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Effect of Different Variation of NH_4^+ Compared to N ($NH_4^+ + NO_3^-$) Fertilization of Tomato (*Lycopersicum esculentum*) Cultivated in Inert Media on the Fecundity of the Aphids *Macrosiphum euphorbiae* (Homptera-Aphididae)

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Abstract: Changes of 0, 20 and 35% of NH_4^+ compared to N (NH_4^++NO_3^-) nutrient solutions of *Lycopersicum esculentum* L. cultivated on inert media on the multiplication of *Macrosiphum euphorbiae* (Aphididae Homomptera) were tested. The use of 0% NH_4^+ decreased 12% the fertility of aphids compared to 20% NH_4^+ and 17% compared to 35% NH_4^+. Reproduction of aphids on the leaves of flowering inflorescence increased 33% compared to that of the fruit setting inflorescence and 40% compared to that of the magnification inflorescence and 52% relative to the fixed level. The interruption of the fertilization of plant and its replacement by water for 4 days decreased 45% the fertility of aphids in the fourth inflorescence and 36% in the seventh inflorescence.

Key words: Aphids multiplication, N (NH_4^++NO_3^-), physiological stages, tomato fertilization, fertilization interruption

INTRODUCTION

Greenhouse aphids are a major challenge to greenhouse crop production. They demand serious attention on the part of the greenhouse grower. Integrated Pest Management (IPM) is an important tool in the management of these pests. It optimizes pest control in an economically and ecologically sound way. Integrated pest management involves the integration of cultural, physical, biological and chemical practices to grow crops with minimal use of pesticides.

Proper fertilization is, sometimes, necessary to give the plants a certain level of resistance against pests (Walters and Bingham, 2007). Several hypotheses have been formulated to understand the dependence of plant resistance on the availability of resources such as N (Dietrich Ploss and Heill, 2004; Hamilton *et al*., 2001; Wilkens *et al*., 1996; Stout *et al*., 1998).

Minkenberg and Ottenheim (1990) find that *Liriomyza trifolii* females that had previously been exposed to plants of high nitrogen content, showed a feeding and oviposition preference for plants of high nitrogen.

This study verified the qualitative variation of total N of nutritive solution destined to tomato plant cultivated in inert media. This was to vary the percentage of NH_4^+ relative to NO_3^-.

We know, moreover, according to Heller *et al*. (1989), an ammoniaco-nitric nutrition increases proteosynthesis, compared to only nitric nitrogen nutrition, particularly among cultivated *Solanaceous*.

The concentration of amino acid nutrition plays a vital role in growth, survival, fecundity of aphids (Srivastava and Auclair, 1974) and in choosing the site where they collect the sap from the host plant (Parry, 1977).

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In this study and in a first test, we used three different nutrients solutions differentiated by the form of N in order to modify the sap composition, hence to influence the aphid's reproduction.

In parallel, we studied the role of the different physiological stages of the leaf support on the fertility of aphids. The principle of this work was to keep the aphids on the leaves of the same physiological stage during the experiment, knowing that the transition from one stage to another takes about 7 days.

In the second test, we observed the effects of two breaks of mineral fertilizer with duration of 4 days each on fertility of aphids. These interruptions took place during the appearance of the fourth and seventh inflorescence. These interruptions were to replace exclusively the nutrition solution by tap water during 4 days. Such an interruption has no effect on the production of tomatoes.

The objective of this study designed to improve the IPM, through the optimal use of mineral nutrient solution of tomato cultivated in inert media against the aphids *M. euphorbiae*.

MATERIALS AND METHODS

The study was conducted in a Greenhouse of the Biology Laboratory of Invertebrates Antibes (Nice) France. That depends on the National Institute of Agronomical Research (Inra) France. It took place from 1/1/2008 to 1/3/2009.

Greenhouse

The test was held in glass-covered greenhouse, with a controlled environment. It is located in National Agronomic Institute Research (INRA) Antibes-France. It has a system of drip irrigation controlled by computer METODYN. The calculation of Potential Evapo Transpiration (PET) is function of global radiation. The irrigation is triggered according to the metabolic needs of the plant.

Plant

The host plant used was the tomato (*Lycopersicum esculentum* L.) variety Prisca at indeterminate growth.

Aphids

The species studied is *Macrosiphum euphorbiae* Thomas. Individuals used in this trial belonged to a clone. It was raised on eggplant, in air-conditioned room with a temperature between 20-22°C and 16 h of photoperiod. This is the method used to raise aphids in identical abiotic conditions. Aphids were reared since their birth in Petri boxes containing a disc of eggplant leaf placed on the agar. Females, thus obtained had the same age. They were reared under identical abiotic conditions.

After that they were transferred simultaneously on tomato in clip-cages and their offspring was used for testing.

Clip-Cages

For the needs of our experience, which consist to encage the aphids, some time on the same leaf, we have made ourselves these clip cages.

These are consisting by two nylon covers, with 3 cm diameter maintained one against the other by a small clamp.

These cages allowed keeping the aphid individually on the leaf and observing its fertility throughout the experiment.

The Crop Steering

The seedlings were made in oven at a temperature between 22 to 25°C in plastic pot containing silica, sand with size between 1 and 3 mm. Irrigation was done with tap-water during the raising of seedlings

During their growth, the seedlings were irrigated by a standard solution, whose composition (meq L^{-1})were: NO_3^- = 11, $H_2PO_4^-$ = 2, SO_4^{2-} = 2, K^+ = 5 Ca^{2+} = 8, Mg^+ = 8. Concerning micronutrients, it has been used 0.1 ml L^{-1} from the solution (Table 1).

The tomato plants were transplanted at 3 true leaves into cubes of rock wool placed in pots containing perlite.

The pH measurements and electro-conductivity of drainage solutions were also carried out every 3 days. They were between 5.8 and 6.5 for the first one and between 1800 and 2500 μS for the second. The input solutions were constant throughout the test. Irrigation was controlled automatically by a system computer-solar meter-metering pump.

The Effect of Different Forms of Nitrogen

Experimental Device

The experimental design was to study simultaneously the effect of different nutrition's of plants and their different physiological stages on the reproduction of Aphids. In this fact, we chose a hierarchical model with 3 classification criteria. This model was adapted to this experimental work. Because, we observed the effect of nutrition of plant on the fecundity of the aphids. On this same plant, we observed the effect of different physiological stages. The first factor is the different solutions. The Statistical assistance used was Assistat 7.5 beta.

The second factor which was subordinate to the first one represented the 3 foliar levels flowering, fruit setting and magnification. These two factors were fixed. The third was a block. It was random.

Different Treatments

In this test, we used 3 solutions differentiated by nutrients nitrogen form provided (Table 2).

- Treatment NH_4^+ (0%): It is composed by 0% of NH_4^+ and 100% by NO_3^-
- Treatment NH_4^+ (20%): It is composed by 20% NH_4^+ and 80% by NO_3^-
- Treatment NH_4^+ (35%): It is composed by 35% NH_4^+ and 65%. by NO_3^-

The main factors influencing the proteosynthesis are total N, $K^+/(Ca^{2+}+Mg^{2+})$ and the amount of NH_4^+ made. The total N was equal to 14 meq $L^{-1}$and it was the same in 3 solutions used. $K^+/(Ca^{2+}+Mg^{2+})$ was equal to 0.67 and is also identical in the 3 solutions. This allows a maximum of absorption of all the macro and micro elements. It also allows a balance between the elements for an optimal assimilation of chlorophyll (Fanasca *et al*., 2006), despite the small difference concentration between K^+, Ca^{2+} and Mg^{2+}.

What differentiates these 3 solutions for proteosynthesis; objective of our work is the variation of NH_4^+ in the total N. It is 0% for the first treatment, 20% for the second and 35% for the third. The

Table 1: Composition (g L^{-1}) of micro-elements of the solution used

Micro-elements	Composition (g L^{-1})
Fe EDTA	6.00
B	2.65
Cu	0.63
Mn	0.27
Mo	0.27
Zn	2.27

Table 2: Composition (meq L^{-1}) of macro elements solutions used

Treatment (%)	K^+	Ca^{+2}	Mg^{+2}	NH_4^+	H^+	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	$K_+/(Ca+Mg)$	$N(NH_4^++NO_3^-)$
	(Meq L^{-1})									
0	7.55	8.20	3.05	0.00	1.60	14.00	1.50	0.00	0.67	14
20	5.47	5.92	2.22	2.80	1.60	11.20	1.50	0.40	0.67	14
35	4.90	5.30	2.00	4.90	1.60	9.10	1.50	3.20	0.67	14

amount of NH_4^+ was lower than NO^-_3. Because an excess of NH_4^+ which behaves as antagonist cations, causes a deficiency of K^+, Ca^{2+}, Mg^{2+} (Heller *et al*., 1989). In this fact, we have used these low concentrations of NH_4^+ regarding to NO_3^-.

Effect of Interruption Fertilization

Two treatments regarding the interruption of the mineral fertilization during four days and its replacement by a water supply were used.

Treatment 1

An interruption of the mineral solution NH_4^+ (20%) took place from 22 to 25/5, the date of onset of the fourth inflorescence. The plants were irrigated with tap-water for four days. Four days prior to the interruption of fertilization, the aphids of fourth larval stage were placed on the feeder leaves of fourth inflorescence which were in bloom.

The adult stage of aphids coincided with the first day of the disruption of fertilization and the first births with the second day. Parallel, as a control, the aphids were deposited on plants which have fertilized normally.

Treatment 2

The suspension of the mineral solution was held from 20 to 23/6; date of onset of the seventh inflorescence.

Seven days before the second mineral interruption of plants, aphids were deposited on the leaves of the seventh inflorescence which was in bloom.

We observed fertility of a series of aphids on these plants before the interruption of fertilization. The progeny of another set of aphids was observed during the interruption of fertilization. Finally, we observed fertility of another series of aphids after the resumption of fertilization.

Experimental Device

The experimental design consisted by 6 blocks. Each block consisted of 5 plants on line. Within each block, we randomly distributed processing of the test 1 and 2 applications of the test 2.

Choice of the Leaves Support of Aphids

The test was conducted on tomato plants. The conduct of these plants was monopodial. The growing axillary in the axils of the leaves were removed when they appear. We have therefore obtained plants consisted of a main stem (sympode). This stem is made up of several elements of sympode or strata. Each of these strata was composed (Fig. 1) as:

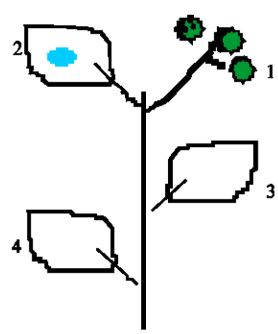

Fig. 1: Scheme of flowering sympode element. 1: Flowering inflorescence, 2: feeder leaf with encaged aphid, 3 and 4: two leaves below the inflorescence

- An inflorescence
- Two leaves below the inflorescence
- A leaf immediately above and opposite to the inflorescence called feeder leaf

The position and the proximity of the feeder leaf enable it to supply mainly the opposite inflorescence. This leaf was used as support for the aphids tested.

The elements used were the sympode flowering inflorescence, fruit setting inflorescence and magnification. The Flowering state was determined by a one to four flowers open.

The magnification stage begins at the end of the fruit of each inflorescence. We chose these strata to determine the role of successive physiological stages on feed aphids and consequently on its fecundity.

Measurements on the Fertility of Aphids

Adult female aphids were placed individually in clip cages on each plant and then collected after they have filed three larvae.

When the larvae had reached the 4th stage, two of them were eliminated. The observation of fertility was conducted on the remaining female. The progeny of a generation was followed.

The young larvae were counted and removed daily. These trials began in the first flower inflorescence.

Sequence for the Effect of Different Forms of Total N

We have maintained individual aphids on leaves of the same physiological stage during the experiment (Fig. 2).

The principle was to encage the aphids on the leaves of flowering stage. Then, we changed sympode when the new inflorescence appears. The same operation was done for aphids encaged on fruit setting leaves and enlargement leaves.

The 1/5, the date of first apparition flowering inflorescence (Fig. 2), we have placed the fourth stage larvae on the feeder leave of the inflorescence on plant tested.

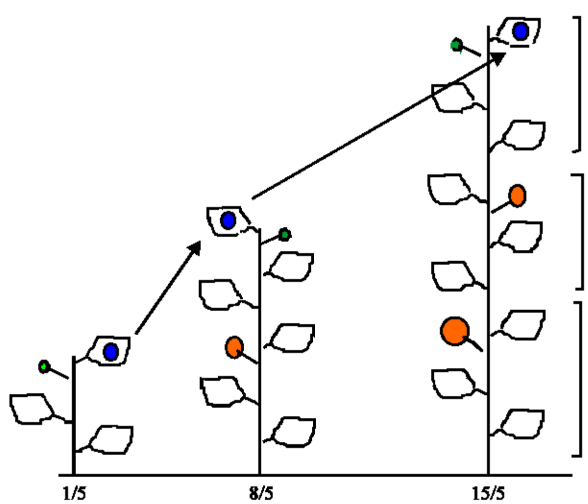

Fig. 2: Scheme of growth of the same plant and moving the clip-cage with aphids on the flowering stage as they appear. 1: Flowering sympode, 2: fruit setting sympode, 3: magnification sympode

The 8/5, the date of flowering of the second inflorescence, the clip cages with aphids are moved on the feeder leaf on this second inflorescence. At the same time, the first inflorescence was originally fruit setting. We have, therefore set a second round of aphids on the feeder leaves of these flowers.

The 15/5, date of third apparition flowering inflorescence (Fig. 2), we have shifted the aphids of first series on the feeder leave of the third inflorescence and the aphids of second series on the second inflorescence, which was in fruit setting stage. At the same time, we introduced a third series of aphids on the first inflorescence which was in magnification.

We also observed the reproduction of aphids on a fixed level. Aphids were placed on 15/5 on the feeder leave of third inflorescence which was in bloom. These aphids were kept throughout the experience on these leaves. They have suffered the effect of the flowering, fruit setting and magnification stage.

Sequence for Effect of Interruption of the Fertilization

This test included two breaks fertilization of plants. The substrates were thoroughly washed the old from the interruption. We used 30 liters of water per plant.

The first interruption of the fertilization took place from 22 to 25/5; the date of onset of fourth inflorescence. The second interruption took place from 20 to 23/6; date of onset of the seventh inflorescence.

Statistical Analysis

At the end of the analysis of variance and when we have been led to reject the hypothesis of averages equality (observed F> theoretical F), we compared these averages. We used the student test.

RESULTS

Effect of Different Fertilization and Foliar Levels on the Reproduction of Aphids

The analysis of variance applied to this test has shown that there is a significant effect of N ($NO_3^- + NH_4^+$) form on the reproduction of aphids than N (NO_3^-) form.The fecundity (Fig. 3) on solution (NH_4^+ 35%) was higher with 17% compared to solution (NH_4^+%) and 12% compared to (NH_4^+ 20%). The contributions of NH_4^+ have had a direct impact on fertility of aphids. This analysis showed, also a very significant effect of the 3 physiological stages of plant on the fecundity of aphids.

Figure 4 shows that fertility gradually decreases from flowering level to magnification level. However, if the difference is significant between flowering-fruit setting and flowering-magnification, it is not between the fruit setting-magnification.

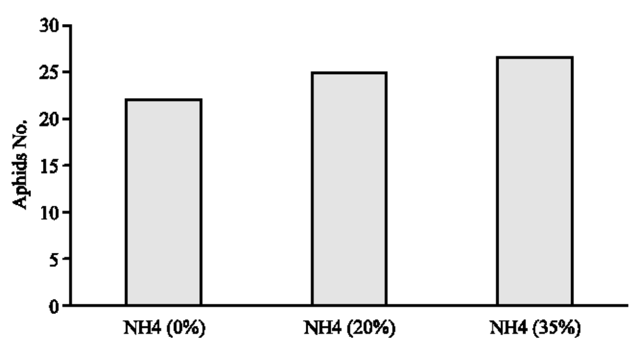

Fig. 3: Aphids numbers born on the plant irrigated by different solutions

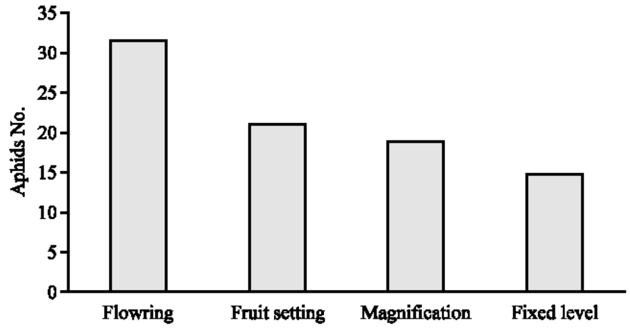

Fig. 4: Aphids numbers born on the leaves of different physiological stages

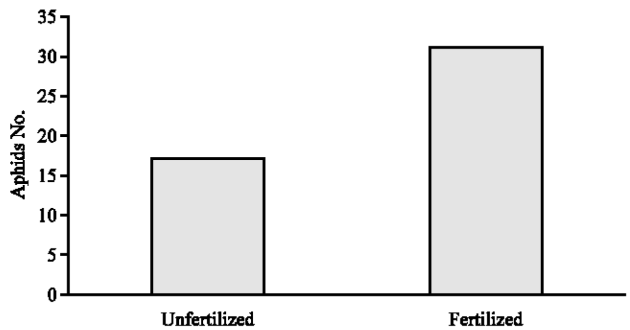

Fig. 5: Aphids numbers born on unfertilized and fertilized plants

We have obtained on the leaves of flowering an increase of aphids births of 33% ($p<0.05$) compared with the fruit setting stage and 40% compared to the magnification stage (Fig. 4).

The number of offspring aphids (Fig. 4) on the flowering leaf stage was significantly higher than that of aphids maintained on leaves of the same inflorescence, throughout the experiment (fixed level). The increase was 52% ($p<0.001$).The comparison of number of births obtained at a fixed level with the fruit setting and magnification level showed no significant difference.

Effect of First Mineral Interruption

The analysis of variance applied to this test has shown a very significant effect of interruption of fertilization on the reproduction of aphids. We note that the number of aphids on plants fertilized was higher than that of unfertilized plants during four days. We recorded a decrease of 45% of births on the plant was irrigated with tap water for 4 days compared to plants that have been fertilized normally (Fig. 5).

Effect of Second Mineral Interruption

We note that the number of aphids was lower during the period of interruption of the mineral fertilizer compared to the periods preceding and following that period (Fig. 6). We found a decrease of 36% in daily fecundity during the fertilization interruption compared to the period prior to the interruption, then an increase of 14% after the plants were fertilized again. We noticed, in this experiment, that just after the mineral break the number of aphids has increased significantly.

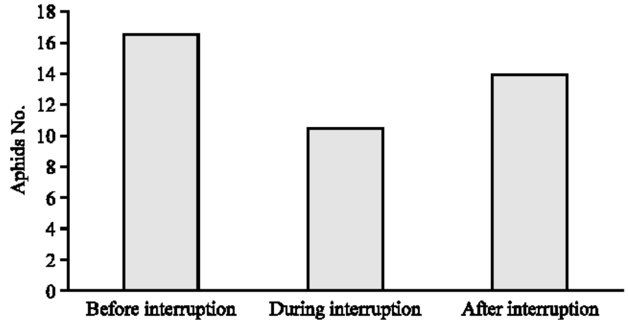

Fig. 6: Aphids numbers born before, during and after mineral interruption

DISCUSSION

In this study, we verified the qualitative variation of total N of tomatoes fertilization on the aphid reproduction. This was to vary the percentage of NH_4^+ compared to NO_3^-.We found that the contribution and the increase of NH_4^+ concentration of total N in a nutrient solution increased significatively the fecundity of *M. euphorbiae*. On solution NH_4^+ (35%) compounded by 35% of NH_4^+ and 65% by NO_3^-, we obtained an increase in aphid fecundity of 17% compared to the NH_4^+ (%) solution which is composed by 100% NO_3^-.The increase of aphids fecundity in NH_4^+ (20%) compared to the NH_4^+ (0%) was 12%

We noted again that, these 3 solutions had the same total concentration of nitrogen. That proved the effect of the NH_4^+ contributions on fertility of the aphid.

Bentz *et al*. (1995) found the higher oviposition on ammonium nitrate treated plants than on no fertilized plant.

Ton and Mauch-Mani (2004) found that a reasonable rate of NH_4^+ in the solution increases proteosynthesis. Hence they revealed that β-aminobutyric acid-induced resistance against necrotrophic pathogens. The observations made on the various physiological stages showed that the level of the same plant and for all fertilizers solutions tested, *M. euphorbiae* reproduces faster on young leaves that on the leaves of fruit setting, magnification and fixed level.

Indeed, we have obtained on the young leaves the offspring higher 30% compared to fruit setting stage, 41.5% compared to the magnification stage and 52.6% compared to the fixed level. On young leaves where the proteosynthesis is important. The flower buds exported to the leaves of the apex a growth substance. These phytohormones are present in the saliva of aphids can play an important role in the development of certain species. The reproduction of *Acyrthosiphon pisum* Harris on *Medicago sativa* L. is more important on flowering stage.

The observations made on plants that have suffered by an interruption of the mineral fertilization during fourth inflorescence showed a low reproduction of aphids compared with plants having been fed normally. This decrease is 45%.

It is important to note that, nutritional stress of plants reduces the osmotic pressure of the sap produced. Because, the removal of sap by aphids depends on the osmotic pressure of this latter (Pollard, 1969).

This interruption of the mineral fertilization had no effect on the production of tomato. Based on these results, we can say that a reasonable contribution of NH_4^+ compared to NO_3^- and a fertilization interruption of 4 days and its replacement by water can significantly reduce the proliferation of aphids.

These results enabled us to establish a relatively high nutrient solution for reducing the proliferation of aphids, with using the variation of ammonium nitrogen in relation to total nitrogen and the break of this fertilization, unlike the variation of only total nitrogen or nitric nitrogen used before.

The use of these 2 factors combined NH_4^+ concentrations in total N and an interruption of the mineral fertilization during 4 days and its replacement by an irrigation with water can be a valuable addition to the use of IPM against aphids *Solanaceae* grown on inert media in greenhouse.

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