commercially produced in Markazi, Lorestan, Fars and Zanjan provinces of Iran. Based on reports by the Iranian Ministry of Agriculture in 2005, overall, this crop is grown on more than 105000 ha annually in Iran [1, 2].

Various pests have negative effects on bean production in Iran which among them web spinning spider mites are one of the most important and destructive group of pests to agricultural crops worldwide. Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch has been considered as a major pest in many bean-growing areas of Iran [3, 4]. TSSM was first described by Koch in 1836 [5]. *T. urticae* is an extremely polyphagous pest that has been reported from more than 900 host species and is described as a serious pest of at least 150 economically important

agricultural and ornamental plants, including many field and forage crops, horticultural crops, ornamentals and weeds. A two spotted spider mite population can expand rapidly with up to 40% per day [6]. The importance of this mite pest is not only due to direct damage to plants including defoliation, leaf burning and even in excessive outbreaks plant death but also indirect damage to plants which decreases in photosynthesis and transpiration [7]. TSSM infests the underside of leaves, where profuse webbing may be present but in severe infestation will occur on both leaf surfaces as well as on the stems and fruits. *T. urticae* feeds on plant juices by using a piercing-sucking process and causes leaf yellowing as well as blistering and deformation of tissue. Because the chloroplasts in leaves are gradually destroyed as the population of feeding mites increases, photosynthesis declines, stomata close and transpiration decreases, leading to reduced production [7, 8]. Infestations are most serious in hot and dry conditions. Because they multiply very fast they are able to destroy plants within a short

period of time. The rapid developmental rate, short generation time and high net reproductive rate of *T. urticae* allows them to achieve damaging population levels very quickly when growth conditions are good, resulting in an equally rapid decline of host plant quality. [9-14].

Dietary nitrogen and carbohydrates impact survival, growth and reproduction of insects [15, 16]. Plant nitrogen fertilization has been shown to modify the dietary nitrogen concentration of the plants for insects to affect their population growth [17, 18, 19]. Numerous investigations were made concerning the influence of the macronutrients N, P and K on the dynamics of pests [20]. Fertilization levels can also influence insect population dynamics and insect pest management strategies. High levels of fertilization may improve the nutritional quality of the plants for phytophagous insects [21]. The positive influence of the level of fertilization on the performance of insect pests is seen as a key factor in the generation of pest outbreaks [17]. In greenhouse ornamental production, fertilization and pesticide application remain as vital agronomic practices for producing high quality and aesthetic crops. Chemical fertilizers elevate plant nitrate and amino acid levels [22] but inadvertently increase the nutritional quality and attractiveness of plants to phytophagous insects [23, 24, 25, 21]. Higher levels of leaf nitrogen may enhance both growth and reproduction of herbivorous insects [26, 27, 28] and reduce their susceptibility to some insecticides [29]. However, the effect of nitrogen fertilization on plants and the related population growth of the pests are still poorly understood. Most studies on the effect of fertilization on pest populations have focused on aphids and on TSSM interactions under field conditions have not been fully investigated. The present study was initiated to determine if different levels of nitrogen fertilization to bean plants grown in field increased two spotted spider mite numbers and spider web production. For this purpose, mites were examined on bean in nutrient solutions with varying amounts of nitrogen under controlled conditions.

MATERIALS AND METHODS

Experimental Plots: This study was conducted over 3 years (2008-2010) at Khomein Bean Research Station, Markazi Province, Iran (49°, 57' longitude and 33°, 39' latitude). This research was studied on six bean (*Phaseolus vulgaris* L.) cultivars including: Chiti beans (Khomein and Ks21189), Red beans (Akhtar and Ks31169) and White beans (Pak and G11867). Bean cultivars were

planted on 5 May 2008, 2009 and 2010 at this station. The plot size was 15 m² (3×5 m) with 2 m (four rows of bare soil) buffer areas between neighboring plots in the same block and each of the four blocks was separated by 3 m of bare soil. Row spacing was 50 cm (six rows in each plot) and the bean seeds dipped 5 cm below the soil and seed spacing was 5 cm (100 seeds on each row).

Residual Soil Nitrogen: Prior to planting, five soil samples (within 15 cm of top soil) across each experimental plot were analyzed to determine residual total nitrogen. Residual nitrogen in soil samples was determined as total Kjeldahl nitrogen and the analysis was performed by the Analytical Laboratory of the Department of Soil and Water Research of Agriculture and Natural Resources Research Center of Markazi Province, Iran, using the standard Kjeldahl procedure with sulfuric acid and digestion catalyst [30, 31].

Nitrogen Treatments: In this study, six bean cultivars with five nitrogen levels were evaluated in a factorial experiment based on randomized complete blocks design with four replications (30 treatments in 4 replications). Treatments consisted of soil applications of 0, 11.5, 23, 46 and 69 kg nitrogen per hectare (as Urea 46% N). The treatments represented sub-optimal, optimal and supra-optimal nitrogen fertility for bean in this field. Soil applications of nitrogen were performed in two stages. The first stage, 50% of each nitrogen concentration applied before planting and the second stage, 50% applied in V3 stage (third trifoliate leaf of bean vegetative stages [32]). All experimental plots were irrigated every 3 days after planting and no fertilizers, insecticides, miticides or any other chemicals were applied to control the pests during the survey period.

Two Spotted Spider Mite Population

Nitrogen Effect: Counting of population dynamics of *T. urticae* were conducted separately for nitrogen treatments on six bean cultivars. Population densities of adult and immature stages of two spotted spider mite were monitored on a weekly basis throughout the bean season (The plant was attacked naturally by TSSM).

Random sampling of various stages of TSSM were initiated in sampling times and numbers were counted from bean leaves in the laboratory. In order to random sampling, for each row in each plot was selected 2 bean plants and for each plant selected 3 leaves from upper, middle and lower portion, randomly. Hence, for each plot was collected 36 leaves.

Numbers of adult and immature stages of two spotted spider mite on the underside of each leaf were counted using a stereo-binocular microscope in the laboratory. All stages of mite, viz. larva, nymph and adult were considered for counting. Different stages were recorded separately. For this purpose, the leaf disc was used. Each leaf disc was 4 cm² of bean leaf center that this unit separated by plastic padding 2 cm×2 cm.

Climatic Effect: To effecting of climatic factors on population density of two spotted spider mite, temperature, moisture, rainfall and solar radiation data were obtained from the Khomein Meteorological Station, 2500 m south east of the research fields at Khomein and were compared with population dynamics of *T. urticae* on six bean cultivars.

Statistical Analysis: Data on different stages of two spotted spider mite and nitrogen effect on TSSM population on six bean cultivars were analyzed with two-way randomized complete block general linear models (GLM) in SAS System Software V6.12 [33]. Means comparison was performed with the Duncan's multiple range test ($P<0.05$) in each year. Also, the combined analysis for means comparison in three years was used in SAS System.

RESULTS

Residual Total Nitrogen in Soil: Total soil nitrogen in all experimental plots, prior to nitrogen treatments, was consistent with a mean level of nitrogen around 0.04% ($P<0.05$). Hence, no significant variations were observed among soil nitrogen in all experimental plots.

Two Spotted Spider Mite Population

Nitrogen Effect: There was indicated a positive response between nitrogen treatments and numbers of adult or immature stages of two spotted spider mite on most sampling dates, during peak population growth. Analysis of variance for numbers of adult or immature stages (Table 1) of two spotted spider mite was showed that significant variations ($P<0.05$) were observed both among nitrogen treatments and among six bean cultivars, in each year (during 2008- 2010).

So, combined analysis for three years are shown in Table 2. These analysis were showed that significant variations ($P<0.05$) were observed in year (Y), cultivar (C), nitrogen (N) and interaction between cultivar and nitrogen (C×N), but no significant variations were observed in other interactions (C×Y, N×Y and C×N×Y).

Table 1: Analysis of variance for *T. urticae* adult and immature stages in various nitrogen levels on six bean cultivars in 2008

Source of Variation	df	Mean Square													
		17-Jun	23-Jun	30-Jun	07-Jul	14-Jul	21-Jul	28-Jul	04-Aug	11-Aug	18-Aug	24-Aug	31-Aug	07-Sep	14-Sep
Replication	3	0.001 ^{ns}	0.026 ^{ns}	0.046 ^{ns}	0.042 ^{ns}	0.019 ^{ns}	0.055 [*]	0.154 ^{**}	0.402 ^{**}	0.565 ^{**}	0.143 ^{ns}	0.622 ^{**}	0.524 ^{**}	0.521 ^{**}	0.503 ^{**}
Cultivar (C)	5	0.035 ^{**}	0.358 ^{**}	1.397 ^{**}	2.297 ^{**}	6.083 ^{**}	10.215 ^{**}	13.843 ^{**}	14.594 ^{**}	18.729 ^{**}	21.707 ^{**}	29.004 ^{**}	36.947 ^{**}	37.823 ^{**}	42.299 ^{**}
Nitrogen (N)	4	0.019 [*]	0.170 ^{**}	0.710 ^{**}	2.050 ^{**}	3.530 ^{**}	6.260 ^{**}	10.374 ^{**}	13.754 ^{**}	15.242 ^{**}	18.172 ^{**}	22.650 ^{**}	26.970 ^{**}	26.731 ^{**}	30.224 ^{**}
C × N	20	0.011 ^{ns}	0.087 ^{**}	0.281 ^{**}	0.334 ^{**}	0.367 ^{**}	0.298 ^{**}	0.245 ^{**}	0.354 ^{**}	0.529 ^{**}	1.099 ^{**}	0.0831 ^{**}	0.516 ^{**}	0.373 ^{**}	0.585 ^{**}
Error	87	0.007	0.016	0.262	0.319	0.015	0.020	0.290	0.035	0.039	0.791	0.053	0.105	0.094	0.086
C.V	18.40	12.95	16.21	17.86	12.40	14.27	17.17	18.80	19.99	18.94	12.31	12.24	13.07	12.93	

df, degree of freedom; MS, mean of squares; ns, *, **, represent non-significant, significant difference at 5 and 1% level, respectively

Table 2: Combined of variance analysis for *T. urticae* adult and immature stages in various nitrogen levels on six bean cultivars during 2008-2010

Source of Variation	df	Mean Square													
		17-Jun	23-Jun	30-Jun	07-Jul	14-Jul	21-Jul	28-Jul	04-Aug	11-Aug	18-Aug	24-Aug	31-Aug	07-Sep	14-Sep
Year (Y)	2	0.00 ^{ns}	0.00 ^{ns}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.50 ^{**}	0.63 ^{**}	0.31 ^{**}	0.50 ^{**}
Error	9	0.01	0.02	0.04	0.04	0.01	0.05	0.15	0.40	0.56	0.16	0.68	0.53	0.55	0.53
Cultivar (C)	5	0.10 ^{**}	1.07 ^{**}	4.19 ^{**}	6.89 ^{**}	18.25 ^{**}	30.64 ^{**}	41.52 ^{**}	55.78 ^{**}	74.18 ^{**}	127.73 ^{**}	136.90 ^{**}	160.17 ^{**}	174.70 ^{**}	188.77 ^{**}
C × Y	10	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.05 ^{**}	0.07 ^{**}	0.10 ^{**}
Nitrogen (N)	4	0.05 ^{**}	0.51 ^{**}	2.13 ^{**}	6.15 ^{**}	10.59 ^{**}	18.78 ^{**}	31.12 ^{**}	47.26 ^{**}	66.72 ^{**}	80.48 ^{**}	118.02 ^{**}	130.51 ^{**}	141.22 ^{**}	152.02 ^{**}
N × Y	8	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.05 ^{**}	0.07 ^{**}	0.09 ^{**}
C × N	20	0.03 ^{**}	0.26 ^{**}	0.84 ^{**}	1.03 ^{**}	1.10 ^{**}	0.89 ^{**}	0.73 ^{**}	1.06 ^{**}	1.58 ^{**}	3.31 ^{**}	1.76 ^{**}	1.75 ^{**}	1.12 ^{**}	1.73 ^{**}
C × N×Y	40	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.03 ^{**}	0.05 ^{**}	0.07 ^{**}
Error	261	0.00	0.01	0.02	0.03	0.01	0.02	0.02	0.03	0.02	0.79	0.05	0.10	0.09	0.08
C.V	18.28	11.99	16.95	17.25	14.58	14.71	14.01	14.82	14.48	17.04	13.92	14.85	14.09	14.31	

df, degree of freedom; MS, mean of squares; ns, *, **, represent non-significant, significant difference at 5 and 1% level, respectively

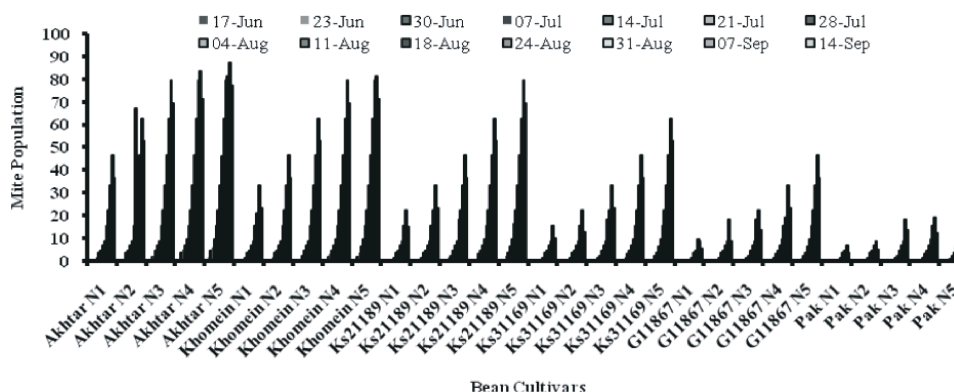


Fig. 1: *T. urticae* population in various nitrogen levels on six bean cultivars in 2008

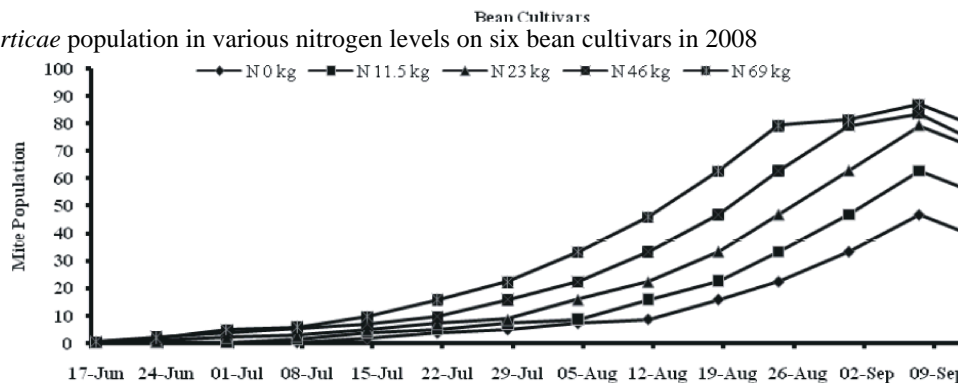


Fig. 2: *T. urticae* population in various nitrogen levels on Red Bean Akhtar in 2008

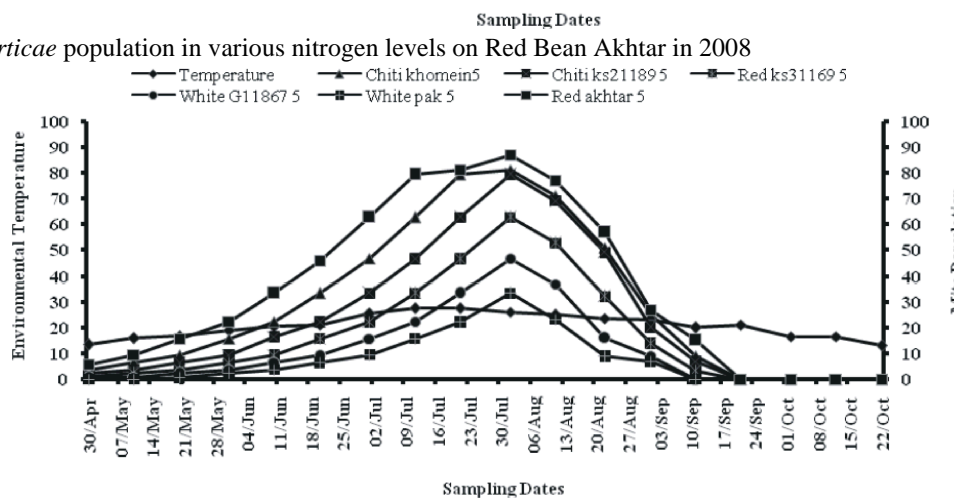


Fig. 3: Population fluctuation of *T. urticae* on six bean cultivars in 2008

The results showed that 69 kg nitrogen per hectare (150 kg ha⁻¹ Urea 46% N) was the most effective on numbers of adult or immature stages of two spotted spider mite (Figur 1 in 2008, the results in 2009 and 2010 were similar). This study indicated that increasing nitrogen level in the range of 0 to 69 kg ha⁻¹ resulted in enhanced mite population on bean leaves in field.

So, the most numbers of adult or immature stages of two spotted spider mite were on Red bean Akhtar. Hence, the Red bean Akhtar had the most susceptible to TSSM

among six bean cultivars. Our findings revealed that White beans (Pak and G11867) were less suitable cultivars, suggesting that they are more resistant to the TSSM than the other cultivars.

On Red bean Akhtar, populations of *T. urticae* began to increase in 15 July and decrease after 7 September. At the highest rate of nitrogen (69 kg ha⁻¹), average adult or immature numbers were 50% greater on 7 September compared to the control (0 kg ha⁻¹) (Figure 2).

Climatic Effect: Interaction between *T. urticae* population and environmental temperature are given in Figure 3. The results of this research were showed that a positive response was between environmental temperature and numbers of adult or immature stages of two spotted spider mite on sampling dates, during mite population growth. Environmental temperature is the most important factor that effect on population dynamic of two spotted spider mite.

TSSM population was appeared on weeds (*Convolvulus arvensis* L., *Althaea officinalis* L. and *Malva neglecta* Wallr) in 15 April (12°C) and was transferred on bean in 12 June (19.5°C). TSSM population was developed and peak population growth was in 24 August to 2 September (29-30°C). Mite populations began to decrease after 7 September to bean harvest. After bean harvest, mite populations were transferred on weeds and soil. Hence, *T. urticae* transfer in soil for survive and diapause.

Finally results were showed that infestations by TSSM populations are most serious in hot and dry conditions, hence, temperature is an important climatic factors on *T. urticae* populations. The best climatic temperature for TSSM populations are 25-30°C that mite populations can expand rapidly.

DISCUSSION

Our studies demonstrated that nitrogen fertilizer treatments resulted in significantly increased densities of both adult and immature *T. urticae* on six bean cultivars in the field (Figures 1 and 2, Tables 1 and 2). This study indicated that increasing nitrogen level in the range of 0 to 69 kg ha⁻¹ resulted in enhanced mite population on bean leaves in field. These results are consistent with findings of other authors for various mites and insects on different hosts treated by nitrogen fertilization [34, 21, 35, 18, 19, 36].

Our findings showed that higher numbers of the adult and immature two spotted spider mite resulted in significantly increasing spider webs production. The rate of change in abundance of *T. urticae* increased with fertilization levels ranging from 0 to 100% of the standard level in the bean field studies. A similar relationship between TSSM densities on bean and spider webs production was reported by other authors [37].

Spider mites were reared individually on leaf discs of apple trees fertilized with three different N levels. The correlation between sex-ratio and respective leaf N

contents proved significant. In a range of 1.8-3.0% N, sex-ratio increased from 0.64 to 0.76. A relationship between increasing fecundity and higher sex-ratio was found. Mortality was not affected [38]. Nitrogen is frequently considered as a limiting resource for insects [39, 40]. The leaf nitrogen content is generally accepted as an indicator of food quality [41] and as a factor affecting host selection by phytophagous insects [39, 40, 42]. Chemical fertilizers are able to modify the nutritive value of plants for phytophagous insects and affect their establishment and population growth [23, 43, 44, 17, 24, 45, 18, 46].

Putnam [47] and Van De Vrie *et al.* [20], reported association between leaf nitrogen level and fecundity or rate of development of European red mite, *Panonychus ulmi* Koch on peach. Leigh [48] has observed that cotton grown in nitrogen-deficient soil frequently demonstrates more severe spider mites injury than plants in adjacent plots receiving adequate or excessive nitrogen. Various studies using living plants, such as those of Storms [49] with apple and Rodriguez *et al.* [50] with strawberry, have indicated that mite populations respond to higher foliar nitrogen, typically by increased fecundity. In leaf disk studies, Wermelinger *et al.* [51] reported a similar response with *T. urticae*.

Our results on the beneficial effect of nitrogen on bean mite density agree with results of Cadena and Cothren [52] and Bondada *et al.* [53]. Many other features of a plant's physiology such as quality and quantity of amino acids [54] and carbohydrate amino acid ratio [55] are known to be correlated with mite population development. It has been suggested that interactions among multiple nutrients be considered in efforts to explain variation in herbivore responses to plant nutrients [56]. The strong influence of nitrogen treatments on cotton carbohydrate metabolism is shown by the significant effect of enhanced foliar photosynthetic rates and stomatal conductance and the altered concentrations of glucose, fructose and sucrose in petioles [57]. Immature developmental time of *Tetranychus pacificus* decreased ($P < 0.05$) as leaf nitrogen increased. There was also a significant ($P = 0.05$) increase in adult fecundity as leaf nitrogen increased. Neither adult longevity, immature survivorship, nor oviposition duration appeared to be influenced by leaf nitrogen content [34].

Many studies have demonstrated that the primary effect of N on crop production is via the formation of leaf area [58, 59, 60]. Many morphological and biochemical characteristics of plant are expected to alter due to varying N supply [61, 62]. The present results indicated

that shoot height and leaf area were significantly increased by nitrogen nutrition. Results indicated that applied nitrogen had effect on seed bean yield, although vegetative bean growth was significantly increased. Several factors may be involved. First, applied nitrogen increased numbers of both adult and immature two spotted spider mite (Figures 1 and 2). Large two spotted spider mite populations can ingest sufficient quantities of cell plant sap to cause severe reductions in yield [63-65]. In summary, nitrogen applied on bean had effect on seed bean yield but resulted in increased TSSM numbers and spider webs production.

There were significant ($P < 0.05$) differences in TSSM population developmental time in 3 years. These differences may have been caused by the higher temperatures that occurred during that year. This would support the observations of Mellors and Propts [66] that larger *T. urticae* populations resulted from 2 to 4°C higher minimum and maximum temperatures. Our mean daily min/max temperatures during 2008 were 14.9/29.5°C, during 2009, 18.6/ 34.7°C and during 2010, 16.4/ 31.7°C. Results indicated that the highest *T. urticae* populations were between August and September that environmental temperature increased. So, the highest *T. urticae* populations were in 2009 because climatic conditions were hot and dry in this year. Leigh [48], suggests that nitrogen-deficient plant growth may result in lower humidity and higher temperatures within the plant canopy which are more favorable to spider mites. *T. urticae* may, in particular, be favored by these conditions. In laboratory studies andres [67], found high temperatures more favorable to this species than to other spider mites.

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