EFFECT OF THE INHERENT VARIATION IN THE MINERAL CONCENTRATION OF ALFALFA CULTIVARS ON APHID POPULATIONS [1]

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ABSTRACT

Plants have inherent variability of mineral content which affects their physiology and consequently the herbivorous insects feeding on them. Besides, insects need considerable amounts of potassium, phosphorus and magnesium in their diets, whereas little calcium, sodium and chloride are required. In this study, the inherent variation on mineral (Ca, S, Mg, N, P, K and also C:N ratio) concentrations and aphid (*Acyrthosiphon* spp., *Therioaphis maculata, Aphis craccivora*) populations on three alfalfa (*Medicago sativa*) cultivars (P3; Crioula, the most widely cultivated in Brazil, and CUF 101, an aphid-resistant) were studied between September / 1997 and August / 1998. A significant variation on mineral concentrations and aphid populations was observed among different sampling times and cultivars. The correlations between C:N ratio, Mg, N, P and S concentrations and aphid density variation suggest that the mineral status affects aphid population dynamics under field conditions.

Key words: Aphididae, insecta, nutrients, resistance, plant-insect relation.

RESUMO

EFEITO DA VARIAÇÃO INATA DA CONCENTRAÇÃO DE MINERAIS EM CULTIVARES DE ALFAFA (*MEDICAGO SATIVA*) EM POPULAÇÕES DE AFÍDEOS (HEMIPTERA: APHIDIDAE)

As plantas têm variação inata do conteúdo de minerais e seu estado nutricional afeta sua fisiologia cloretos. A variação inata na concentração de minerais (Ca, S, Mg, N, P, K e também a razão C:N) e na população de afídeos (*Acyrthosiphon* spp., *Therioaphis maculata, Aphis craccivora*) em três cultivares de alfafa (*M. sativa*) - P3; Crioula, as mais cultivadas no Brasil, e CUF 101, resistente a afídeos - foi estudada entre setembro / 1997 a agosto / 1998 neste trabalho. A concentração de minerais e as populações de pulgões variaram significativamente entre os diferentes períodos de coleta e cultivares. As correlações encontradas entre as concentrações de Mg, N, P, S e a razão C:N e a variação no número de pulgões sugerem que os minerais da planta afetam a dinâmica populacional dos pulgões em campo.

Palavras-chave: Aphididae, insecta, nutrientes, resistência, relação planta-inseto.

([1]) Received for publication in May 21, 2004 and accepted in February 25, 2005.

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1. INTRODUCTION

The term nutrition involves a qualitative aspect and despite considerable phylogenetic differences and distinct feeding habits, insects have qualitative needs for most nutrients (except for sterols) similar to those of vertebrates.

The main nutritional needs of insects are amino acids, vitamins, minerals, carbohydrates, lipids and sterols, which should be appropriately balanced, especially in the case of the sugar:protein ratio. Another nutritional aspect considers the quantity, i. e., the nutrients effectively ingested, digested, assimilated and converted into tissues during development (DADD, 1985).

Insects have a great variation in their quantitative need for nutrients. This variation may reflect differences in metabolism, as well as accumulated reserves from a previous stage, or association with other organisms which synthesize some nutrients (HOUSE, 1962). But some aspects of insect mineral nutrition are not easily studied due to difficulties in manipulating simple radicals in diets (HOUSE, 1962, DADD, 1973).

Despite of that, insects need considerable amounts of potassium, phosphorus, magnesium and small amounts of calcium, sodium and chlorides during their development. Minerals are important for ionic balance, membrane permeability and enzyme activation (DADD, 1973).

Mineral concentration is related to nutritional status of plants affecting their physiology and the herbivorous insects that feed on them in positive, neutral or negative ways (DALE, 1988). Several research papers have been published on the effect of mineral use (supplementation or deprivation) and its impact on insect biology (BARKER and TAUBER, 1957; TAYLOR et al., 1952; KINDLER and STAPLES, 1970; MALBRY et al., 1997; BUSCH and PHELAN, 1999; JANSSON and EKBOM, 2002). But there is little information on the effect of inherent concentration of minerals on aphid population dynamics under field conditions.

Certain species and cultivars growing under the same conditions may differ in their ability to use mineral elements available in the soil (PAINTER, 1954). Plants also have inherent variability in nutrient levels (MATTSON and SCRIBER, 1987; EASTON et al., 1997). In this work, we investigated the relationships between inherent variation in the Ca, S, N, Mg, P and K concentration of three alfalfa (*Medicago sativa*) cultivars and variation in aphid populations on these plants in different sampling period.

2. MATERIAL AND METHODS

Plant material of each cultivar, P3; Crioula, the most widely cultivated alfalfa cultivar in Brazil; and CUF 101, an aphid resistant cultivar, (replicates from 3 plots of 3 x 2 m) was sampled from September / 1997 to August / 1998 (12 samplings-15 / 09 / 97; 10 / 10; 10 / 11; 08 / 12; 05 / 01 / 98; 30 / 01; 02 / 03; 01 / 04; 30 / 04; 01 / 06; 03 / 07; 07 / 08), at the pre-bloom stage. The three alfalfa cultivars were cultivated at the Canchim cattle farm of Embrapa (Brazilian Federal Agricultural Research Agency) (22^0 01'S and 47^0 54'W) near São Carlos, São Paulo state, Brazil. The soil was fertilized (180 kg ha^{-1} of P_2O_5; 150 kg ha^{-1} of K_2O; 30 kg ha^{-1} of FTE BR 12; plus 30 kg ha^{-1} of K_2O after each harvest) and the fields were irrigated (15 mm twice weekly) in the dry season. In June, CUF 101 plant samples were not included in the analysis because of ants damages.

One hundred shoots, collected individually once per sampling from each alfalfa cultivar, were placed in plastic bags (1 shoot per bag) and stored in common freezers (-20°C). A soap solution (500 mL) was added to each bag, which was then shaken for a few seconds. The shoot was removed and the solution filtered. Aphids retained on the filter were transferred to Petri dishes and the species *Therioaphis maculata*, *Aphis craccivora* and *Acyrthosiphon* spp. were separated and counted using a stereomicroscope and expressed as aphid density. Both species of *Acyrthosiphon* (*A. pisum* and *A. kondoi*) were considered as one (*Acyrthosiphon* spp.) due to difficulties in identifying early instars.

Plant material (500 g) was dried at 60°C in a forced-air drying oven, until constant weight was reached. Each sample was ground in a Wiley-type mill equipped with a 20 mesh sieve (SARRUGE and HAAG, 1974). Nitrogen concentration was determined by a microkjeldahl method (AOAC, 1995), after sulfuric digestion. After nitroperchloric digestion, the calcium and magnesium concentrations were determined by titration with EDTA; phosphorus concentration was determined by colorimetry and potassium by flame photometry (MALAVOLTA et al., 1989). Carbon content was determined after calcination in a muffle furnace at 550°C-600°C (SILVA, 1981).

Statistical analyses were performed using Sigma Stat 2.03 (1992-1997 SPSS Inc.). Two-Way ANOVA was used to study the effects of sampling time and cultivar on the concentration of six different minerals and also C:N ratio (3 replicates / sampling / cultivar). Nonparametric analyses with two or more factors are not generally acceptable (ZAR, 1999) and therefore ANOVA on Ranks was used to study the variation in the aphid population (100 replicates /

sampling / cultivar). Multiple comparisons among data were performed using the Dunn´s test. Spearman rank order correlation was used to test for correlation between aphid populations and mineral variation.

3. RESULTS

Two Way ANOVA tests indicated differences among mineral concentrations for variables sampling and cultivar, but no significant interaction (sampling x cultivar) was found.

Mineral concentration in alfalfa cultivars had significant ($p<0.05$) variation in different sampling times (Fig. 1). No significant differences were found among phosphorus concentrations during the first five sampling times ($p<0.001$), but a significant decrease in the 6th sampling was detected ($p<0.001$). After a decrease on the 9th sampling, nitrogen concentration increased significantly ($p<0.001$) and was the highest at the 11th sampling. Calcium concentration was the highest in the 1st and 12th samplings ($p<0.001$). There was a significant increase in sulfur concentration from the 1st to the 6th samplings ($p<0.001$). The lowest potassium concentration was found at the 4th, 5th, 8th and 12th samplings ($p<0.001$). Magnesium concentration was the highest at the 8th sampling ($p<0.001$). C:N ratio was the highest from the 1st to the 4th samplings and at the 9th ($p<0.001$)(Figure 1).

Phosphorus concentration was the highest in P3 cultivar ($p<0,001$). C:N ratio , magnesium and calcium concentrations were the highest in Crioula cultivar ($p=0,002$; $p<0,001$ and $p=0,003$, respectively). Nitrogen concentration was the highest in CUF 101 ($p=0,01$) (Table 1).

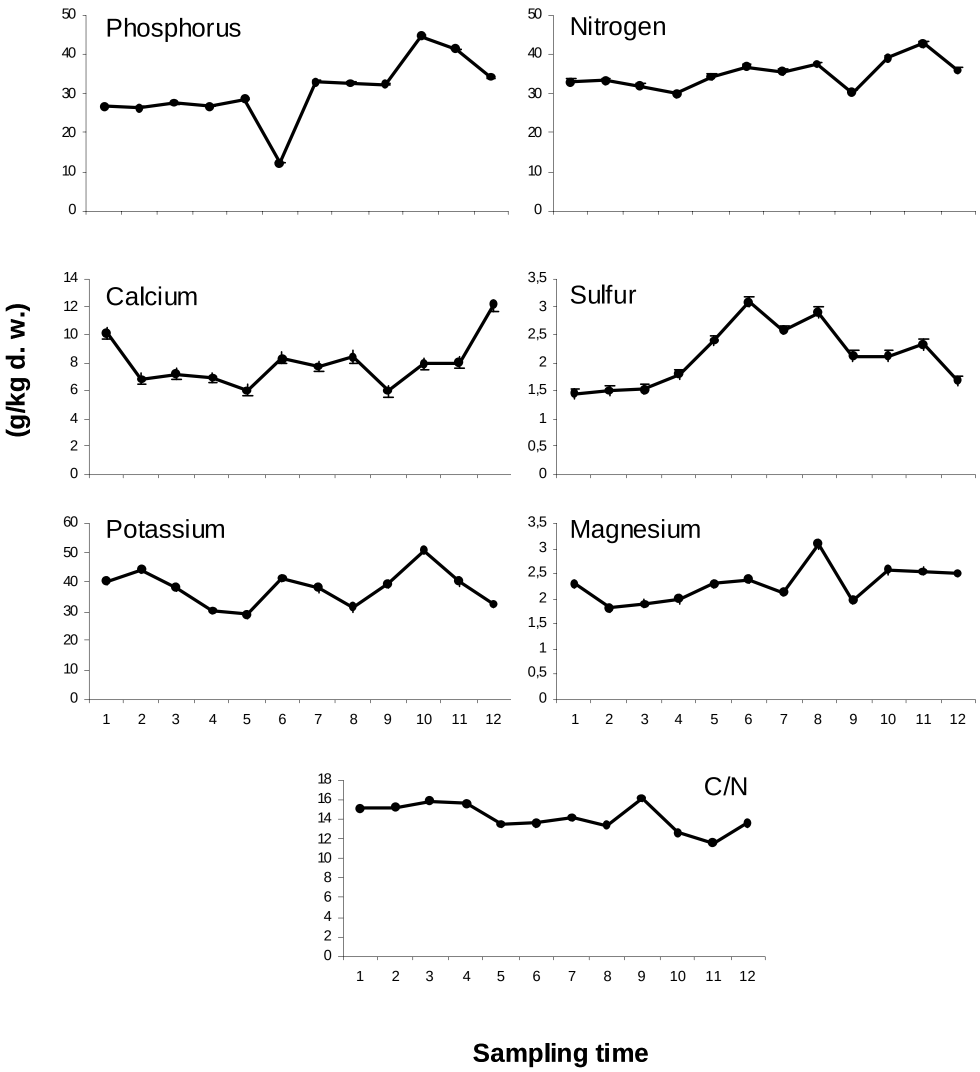

Figure 1. Variation in the mineral concentrations in alfalfa (*Medicago sativa*) cultivars on different sampling times. (mean ± s. e.)
Data for sampling in Two Way Anova (sampling x cultivar).

Table 1. Concentrations (g kg^{-1} d. w.) of different minerals in three alfalfa (*Medicago sativa*) cultivars. (mean ± s.e.)

Mineral	Cultivar		
	P3	Crioula	CUF 101
Phosphorus	33.2 ± 0.09^a	27.6 ± 0.09^b	28.0 ± 0.09^b
Nitrogen	34.0 ± 0.3^a	33.6 ± 0.3^a	35.2 ± 0.3^b
Calcium	7.6 ± 0.2^a	8.6 ± 0.2^b	7.6 ± 0.2^a
Sulfur*	2.1 ± 0.04	2.1 ± 0.04	2.1 ± 0.04
Potassium*	38.3 ± 0.7	36.3 ± 0.7	38.5 ± 0.7
Magnesium	2.1 ± 0.04^a	2.5 ± 0.04^b	2.2 ± 0.04^a
C:N	14.4 ± 0.1^a	14.9 ± 0.1^b	13.9 ± 0.1^a

* Indicate no significant ($p>0.05$) differences for a mineral among cultivars. Different letters indicate significant ($p<0.05$) differences for a mineral in a row. Data for cultivar in Two Way Anova (sampling x cultivar).

There was a significant variation on aphids density on different samplings and cultivars ($p<0.05$). *Acyrthosiphon* spp. density was the highest on the 3 rd and 4th samplings and peaked at the 9th. The highest density of *Acyrthosiphon* spp. observed was found on the resistant cultivar CUF 101. *Therioaphis maculata* density was the highest from the 1st to 3rd samplings and also at the 9th and 12th. CUF 101 had the lowest density of this aphid species. *Aphis craccivora* was found from the 3rd to the 6th samplings and also at the 9th but, it was almost absent at the other samplings. Also, CUF 101 had the lowest density of this aphid species. Total aphid density was high from the 1st to 4th samplings and also at the 9th. Total aphid density was usually lower on the resistant cultivar CUF 101 than the others (Figure 2).

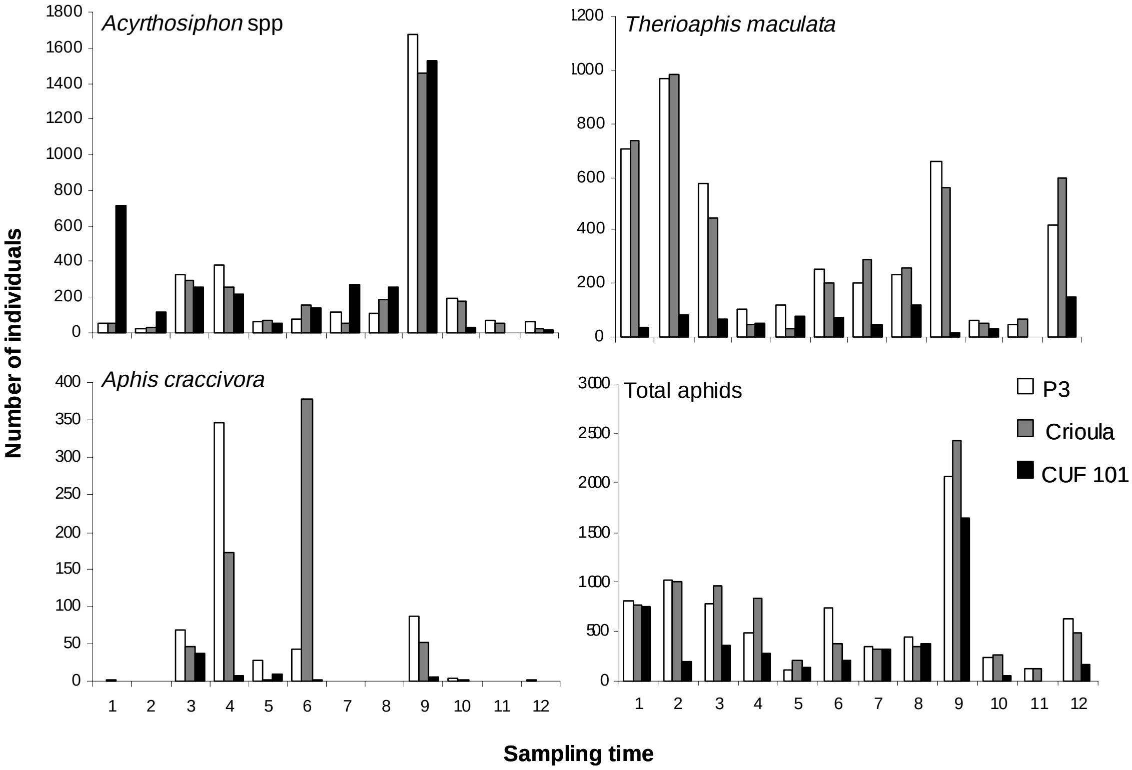

Figure 2. Variation in the aphid populations on three alfalfa (*Medicago sativa*) cultivars in different sampling times. Anova on Ranks (Kruskal-Wallis test) – 100 replicates. Data presented as total number of individuals in each sampling for each cultivar.

C:N ratio was positively correlated to the variation of *Acyrthosiphon* spp. and *T. maculata* populations (r=0.6, p=0.03; r=0.63, p=0.02, respectively) and also to total aphid density variation on all cultivars (Crioula- r=0.9, p<0.001; P3- r=0.8, p<0.001 and CUF 101- r=0.6, p=0.03). There was a negative correlation between nitrogen and magnesium concentrations and total aphids density on P3 and Crioula (N- r= -0.63, p=0.02; r= -0.79, p<0.001; Mg- r= -0.59, p=0.04; r= -0.7, p=0.009; respectively). Total aphid density on P3 was also negatively correlated to phosphorus concentration (r= -0.63; p=0.03) and total aphid density on Crioula was negatively correlated to sulfur concentration (r= -0.58; p=0.04).

4. DISCUSSION

Mineral analysis of alfalfa plant tips in this work was made during the pre-bloom stage and therefore, the mineral variation found was not related to growth stage. Despite of that, Abrahamson and Mc Crea (1985) found that most seasonal changes in the nutrient content and also the highest mineral content in *Solidago altissima* were associated with young, actively growing plant parts and inflorescences. Aiazzi et al. (1999) found that seasonal differences in the mineral content of *Atriplex cordobenses* were related to the growth stage (vegetative or reproductive) with accumulation of some minerals in the reproductive structures. As the alfalfa cultivars had a very short cycle between vegetative and reproductive stages (about 30 days), so the observed variation in the mineral concentration during pre-bloom might have resulted from factors affecting soil mineral uptake on different samplings.

Significant differences in mineral concentration among different alfalfa cultivars were found in this work (Tab. 1), which implies genotypic differences among cultivars. Easton et al. (1997) reported genetic variation in the concentration of macro and micro minerals in ryegrass (*Lolium perene*).

Mineral status affects plant's physiology and the herbivores feeding on them, but mineral ions are also important to insect's physiology in at least three major processes: enzyme activation (K, Mg, Fe, Co, Mn), trigger and control mechanisms (Na, Ca, K), and structure formation (Mg). Insect's tissues have large quantities of three major mineral elements: P (10 g kg^{-1} d.w.), K (1 g kg^{-1} d.w.) and Mg (2 g kg^{-1} d.w.) Therefore, it is expected that herbivore interaction with host plants would be at least partially mediated by an interaction between minimal optimum nutrient requirements and the inherent variability of the plant contents of these nutrients (Mattson and Scriber, 1987).

Several investigators (Auclair, 1965; Dadd and Mittler, 1965; Dadd, 1967; Akey and Beck, 1972; Auclair and Srivastava, 1972) did show that macro and micro minerals are essential for aphid development, affecting significantly their biology on artificial diets. Mineral concentrations above optimum thresholds are usually toxic to aphids, e. g., high boron and molybdenum (Auclair and Srivastava, 1972), phosphorus and potassium (Auclair, 1965) to *A. pisum*; magnesium to *A. pisum* (Auclair, 1965) and *M. persicae* (Dadd and Mittler, 1965) and nitrate to *S. graminum* (Salas et al., 1990)

The concentration of individual minerals (e.g., P, Mg, S), also their ratios in alfalfa tissues, was negatively correlated to aphid population variation in the present work, suggesting that they affected aphid biology. Jansson and Ekbom (2002) found negative correlations between both magnesium and sulfur concentration in *Petunia* leaves and fecundity and longevity of the aphid *Macrosiphum euphorbiae* and Busch and Phelan (1999) found that high phosphorus concentrations in soybeans resulted in longer developmental time of soybean looper (*Pseudoplusia includens*) and mineral proportions, such as high P:S ratio decreased pupal mass of this insect, but the opposite response was measured with a high S: P ratio.

In this work, a positive correlation of C:N ratio and a negative correlation of N between aphid population were found. Tripp et al. (1992) related fewer whiteflies on tomatoes with high C:N ratios. A decline on the performance of the aphids *M. euphorbiae* and *Myzus persicae* was found on potato tuber-filling leaves with a high C:N ratio, but C:N ratio on leaves was not correlated to sugar: amino acid ratio (Karley et al., 2002). Although most sucking insects respond positively to N fertilization (Jansson and Ekbom, 2002), after reviewing several papers on N supplementation, Van Emden (1966) related that in 36% of the cases aphids responded negatively to N fertilization, e.g., on barley (Salas et al., 1990) and *Polygonum pensilvanicum* (Malbry et al., 1997).

Salas et al. (1990) related nitrogen supplementation was positively related to alkaloid (gramine) concentration and was negatively correlated to aphid (*Schizaphis graminum*) performance on barley. Alkaloids are also present in alfalfa (Connor et al., 1973; Phillips et al., 1992; Wiehler and Marion, 1958) and are known to be related to root symbiont biochemical and ecological relations on alfalfa (Phillips et al., 1992). Present data, supported by negative correlations between aphid density and nitrogen concentration, highlights a possible relation between alkaloids and aphids on alfalfa. A significant higher concentration of nitrogen on the aphid resistant cultivar was also detected.

No significant correlations between aphid population variation and minerals, except for C:N ratio, were found for the resistant cultivar CUF 101, possibly due to its negative effect on *T. maculata* population size. BUSCH and PHELAN (1999) argued that responses to mineral concentration varied within species, also within cultivars, and were species-specific such as found in this work.

That would explain differences in the results of different researches on mineral effect on the same plant and aphid species, e.g., peas and pea aphids (BARKER and TAUBER, 1957 and TAYLOR et al., 1952).

Interestingly, the number of *Acyrthosiphon* spp. on the resistant cultivar was as high as or even higher than in the susceptible cultivars. Apparently, *Acyrthosiphon* spp. overcomes the resistant characteristics of CUF 101. The existence of "resistant" *A. pisum* (BOURNOVILLE et al., 2000) and *A. kondoi* (ZARRABI et al., 1995) biotypes to CUF 101 was already reported.

Undoubtedly, there are other factors affecting aphid population dynamics in alfalfa under field conditions such as climatic conditions, mainly, temperature and rain (BERBERET et al., 1983; CARVALHO et al., 1996) and also qualitative changes induced by crowding that are the most probable regulating factor for aphid populations according to DIXON (1977).

Correlations found between inherent mineral concentration and aphid populations on alfalfa suggest that mineral variation is related to aphid population dynamics under field conditions. Whether minerals affect aphid biology directly or indirectly through their effect on plant physiology remains to be determined.

5. CONCLUSIONS

1. Mineral concentration and aphid abundance in alfalfa vary with sampling time and cultivar.

2. Variation in aphid abundance along different sampling times is correlated to C:N ratio, N, Mg, P and S, but correlations vary with cultivar and aphid species.

ACKNOWLEDGEMENTS

The authors thank CAPES for financial support and Dr. Pitágoras da Conceição Bispo for statistical debates.

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