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# FITOSSANIDADE

## WEATHER, CULTIVAR AND DENSITY-DEPENDENT PROCESSES INFLUENCE ON APHID IN ALFALFA <sup>(1)</sup>

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It is important to understand the effects of weather, insect density and plant cultivar on insect abundance to predict and prevent crop production loss. The present work investigated the influence of such factors on aphid in alfalfa during one year (short term). Data was collected from September/1997 to August/1998 at Canchin Farm (CCPSE-EMBRAPA), São Carlos, State of São Paulo, Brazil. Weather conditions had, in general, little effect on the variation of aphid populations, but *Therioaphis maculata* abundance was negatively correlated to humidity. Moreover, high maximum temperatures and low levels of rainfall possible favored *T. maculata* high abundance on Crioula and P3 cultivars. Therefore, appropriate management of this species is required during hot and dry periods. Population variation of *Acyrtosiphon* spp., *T. maculata* and *Aphis craccivora* seems to be regulated by density dependent processes. Resistant cultivar CUF 101 had a lower abundance of *T. maculata* and *A. craccivora* and a narrower population variation than the other cultivars and may reduce their abundance on field. Long-term studies on population dynamics including the effect of climatic conditions and density-dependent factors on plant quality will contribute to pest management in alfalfa fields.

**Key words:** Herbivory, Hemiptera, *Medicago sativa*, resistance, population dynamics.

### RESUMO

#### INFLUÊNCIA DO CLIMA, DA CULTIVAR E DE PROCESSOS DEPENDENTES DA DENSIDADE SOBRE AFÍDEOS EM ALFAFA

É importante entender os efeitos do clima, da densidade dos insetos e da cultivar sobre a abundância de insetos para se prever e prevenir perdas na produção das culturas. O objetivo do presente trabalho foi analisar a influência desses fatores sobre afídeos em alfafa no período de um ano. Os dados foram coletados de setembro/1997 a agosto/1998 na Fazenda Canchin (CPPSE-EMBRAPA), São Carlos, São Paulo, Brasil. As condições climáticas, em geral, tiveram pequeno efeito sobre a variação populacional dos afídeos, mas a abundância de *Therioaphis maculata* esteve negativamente correlacionada à umidade. Além disso, as altas temperaturas máximas e os baixos níveis de precipitação pluvial possivelmente favoreceram a alta abundância dessa espécie nas cultivares Crioula e P3. Portanto, torna-se importante o manejo desse afídeo durante períodos quentes e secos. As variações populacionais de *Acyrtosiphon* spp., *T. maculata* e *Aphis craccivora* parecem ser reguladas principalmente por processos dependentes da densidade. Na cultivar resistente CUF 101 observou-se menor abundância de *T. maculata* e *A. craccivora* e uma variação populacional menos pronunciada do que nas demais cultivares, podendo ser utilizada para redução da população dessas espécies em campo. Estudos de longa duração, incluindo os efeitos do clima e da densidade sobre a qualidade da planta, vão contribuir para o manejo de afídeos nos alfafais.

**Palavras-chave:** Herbivoria, Hemiptera, *Medicago sativa*, resistência, dinâmica populacional.

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## 1. INTRODUCTION

Alfalfa (*Medicago sativa*) is the most studied livestock feed plant; has a great potential for human nutrition and reduces the ecological costs of agriculture (SMALL, 1996).

The Centro Nacional de Pesquisa de Pecuária do Sudeste (CPPSE-EMBRAPA) has been breeding several alfalfa cultivars in a central region of São Paulo State (São Carlos City) since 1991. The aim is to be investigating basic problems concerning the cultivation of alfalfa under the climatic conditions of the region (RASSINI, 1998). Insect herbivory is one of these problems, and may seriously decrease the forage yield, e.g., the economical loss of 81 million dollars caused by the spotted alfalfa aphid, *Therioaphis maculata*, from 1954 -56 in the USA (LARA, 1991). CARVALHO et al. (1996) argued that aphids, mainly *T. maculata*, are possibly the most important alfalfa pests in Brazil. *Acyrtosiphon pisum*, *A. kondoi*, *T. maculata* and *Aphis craccivora* were the aphid species registered on alfalfa in São Carlos (SOUSA-SILVA et al., 1998)

Abiotic factors, mainly temperature, affect aphid biology (NIELSON and BARNES, 1961; GRAHAN, 1959) but responses may vary depending on the plant cultivar (MORGAN et al., 2001). Aphid population variation on field seems to be affected by both climatic conditions (CARVALHO et al., 1996; BERBERET et al., 1983; ARAUJO and SALES, 1985) and density-dependent factors, such as crowding (DIXON, 1977).

Studies on the factors influencing insect abundance on short and long term are important to predict and prevent serious production loss due to their large populations. Therefore, this work investigated the effects of weather (temperature, humidity and rainfall), aphid abundance and alfalfa cultivars on the variation and abundance of different aphid species populations on these plants during one year (short term).

## 2. MATERIAL AND METHODS

Alfalfa cultivars Crioula and P3, most cropped in Brazil and the aphid resistant CUF 101 were cultivated at Canchin Farm of EMBRAPA in São Carlos, São Paulo State, Brazil (21°10'S and 47°53'W). Plots (1,5 x 5 m) were arranged in a randomized block experimental design with three replicates per cultivar. Plants were fertilized (180 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 150 kg ha<sup>-1</sup> of K<sub>2</sub>O, 30 kg ha<sup>-1</sup> of FTE BR 12, plus 30 kg ha<sup>-1</sup> of K<sub>2</sub>O after each harvest).

Aphid populations were evaluated once monthly from September/97 to August/98 collecting

randomly 100 individual shoots per sample from each alfalfa cultivar on the three plots. Shoots were placed in plastic bags and then stored in freezer (-20 °C). These were later thawed and then shaken for a few seconds in 500 mL of a soap solution added to the bags. Subsequently, the shoots were removed and the solution was filtered using a fine-mesh cloth. Aphids retained in the filter were transferred to petri dishes, the species *T. maculata*, *A. craccivora* and *Acyrtosiphon* spp. were identified according to the descriptions of SOUSA-SILVA et al. (1998) and the insects were counted using a stereomicroscope. The two species of *Acyrtosiphon* found, *A. pisum* and *A. kondoi*, were counted together as *Acyrtosiphon* spp. due to difficulties to sort early instars.

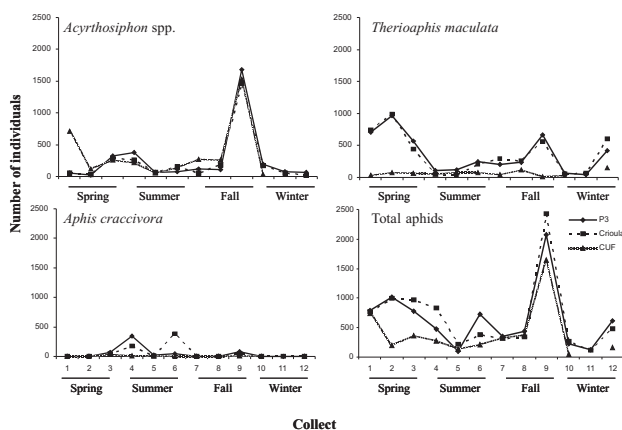
Weather (maximum, minimum and average temperature, humidity and rainfall) data used in this work were provided by the Agrocimatological Station of CPPSE-EMBRAPA and included the period between each sampling, e. g., for the 2<sup>nd</sup> sampling, weather data from 15/09/97 (1<sup>st</sup> sampling) to 10/10/97 (2<sup>nd</sup> sampling).

Nonparametric analyses with two or more factors are not generally acceptable. Therefore ANOVA on Ranks (Kruskal-Wallis) using H critical values distribution for ranks was used to study the variation in the aphid abundance (100 shoots/sampling/cultivar) among different sampling times and among cultivars. Multiple comparisons among data were performed using the Dunn's test and expressed as aphid abundance (number of individuals) collected. Correlation among variables (climatic and aphid abundance) was estimated using backward stepwise regression (SigmaStat 2.03 - 1992-1997 SPSS Inc.). Density dependence, i.e. relative growth rate dependence upon population size (POLLARD et al., 1987), was detected testing for correlations among dissimilarity (Euclidian distances) matrices of  $x_i$  ( $\log_e N_i$ ) and  $d_i$  ( $x_{i+1}-x_i$ ) of different aphid species on different alfalfa cultivars using Mantel test (NTSYSpc 2.10s, Applied Bioestatistics Inc.); where  $N_i$ =population size ( $i= 1, 2, 3...$  samplings) and  $d_i$  = relative growth rate.

## 3. RESULTS AND DISCUSSION

Total aphid abundance on alfalfa varied significantly ( $H=899.3$ ;  $P < 0.001$ ) among sampling times on the same growth stage of alfalfa (prebloom). On the population dynamics of specific aphid species, *Acyrtosiphon* spp. had an outbreak in the 9<sup>th</sup> sampling, but densities remained bellow two individuals per shoot most of the time ( $H= 1061.5$ ;  $P < 0.0001$ ). The highest abundance of *T. maculata* was

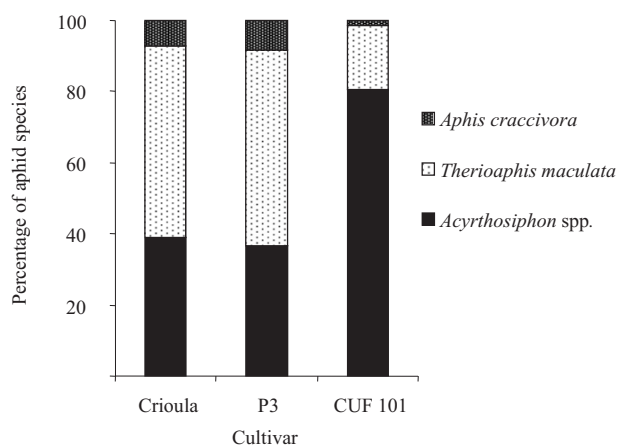
in the spring (1<sup>st</sup> to 3<sup>rd</sup> samplings), but this species had more than two individuals per shoot during several months, except on CUF 101 cultivar ( $H=800.2$ ;  $P < 0.0001$ ). *A. craccivora* was present only in a few samples, mainly in the summer, in a very low abundance ( $H=378.7$ ;  $P < 0.0001$ ) (Figure 1). Growth stage affected the percentage of infected shoots on peas, but was not correlated to *A. pisum* abundance (McVEAN et al., 1999). Long population growth duration periods are related to the aphid *Metopolophium dirhodum* on cereals (HONEK, 1991) maximum abundances, but in the present work, aphids were sampled on very similar time intervals, therefore, maximum abundance of aphids in the present work are not related to population growth duration. Aphid population variation in alfalfa seems to be related to plant quality, e.g., mineral concentration, such as magnesium, sulfur and phosphorus were negatively correlated to total aphid abundance (SILVA et al., 2005a) and amino acids, mainly alanine that was positively related to the increase of aphid number on low abundance (up to 4 individuals/shoot) (SILVA et al., 2005b)



**Figure 1.** Aphid abundance (number of individuals per 100 shoots) in three cultivars of alfalfa cropped at São Carlos, SP, Brazil. September 15/1997 to August 8/1998.

There was a significant lower number of total aphids ( $H=258.3$ ;  $P < 0.001$ ), *T. maculata* ( $H=467.4$ ;  $P < 0.001$ ) and *A. craccivora* ( $H=73.0$ ;  $P < 0.001$ ) on CUF 101 than on other cultivars, but a higher abundance of *Acyrthosiphon* spp. ( $H=159.4$ ;  $P < 0.001$ ) (Figures 1 and 2). Different cultivars (MORGAN et al., 2001) affect several biological parameters of aphids, leading to differences in population size. CUF 101 is a multiple resistant cultivar to *A. pisum*, *A. kondoi* and *T. maculata*. However, the occurrence of *A. pisum* (BOURNOVILLE et al., 2000) biotypes able to overcome CUF 101 resistance

has been already described. Despite that, resistant cultivar affected aphid abundance on alfalfa significantly, mainly for *T. maculata* and *A. craccivora* and is a possible alternative to reduce their abundance in the field.



**Figure 2.** Percentage of aphids species on different alfalfa cultivars cropped at, São Carlos, SP, Brazil. September 15/1997 to August 8/1998.

Interestingly, CUF 101 had the lowest total aphid (Figure 1), but its annual production (ton. of dry matter) was lower than on Crioula or P3 (RASSINI, 1998), suggesting that these two cultivars are tolerant to aphids or that resistance, such as antibiosis on CUF 101, has a metabolic cost, consuming metabolic resources, e.g., carbon and nitrogen compounds, primary used for plant growth (EDWARD and WRATTEN, 1981).

There were just a few significant relationships between aphid number and weather variables. Humidity and minimum temperatures were negatively related to *T. maculata* population abundance on Crioula ( $r^2_{adj} = 0.517$ ;  $F=6.89$  and  $P=0.017$ ), but only humidity on P3 ( $r^2_{adj} = 0.502$ ;  $F=12.4$  and  $P=0.006$ ). Abiotic factors, mainly temperature (SIDDIQUI et al., 1976), also humidity (GRAHAM, 1959) affect alfalfa aphid biology under controlled conditions. However, under field conditions in the present work, it seems that aphid population dynamics is not correlated to most of the climatic factors evaluated. The same was found by other researchers on long-term (JAROSIK and DIXON, 1999; WOOL, 2002) and short term (PINHEIRO et al., 2002) studies of insect population variation. The effect of other variables not evaluated in the present work, such as solar radiation and insolation, may not be neglected because they affected aphid abundance on cotton plants (ARAUJO and SALES, 1985). Moreover, abiotic factors may affect aphid biology differently on different cultivars (MORGAN et al., 2001).

**Table 1.** Climatic data (average) of different sampling periods from September 15/1997 to August 8/1998 recorded by the Agroclimatological Station of CPPSE-EMBRAPA (21°10'S and 47°53'W), São Carlos (SP), Brazil

Sampling	Mean temperature			Rainfall	Humidity
	Maximum	Average	Minimum		
		°C		mm	%
1 <sup>st</sup> (15/9/97)	29.2	21.8	13.5	23.0	54.5
2 <sup>nd</sup> (10/10/97)	28.0	20.6	10.0	46.6	70.0
3 <sup>rd</sup> (10/11/97)	31.0	23.9	18.0	199.0	84.0
4 <sup>th</sup> (08/12/97)	28.0	22.9	18.5	247.9	92.0
5 <sup>th</sup> (05/1/98)	30.0	24.4	18.5	121.8	88.0
6 <sup>th</sup> (30/1/98)	30.0	24.3	19.0	160.8	86.0
7 <sup>th</sup> (02/3/98)	30.0	24.8	19.5	307.7	87.0
8 <sup>th</sup> (01/4/98)	30.0	24.0	18.7	106.2	84.0
9 <sup>th</sup> (30/4/98)	27.5	22.3	17.5	105.2	80.0
10 <sup>th</sup> (01/6/98)	24.3	18.9	13.7	86.4	77.5
11 <sup>th</sup> (03/7/98)	23.4	17.5	11.7	5.0	83.0
12 <sup>th</sup> (07/08/98)	25.0	18.9	11.5	23.2	69.0

**Table 2.** Pearson's correlations for aphid species abundance on different alfalfa cultivars

Aphid species	P3	CUF 101	CUF 101
	x Crioula	x Crioula	x P3
<i>Acyrtosiphon</i> spp.	0.92*	0.50	0.59*
<i>Therioaphis maculata</i>	0.90*	0.01	0.02
<i>Aphis craccivora</i>	0.88*	0.13	0.01
Total ( <sup>1</sup> )	0.92*	0.81*	0.79*

\*Indicate significant correlations ( $p < 0.05$ ). (<sup>1</sup>) total aphid abundance on the specific cultivar. N=12.

Despite that, the negative relation between *T. maculata* population variation and humidity is in agreement to the results of GRAHAM (1959) that related usually lower longevity, fecundity and reproductive periods of this aphid species at high humidity (85-90%) than at intermediate (65-70%) and low humidity (25-30%). Indeed, high abundance of *T. maculata* was recorded when humidity was close to 73%. NIELSON and BARNES (1961) related an increased mortality of this aphid species during high humidity periods by the fungus *Entomophthora exitialis*. Interestingly, humidity was not correlated to *T. maculata* population variation on CUF 101, a possible result of the resistant features of this cultivar upon this aphid species affecting population variation differently from the other cultivars (Table 2)

Prevalence of *T. maculata* among other aphid species (Figure 2) in the present work might be related to maximum average temperatures (Table 1) because high temperatures and also dry weather are suitable to *T. maculata* (BERBERET et al., 1983). The upper limit

of development for this species range from 35-37 °C, the intrinsic rate of increase is highest at 30 °C and low humidity (25-30%) (GRAHAM, 1959). Maximum temperature recorded in several sampling periods in the present work were possible deleterious for the other aphid species, because temperatures ranging from 26.4 °C (MORGAN et al., 2001) to 30 °C (SIDDIQUI et al., 1976) for *A. pisum* and higher than 25 °C for *A. kondoi* (BERBERET et al., 1983) exceed the limit for development of these species. KODET and NIELSON (1980) showed that temperatures between 20-25 °C suppressed the alata production in *A. kondoi* possibly decreasing its dispersal potential.

Total aphid population variation was correlated among all cultivars and between P3 and Crioula for all aphid species, but not for CUF 101 and other cultivars, except for *Acyrtosiphon* spp. on P3 (Table 2), suggesting that aphid population dynamics is disrupted by plant cultivar.

Present results indicate that aphid populations on alfalfa were negatively density-dependent (i. e., relative growth rate decreases as population increases) (Figure 3). Despite that, linear models to evaluate density dependence were unreliable with increased data variation and decreased census points (POLLARD et al., 1987). Alternatively, Mantel's test was performed to detect density dependence and significant ( $P < 0.05$ ) correlations between growth rate and aphid abundance after data permutation were found for total aphids on all cultivars, also for total *Acyrtosiphon* spp. and *A. craccivora* and also for some aphid species on specific cultivars (Table 3).

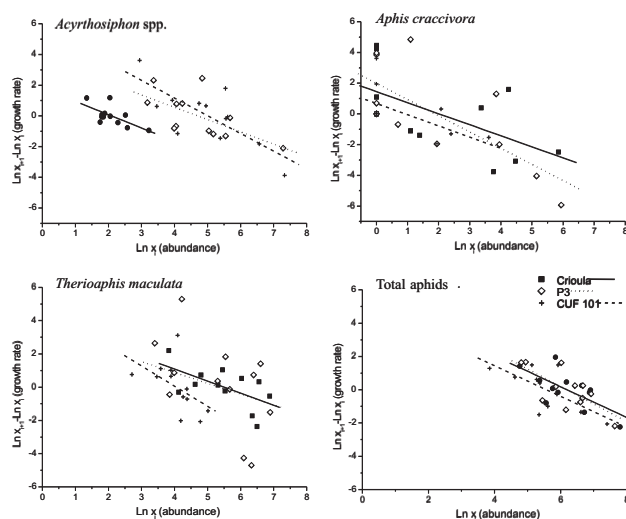
**Table 3.** Results (r) of Mantel test for detection of density dependence (population size X growth rate) in aphid populations on different alfalfa cultivars

Aphid species	Cultivars			Total <sup>(1)</sup>
	P3	Crioula	CUF 101	
<i>Acyrtosiphon</i> spp.	0.30*	0.40	0.58*	0.47*
<i>Therioaphis maculata</i>	0.14	0.23	0.47*	0.16
<i>Aphis craccivora</i>	0.47*	0.19	0.18	0.37*
Total <sup>(2)</sup>	0.50*	0.41*	0.36*	-

\*Indicate significant correlation [p (Z random = Z observed) < 0.05] for 5000 permutations

<sup>(1)</sup> Total aphid abundance of a specific species.

<sup>(2)</sup> Total aphid abundance of a specific cultivar. N= 12.



**Figure 3.** Growth rate vs. population size of different aphid species on alfalfa cultivars cropped at São Carlos, SP, Brazil. September 15/1997 to August 8/1998. Different lines are the linear fitting of scatter plots.

Interactions among different aphid species populations on all alfalfa cultivars suggest that total aphid abundance was regulated by density-dependent mechanisms. Noticeably, density-dependent regulation on CUF 101 occurred at high densities for *Acyrtosiphon* spp. and low densities for *T. maculata* and *A. craccivora* on P3 in a short time scale (i. e., within a year) (Table 3). SEQUEIRA and DIXON (1997) related density dependence in population regulations occurring on different time scales (i. e., within and between years). JAROSIK and DIXON (1999) also related a density-dependent regulation at low densities for the Turkey oak aphid (*Mizocallis boernerii*). DIXON (1977) argued that qualitative changes in aphids induced by crowding appear to be the most important population regulation factor. McVEAN et al. (1999) related that high

densities of *A. pisum* followed by a population crash, similar to the results for *Acyrtosiphon* spp. in the 9<sup>th</sup> sampling in this work (Figure 1), resulted from increased alata production suggesting a density-dependent regulation. Summer declines of the Turkey oak aphid (*M. boernerii*) were also related to migration, increasing linearly with aphid density (KINDLMANN and DIXON, 1996).

It is important to highlight that meteorological factors, similar to density-dependent factors, do not manifest themselves directly but through their effect on plant quality (JAROSIK and DIXON, 1999). Indeed, previous works related correlation among plant quality, i.e., mineral (SILVA et al., 2005a) and amino acids (SILVA et al., 2005b) on alfalfa to aphid variation, but meteorological effects on plant quality were not evaluated. Long-term studies on alfalfa aphid population dynamics including weather conditions and density-dependent factors and their effect on plant quality will certainly contribute to improve pest management programs on alfalfa fields.

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