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Study of the Impact of Water and Nitrogen Resources on Aphid Infestations and Growth and Production Parameters in the Orchard of Peach

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Abstract: ***This work aims at developing an integrated and ecological control strategy of pest management to assess the impact of water and nitrogen resources on aphid infestations and growth and production parameters in the orchard of peach. The results obtained in the study of the green peach aphid show a significant effect on the availability of water and nitrogen on the level of infestation of green peach aphid. Indeed, the most favorable fertilization regime (T1 = 100%) has proved much more favorable to a prominent development of aphid populations, while deficit procedure (T2 = 75%) could minimize infestations thereof. Parallel to this finding and regarding growth and production parameters of the trees, reducing water and nitrogen intake does not affect their evolution. Instead, foliar analyzes and analyzes of fruit from both fertilization regimes have shown no significant difference with regard to their physical and chemical characteristics.***

Keywords: water, nitrogen, *Myzus persicae, peach, growth parameters, production parameters, fertilization, infestation.*

1. Introduction

Plants need, primarily for their growth and development, water, mineral soil substances, CO2 and O2 atmosphere. Indeed, the fresh material of plants is 70 to 80% water (Heller et al., 1993).

And since water plays a crucial role in the performance and quality of agricultural production, irrigation proves to be an essential tool to compensate for the lack or deficiency of this essential element in the obligation climate context (Kotchi, 2004).

The modernization of the sector and arboreal non-intensification requires the control of all factors of production like irrigation, fertilization and plant protection. Among the pests to fear in peach aphid is the Myzuspersicae; this pest can develop fairly rapidly under favorable conditions and is considered the second important pest after the scourge of peach fruit fly.

It is known by its polyphagia and mainly its resistance of several active substances such as pyrethroid and neonicotinoid (SEVERINE & LAETITIA, 2012), which pose many control problems for farmers. The objective of this study is to establish an integrated and ecological control strategy of pest management to assess the impact of water and nitrogen resources infestations aphid and growth parameters and production in the orchard of peach.

2. Working Methodology

The study has been conducted in two tree orchards in the area of Sais, and it has the aim to follow the fluctuations of aphid populations and the evolution of growth and production parameters in peach trees under two systems of different fertilization; an experimental device was developed (Figure 1).

The first system of fertilization T1 being favorable to the normal development of the fruit trees as it contains sufficient quantities (100%) of water and nutrients (especially nitrogen) is made through two emitters, each with a rate of 8 liters per hour, whereas the second speed (T2) is rendered deficient in these elements (75%) due to a restriction of the amounts of water and nitrogen made while changing one of the emitters of 8 liters / hour at ….of the sheath by another dripper a rate of 4 liters / hour in order to reduce water and nitrogen resources made trees 25%.

At a parcel of peach, ten trees have been selected; while avoiding the edge effect of the orchard, five of which have been awarded in the first fertilization system (T1) and the other five trees have been assigned to the second procedure (T2) (Figure 1). It should be noted that the study plot has been spared of any chemical treatment throughout the study period. In each tree among the five selected, the four cardinal positions of the shaft have been considered and in each direction, two types of branches (vegetative and fruit) infested with aphids have been tagged and monitored during the entire period of the study. For each of the two types of shoots selected at each cardinal direction, three other

infested twigs have also been marked relief branches if a possible loss.

Two types of data have been collected through this experimental device, the first type is related to the species M. persicae, whereas the second type of data is concerned with the measurement of certain growth parameters and production of both fruit species in question. These data have been collected through a pre Scorecard, and for the rating of aphids (Annex 1) and another sheet for various measurements of the tree (Appendix 2).

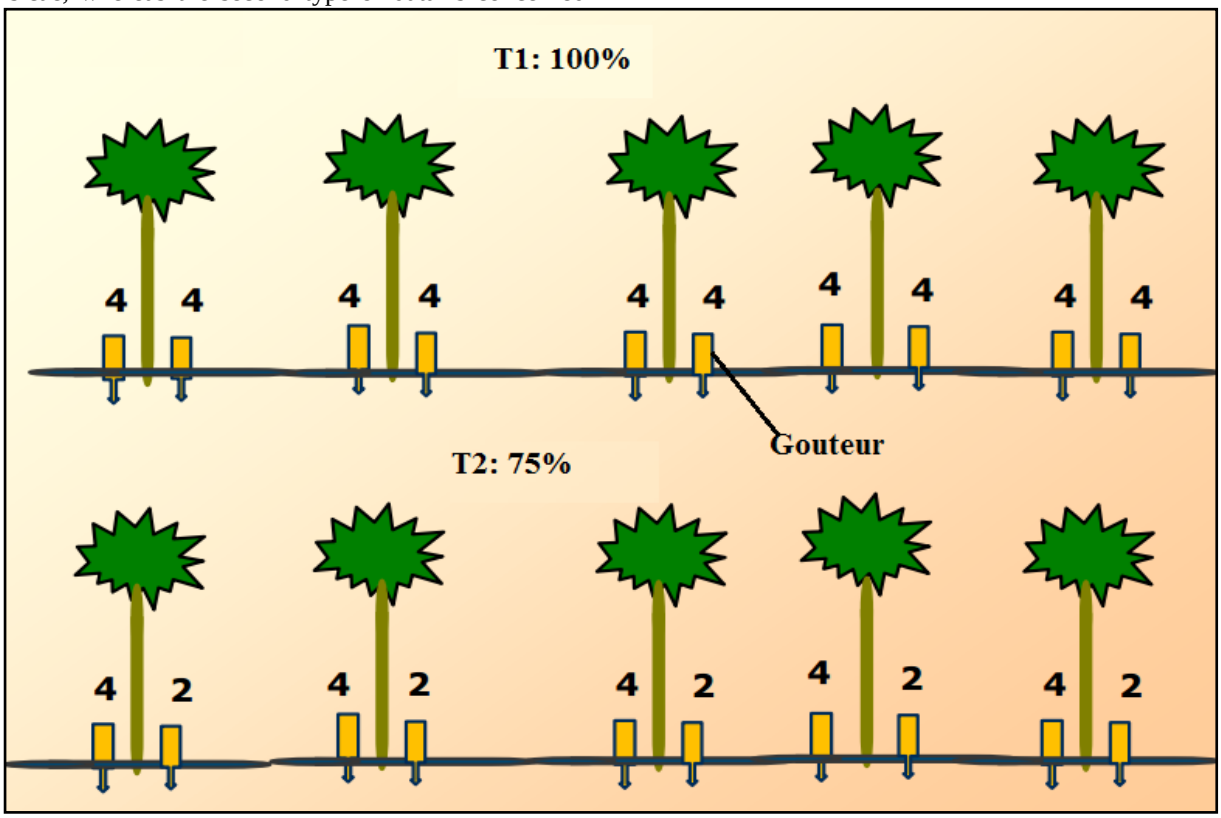


Figure 1: Illustration of the experimental device in split-plot for testing installed at peach.

Monitoring aphid populations

To assess the degree of infestation of wingless aphids on trees, monitoring has been performed weekly on mixed branch where the infestation is noted for each type of branch (vegetative and fruiting) at four cardinal directions and two treatments according to the classification of fertigation LECLANT (1970) (table 1).

Table 1: Classification of degrees of infestation of aphids (LECLANT, 1967)

Infestation degree	Aphid nombre	Medium nombre in class
0	0	0
1	5^0 à 5^1 = 1 à 5	$5^{0,5}$ = 2,2
2	5^1 à 5^2 = 6 à 25	$5^{1,5}$ = 11,2
3	5^2 à 5^3 = 26 à 125	$5^{2,5}$ = 55,9
4	5^3 à 5^4 = 126 à 625	$5^{3,5}$ = 279,5
5	5^4 à 5^5 = 626 à 3125	$5^{4,5}$ = 1397

In reporting on the scale infestation of the tree (number of infested shoots) and the degree of infestation (number of individuals per shoot), an infestation index has been calculated using the following formula:

IF = Σ (d fd x) / (x Σfd 5)

D = degree of infestation between 0 and 5 and fd = shaft frequency shoots with the degree of infestation between 0 and 5.
This index follows a normal distribution (Normality Test Shapiro-Wilk) facilitating analyzes.

As for adults, they are counted directly to the naked eye on each branch and marked in each direction at the same time of the rating of the degree of infestation of all wingless throughout the test period.

Monitoring certain growth parameters and production of trees

The establishment of large populations of aphids in the trees has created serious distortion of twigs, and a winding is followed by severe leaf drop and an important fall fruits. This has caused, no doubt, loss of production and an adverse effect on the vigor of the trees in the short, medium and long term due to the disruption of their normal development. In this regard, certain growth parameters and other production trees have been followed over time to highlight the aphid effect on these parameters in normal conditions and in case of water and nitrogen restriction 25%.

Thus, seven parameters have been measured over time during the months of May and June and the first two weeks of July. These actions have concerned both types of branches (vegetative and fruit) to be located at each

orientation of each of the five trees assigned to each modality for the fertilization of plot peach. Indeed, the diameter is of the vegetative branches and fruit, and the fruits have been recorded using a caliper (Figure 2), while the length of both branches has been measured by a tape measure. The number of sheets has been visually counted only on the vegetative branches as their evolution is slow on the fruit branches.

Figure 2: instrument used for the different measures: Foot coulisse digital.

Analysis of fruit and leaves

Given the importance of fruit and leaf analysis in contributing to the achievement of results and for full information on the basis of the results obtained during the first year of study, nitrogen export analysis per sheet is recorded via the method of Kjeldal (Annex 3) and determination of physical and chemical characteristics of the fruits is achieved via the analytical protocols presented in Table 2

The analyzes have been performed in the lab of basic science department at the National School of Agriculture of Meknes and the Official Laboratory of Chemical Analysis and Research in Morocco (Morocco LOARC).

Statistical analysis

An infestation index has been calculated from the different levels of aphid infestation recording each statement during the study period, then a Shapiro-Wilk normality test has allowed us to verify the normality of this index before submitting it to the analysis of variance.

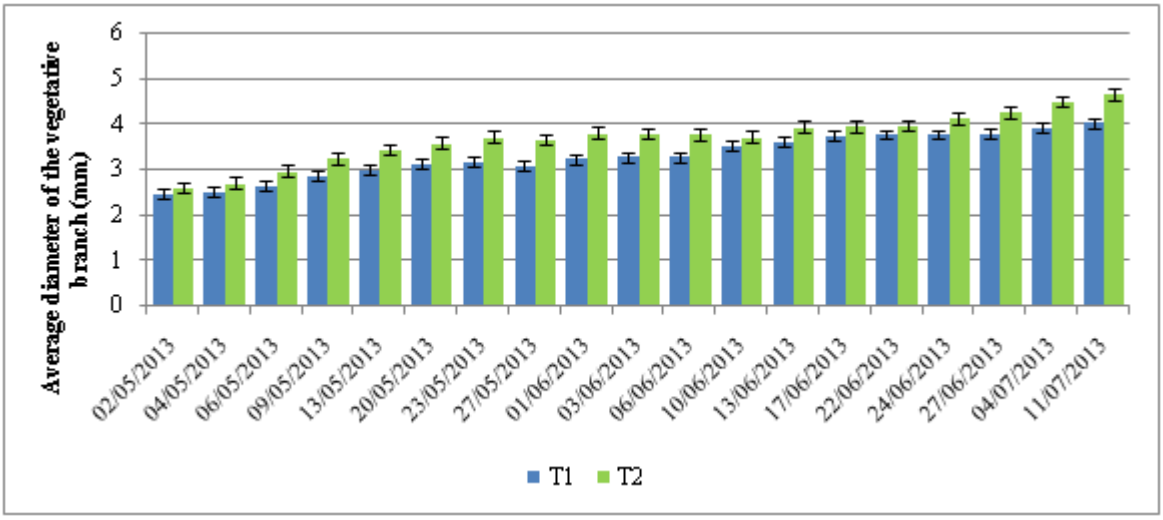
Other measures on growth parameters and production trees have been subjected to the tests without any processing or computation of synthetic variables. The analyzes have been performed for each date readings throughout the study period.
All the data collected is processed and analyzed by software SAS, MINITAP, SPSS Version 20 and Excel 2010 version.

3. Results and Discussion

Part I: Effect of water and nitrogen regimes on growth parameters and production of peach

1. Development of the diameter of the vegetative branch, according to each fertilization system.

Having been measured twice a week for two months for a digital caliper, the diameter of the vegetative branch is followed in order to highlight the relationship of the development with different degrees of infestation recorded at each of the two regimes fertilization in question. The results obtained are shown in Figure3.



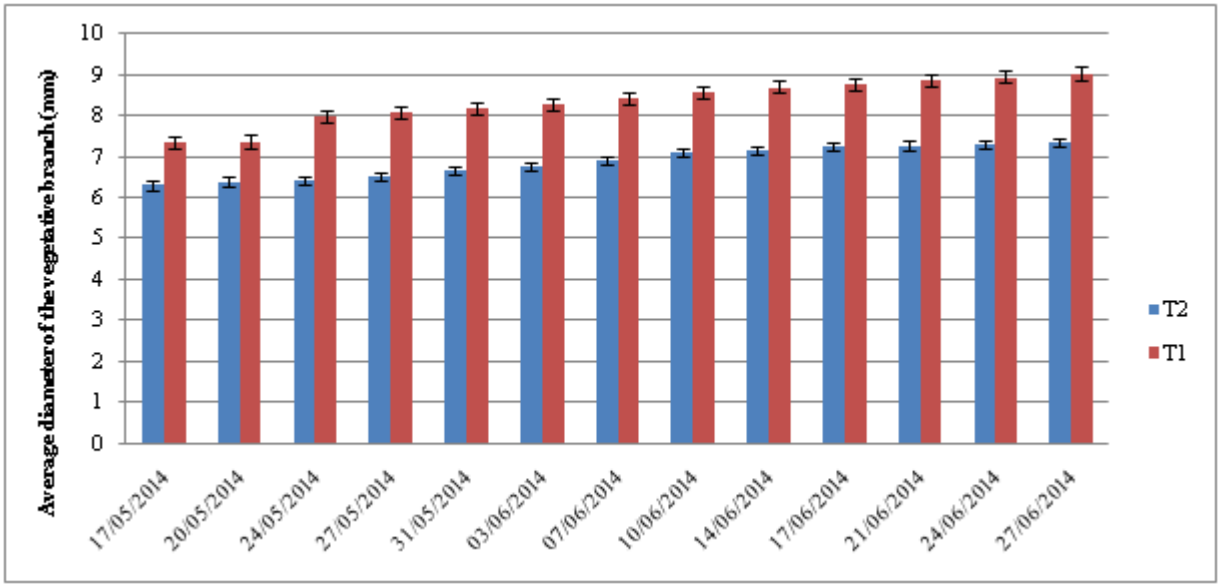
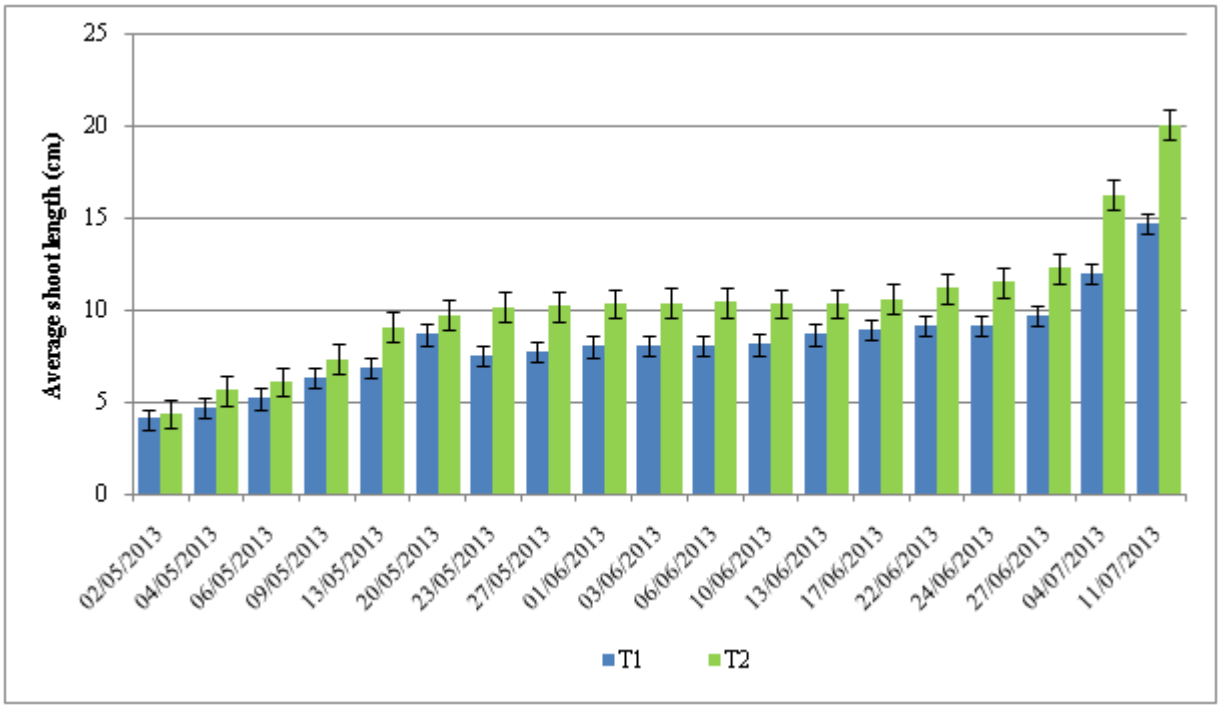


Figure 3: Comparison of the diameter of the vegetative branch (mean ± standard error) between the two regimes T1 and T2 fertilization in each follow-up date

No significant differences between the diameter of vegetative branches of trees submitted to T1 scheme and that of vegetative branches of trees submitted to T2 regime are noticed for the first year of study and at the end of May. These differences in diameter between the two regimes concerned continue to increase until the end of June, with a relative increase in this difference during mid June. Also for the second year of study the diameters spreads continue to rise in early June to late June. Thus, it is clear that the changing diameter of the vegetative branches in both regimes adopts the same following pace throughout the study period (Figure 3).

Change in the length of vegetative branches as a result of both T1 and T2 diets.

After the diameter of the shoots, the length thereof is the second parameter studied to highlight the water reduction effect and nitrogen intake on changes in length of the vegetative branches in both regimes T1 and T2. Indeed, it has been measured twice weekly throughout the study period. The measurements are performed on the four cardinal directions of the five trees in each fertilization system. Figure 4 shows the main results.



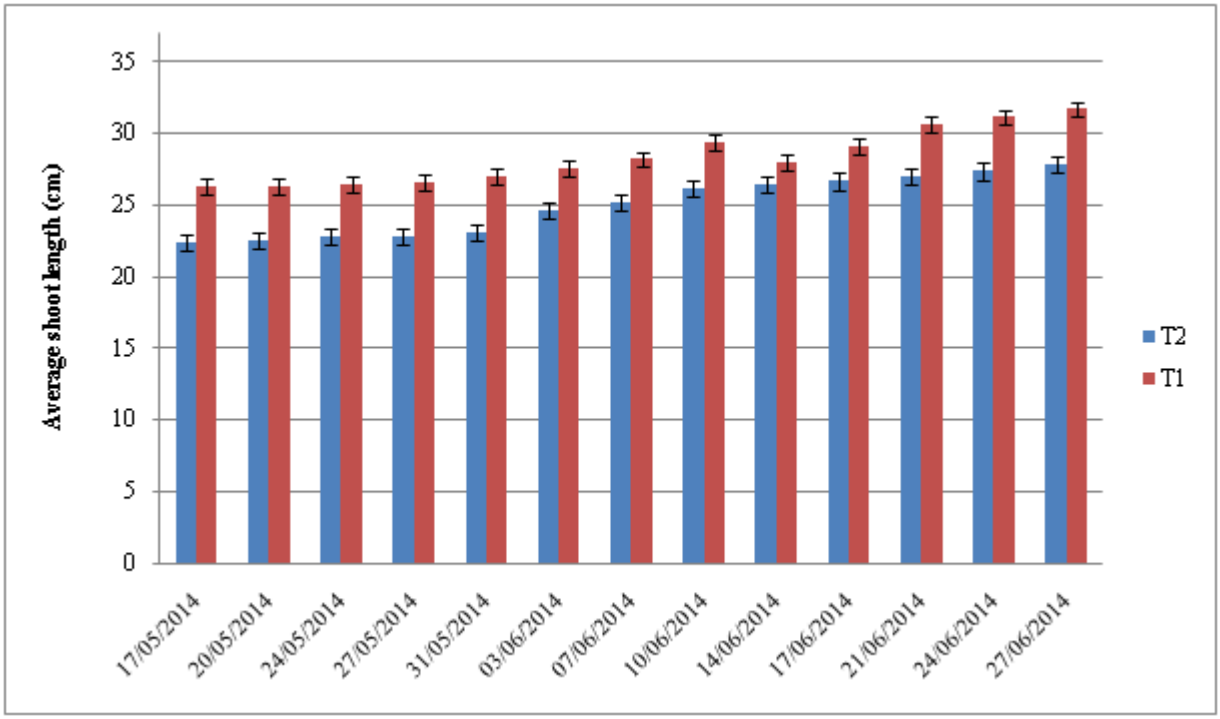


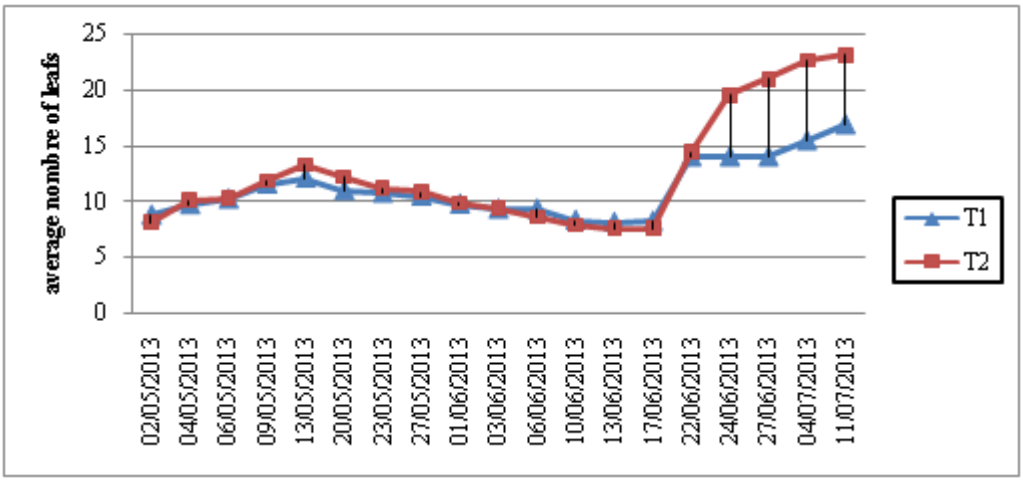
Figure 4: Differences in average length (mean ± standard error) shoots between T1 and T2.

Unlike the diameter of shoots, length of shoots at the two types of plans remained significantly different during the entire period of study with, however, a surprising increase in the difference between the vegetative branches of both plans in a late time in May for the first year of study. Indeed, an enough change which is important and similar to the first year shoot length has been observed at the two regimes during the second year of study (Figure 2).

Evolution of the number of leaves of vegetative branches in the T1 and T2 diets

The green peach aphid attacks ,in the first place, the underside of the leaves on which it forms large colonies, feeding from the phloem, a winding which causes yellowing of the leaves is followed by their fall in case severe outbreaks. Following the number of leaves of vegetative branches over time in four branches of each tree among the five subjects in question in each of the two systems of fertilization, the objective is to highlight the scheme in which the number of leaves is significantly more affected by infestations of the green peach aphid.

The results obtained from the beginning of the study passing through the firstof May to the 10thof June 2014 areidentical in the two fertilization plans, regarding the number of leaves of vegetative branches. However, for the first year of study, the number of sheets of evolution of fluctuations has been found and it is for the period from May the 2nd to May the 15th, from the 20thof May to the 22th of June at the graduates of fluctuation; this is explained by the degree of infestation by aphids for the two years of study (see Part interaction behavior of aphids and physiological capacities of plants) (Figure 5)



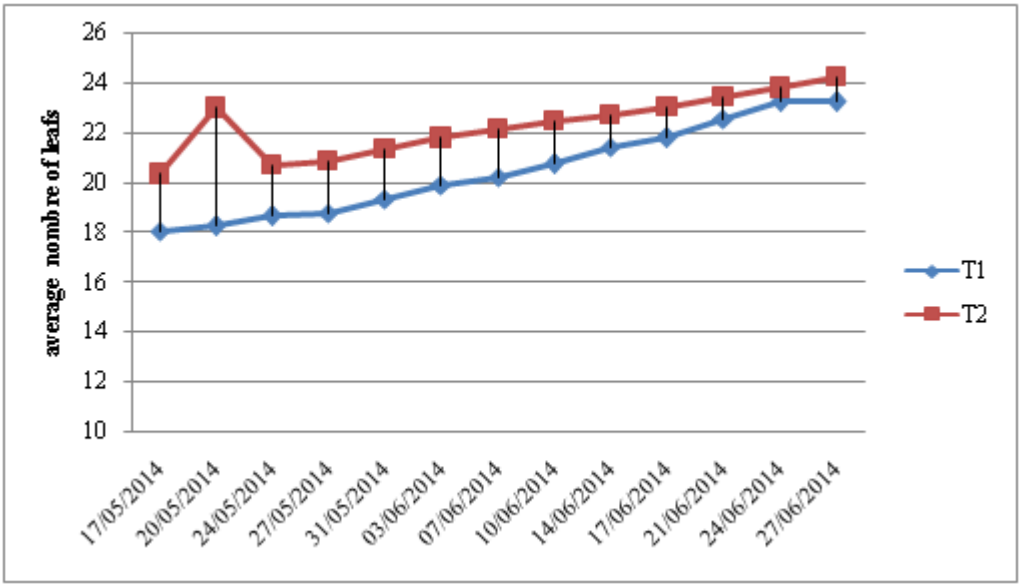
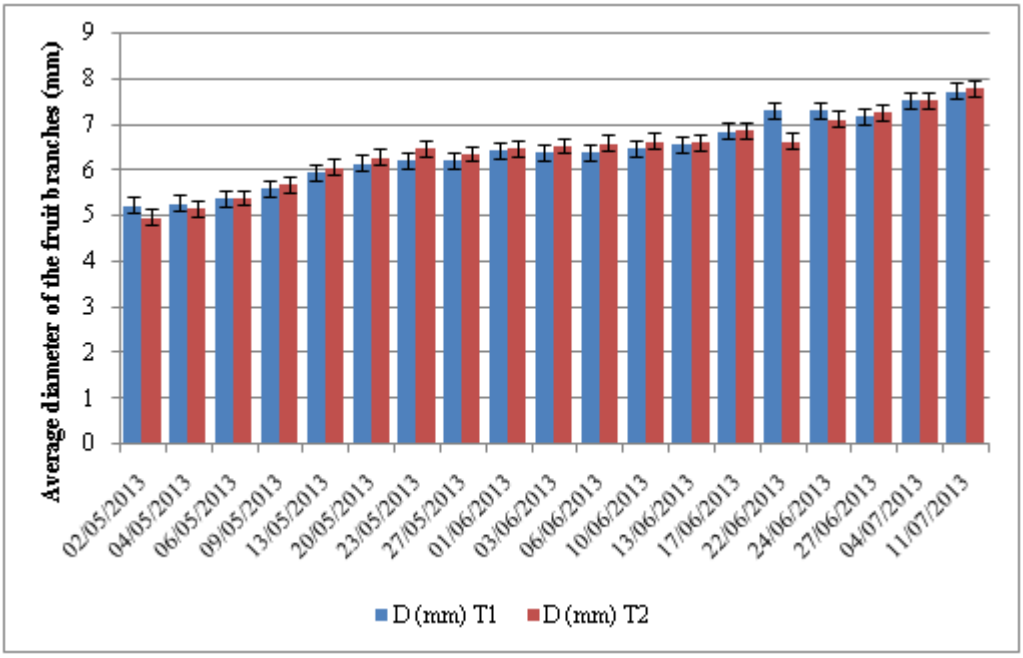


Figure5: Development of the average number of leaves (mean ± standard error) of vegetative branches in the trees submitted to T1 regime and in those submitted in Q2 regime.

2. <u>Evolution of the diameter and length of fruit branches depending on the level of infestation of *M. persicae* in each of the two systems of fertilization (T1 and T2).</u>

Because of the strong relationship between these two parameters for vegetative branches, we have decided to study them simultaneously for fruit branches. Indeed, measurements of these parameters are performed in the same way as that of the vegetative branches throughout the period of this work. The aim is always to see the impact of aphid attacks on the growth of shoots and fruit to highlight the scheme under which these branches were severely affected and also to know to what degree the reduction in water and nitrogen affects physiological capabilities of peach.

Furthermore the diameter of the fruit branches depicts the observation of a normal evolution during the first two thirds of May at the two regimes fertilization. However, stabilization of the development of this parameter has been observed from the last third of May until the end of the study, taking for granted the two years of study.



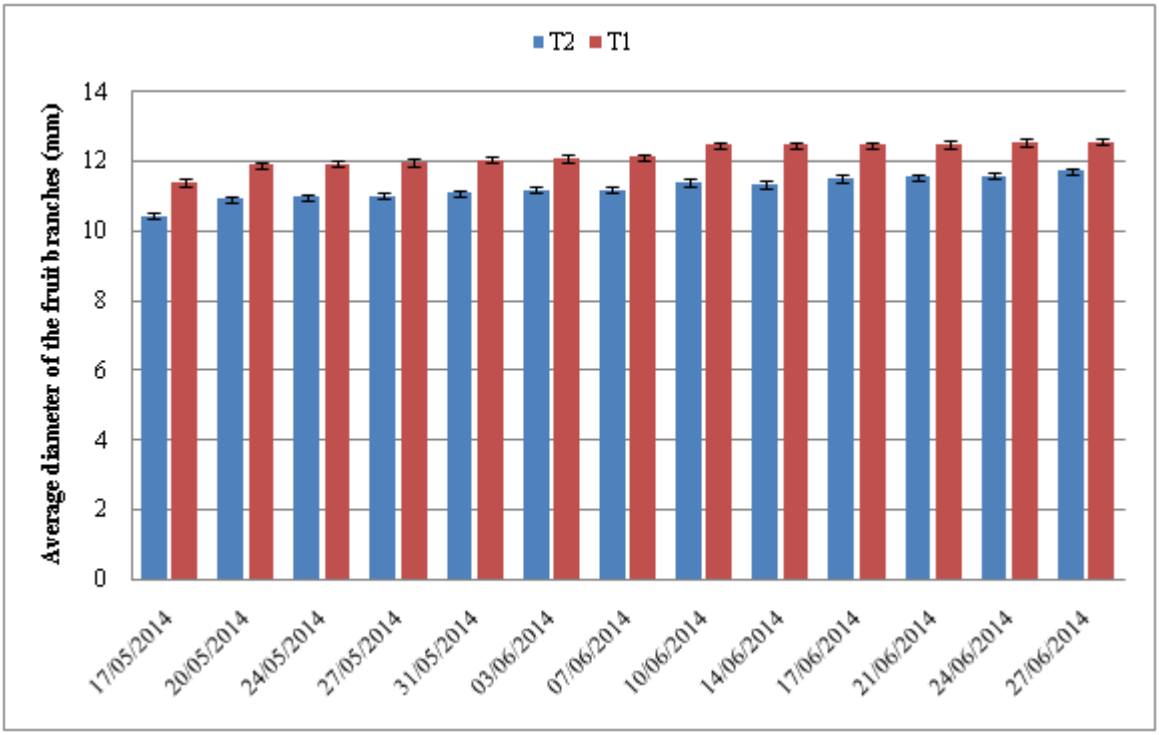
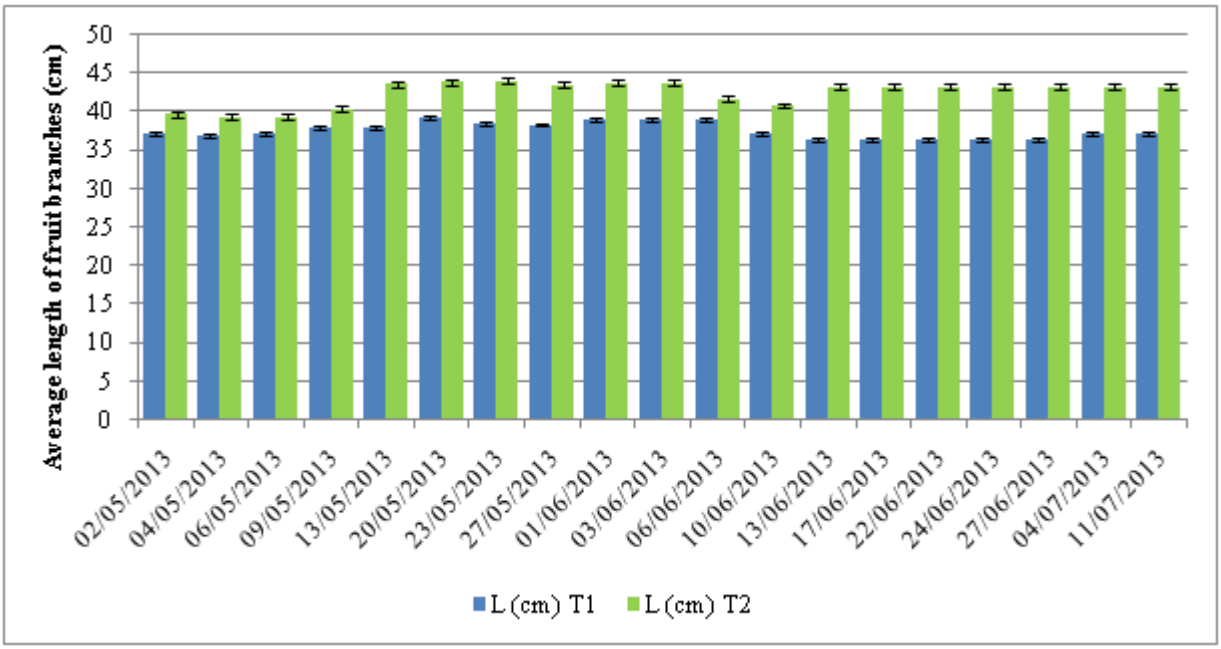


Figure 6: Comparison of the mean diameter (mean ± standard error) of fruit branches between the two regimes fertigation T1 and T2.



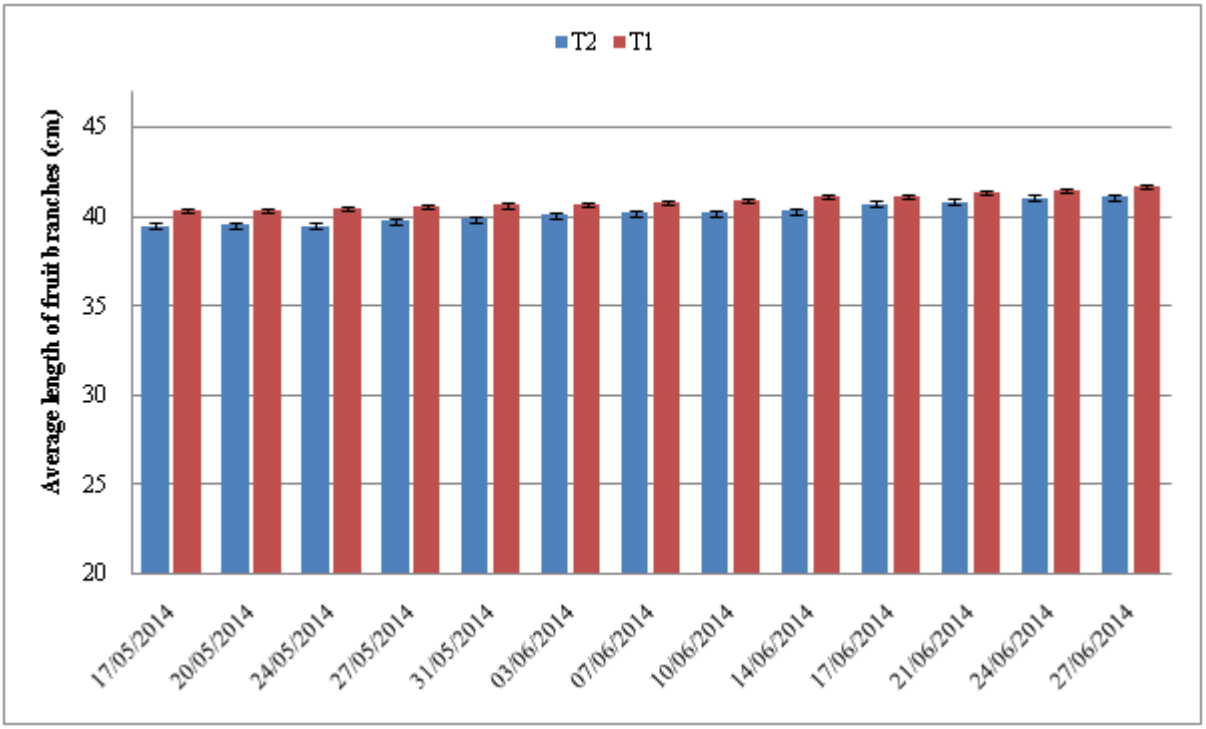
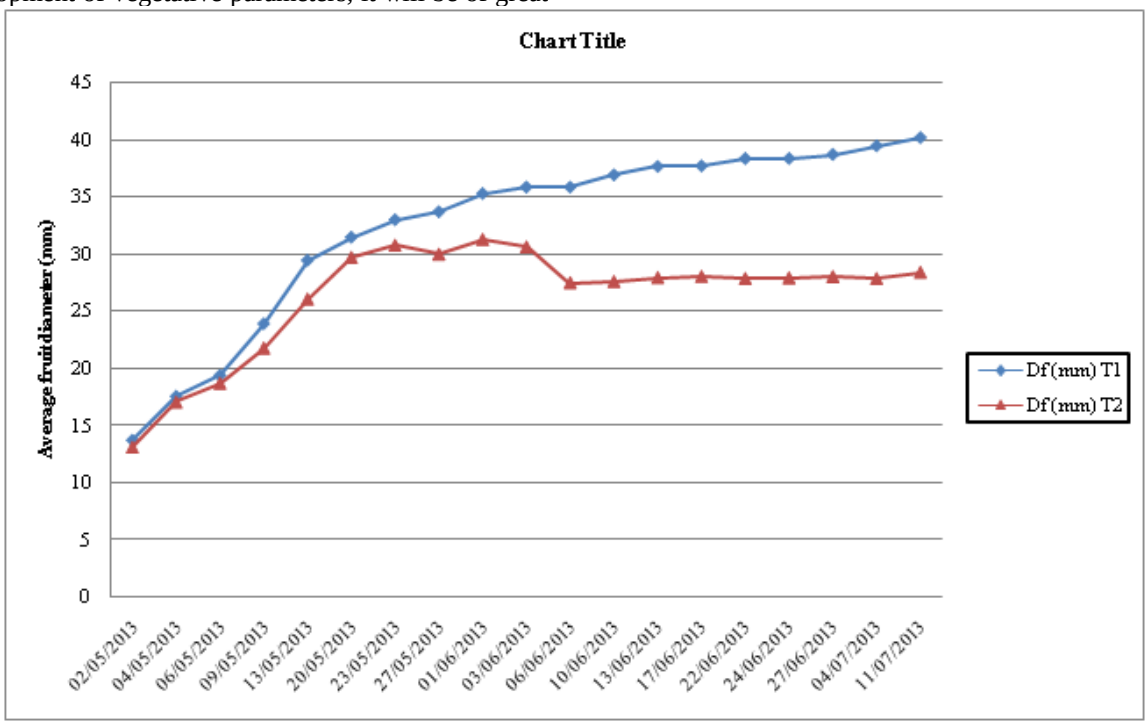


Figure 6: Comparison of average length (mean ± standard error) of fruit branches between the two regimes fertigation T1 and T2.

As for the length of the fruit branches, no difference is recorded between the branches of the two schemes with a less significant development at the second adopted regime and for the duration of the work (mid-May to early July) for the second year of study and from early May to early July (Figure 5).

3. Effect of water reduction and nitrogen input on the production parameters of peach in two regimes of T1 and T2 fertilization.

After seeing water reduction impact and nitrogen input on the development of vegetative parameters, it will be of great importance to also have their effect on the behavior of the development of the fruit diameter. In this regard, two fruits per branch and cardinal position of each shaft from the ten shafts of the experimental apparatus have been selected and monitored from mid-May (setting) until early July (fruit coloring beginning). Their diameter has been measured regularly for a digital caliper to follow, with maximum precision, this parameter. Thus, the results collected from two fertilization systems have been combined, averaged and shown in Figure 7.



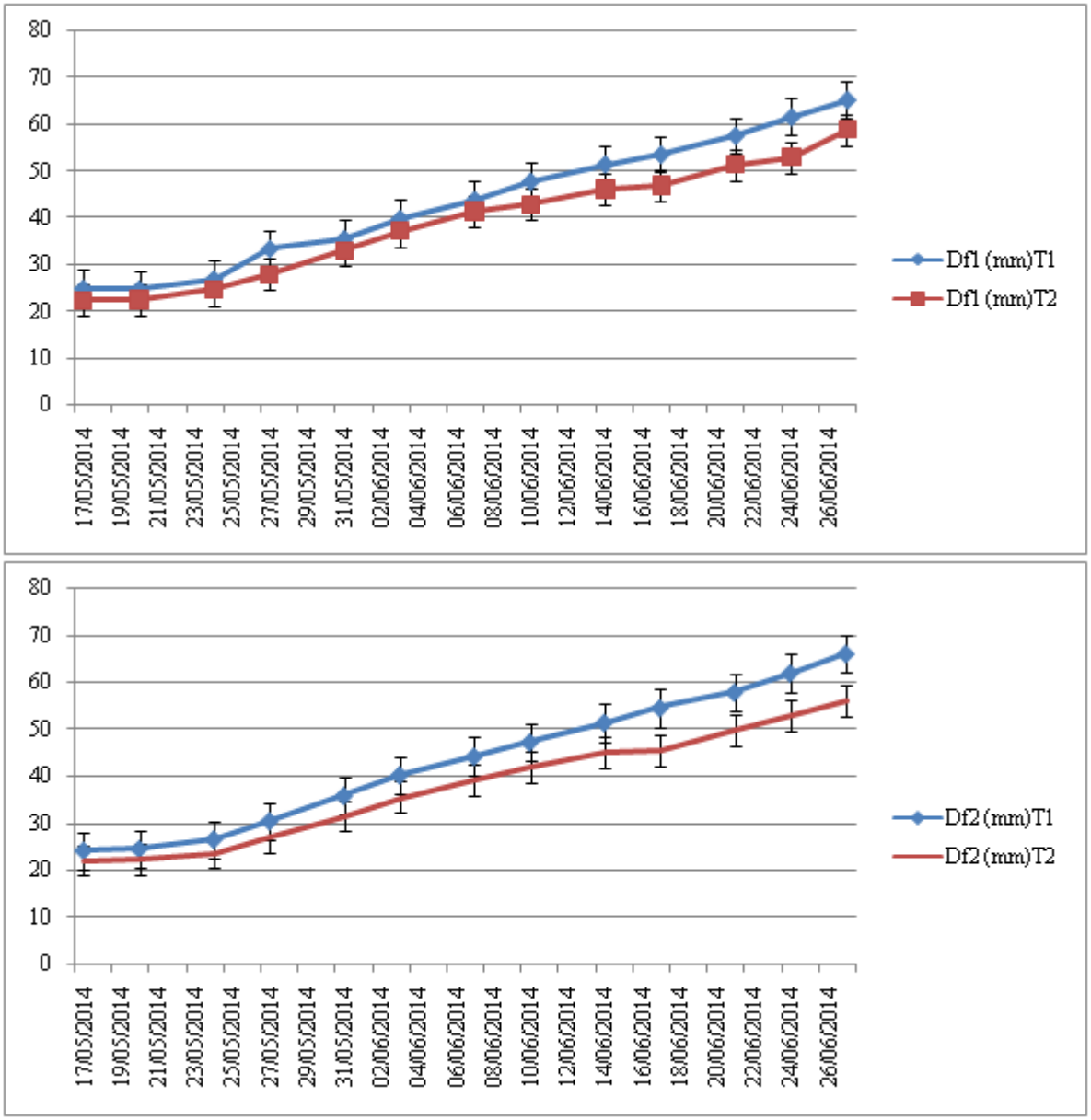


Figure 7: Evolution of the average diameter (mean ± standard error) of fruit depending on the fertilization system (T1 and T2).

At the end of the results of Figure 7, there is a normal evolution of the fruit diameter at both plans of fertilization (T1 and T2) during the first two thirds of May; in the same direction, no statistical distinction between fruit diameters of these two schemes has been achieved during this period. However, a small slowdown in the development of this diameter has been observed at the level of favorable treatment (T1) from 14 June until 18 June, while at the level of the deficit procedure (T2) there has been a natural development of this parameter during the measurement.

From the second week of June and through the early July, we have noticed a slight change in diameter for fruit trees submitted to the first procedure (T1) against a stabilization of the evolution of this parameter for trees assigned to the second regime (T2), always with fruit sizes in the treatment of fertilization T1 higher than those encountered in T2 fertilization treatment. It is not until the 4th of July that fruit sizes, at the deficit procedure (T2), will begin to increase slightly.

However, with the exception of the first year of study only one difference is marked and the degree of development of the outcome of fruit caliber deficit diet; this difference can be explained by the degree of aphid outbreaks during the two year of study.

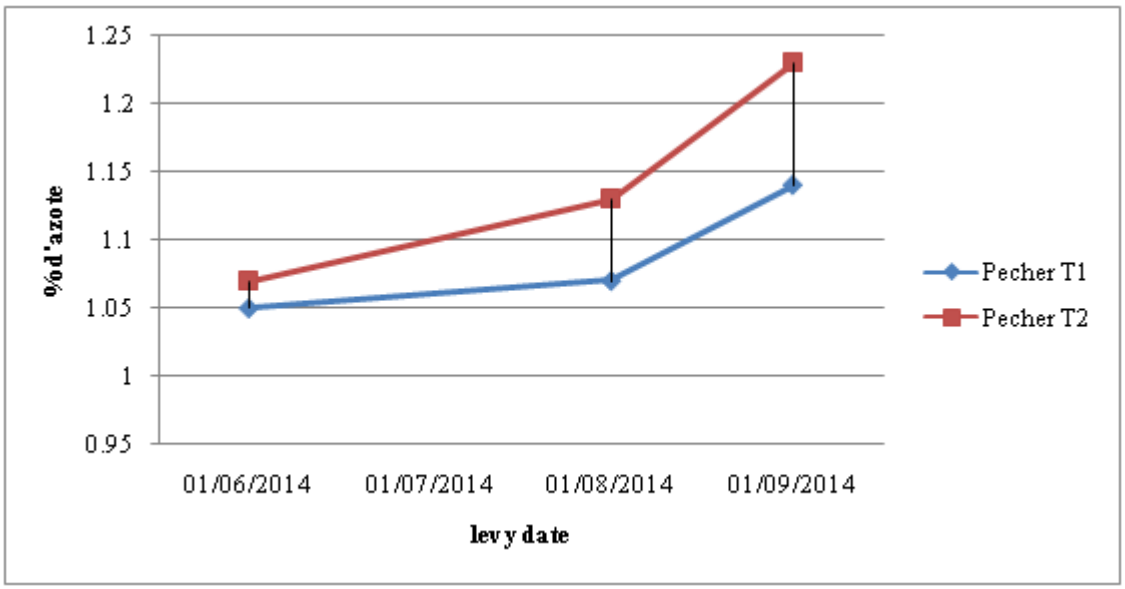
In conclusion, the results obtained for fruit calibers are similar to those taken down in growth parameters. Indeed, for the vegetative parameters are able to show a good vegetative growth in affected trees to deficit regime at the level of those assigned to favorable treatment, although the fruit calibers obtained at the trees subjected of treatment T1 and T2 have followed the same pace from the start until the end of the measurement period.

Table 2: The fruit test results and leaves from both plans (Appendix 6and 7).

measured parameter	T1	T2
Brix (%)	13,6	13,3
Fructose	2,2	2
Glucose	3,1	3

Saccharose	6,1	6
Crude proteins(%)	0,8	0,8
Formol index	19,8	19,6
Acidité	79,4	77,6
PH	4	41

humidity(%)	85,1	84,7
ashs(%)	0,46	0,46
organic matter	14,4	14,8
Total nitrogen	0,9	0,9



The test results clearly show the lack of difference between fruit from the two systems. However the nitrogen export by the Kjeldahl method (Appendix 5) has revealed an export difference between the two systems, while the amount of nitrogen on T2 is important; this can be explained by the spectacular attack by aphids; we can deduce that there is a positive correlation between the nitrogen content and the proliferation of aphids.

Part II: study of the effect of water stress and nitrogen on the level of infestation of aphid populations peach

The second part of this work is devoted to studying the effect of water and nitrogen stress on the level of infestation of the green peach aphid in relation to the growth of shoots and fruits.

1- Effect of water stress and nitrogen on the infestation of wingless populations of green peach over the two years of study.

The variance analysis ANOVA depicts a significant effect of treatment of fertigation on the infestation of aphids that from the first week of June (01/06/2013 and 09/06 / 2014) during the two years of study. This highly significant effect (P <0.01) has helped to highlight two distinct groups where the infestation at the T1 system is significantly higher than that of T2 regime. This significant difference in the infestation is gone 15 days (around mid-June) and, from that date and until the end of July (total migration of aphids to secondary hosts), infestations recorded in both regimes of fertigation are substantially similar. Unlike the second year of study, the infestation continues to increase until the 4th ofJuly.

There is a lack of significant differences between the two systems of fertigation, regarding the degree of infestation of the green peach aphid, throughout the month of May and during the second half of June. And the reduction of infestation during the first half of June by reducing amounts of water and nitrogen introducedis an important element which could be incorporated into an integrated strategy against this aphid species and also a good alternative to theprotection of the plant products that continues to cause resistance in the species in question. From an economic perspective, the reduction of water and nitrogen inputs can reduce costs for treatments against aphids in the absence of side effects of this reduction on the growth parameters and production of trees (Table 4 and Figure 4).

In the first year

Table 3: Analysis of variance of the index of infestation 01/06/2013 (** indicates a highly significant effect).

Source	DDL	Type III SS	Squared mean	Value F	Pr > F
Fertigation	1	0.24025	0.24025	9.95	0.0034**
Blocs	4	0.1385	0.034625	1.43	0.244
Error: MS(Error)	34	0.821	0.024147		

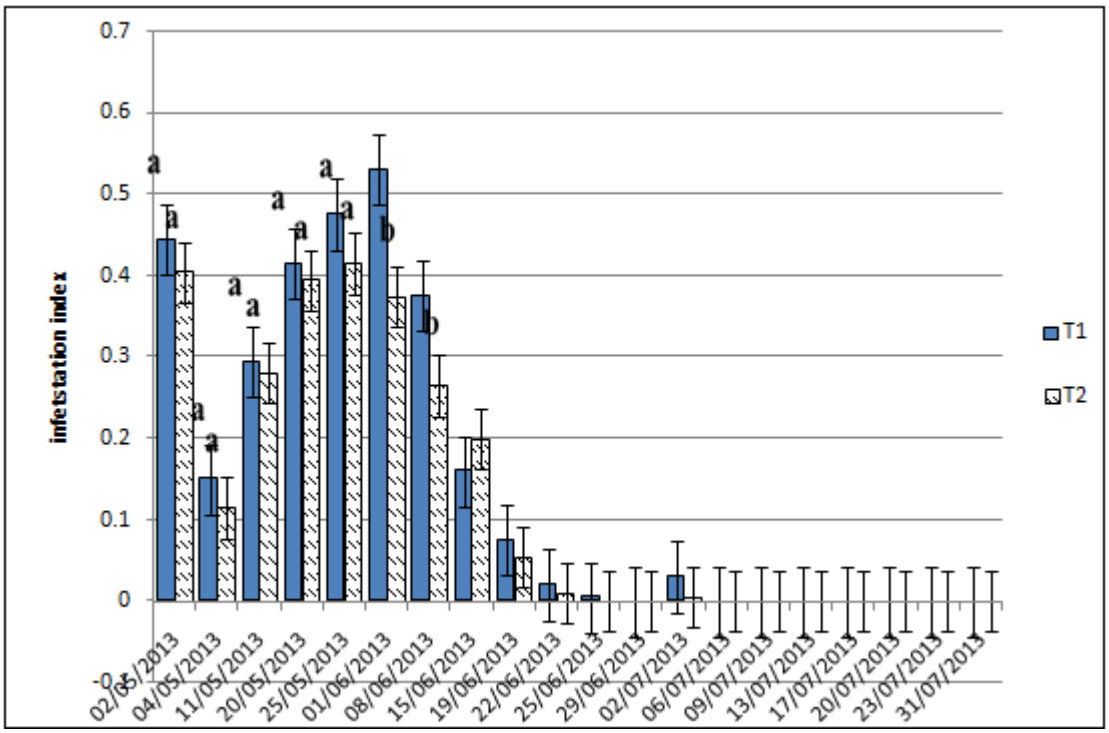


Figure 8: Comparison of the mean infestation index (mean ± standard error) of the populations of Myzus persicae on peach for two systems of fertigation (T1 and T2).

Table 4: Analysis of variance of the index of infestation 09/06/2013 (** indicates a highly significant effect).
In second year

Tests of inter-subjects effects						
Variable dépendante: if						
Source		Somme des carrés de type III	ddl	Moyenne des carrés	D	Sig.
Ordonnée à l'origine	Hypothèse	,020	1	,020	81,000	,001
	Erreur	,001	4	,000[a]		
Trt	Hypothèse	,001	1	,001	16,000	,016
	Erreur	,000	4	6,250E-005[b]		
Bloc	Hypothèse	,001	4	,000	4,000	,104
	Erreur	,000	4	6,250E-005[b]		
trt * bloc	Hypothèse	,000	4	6,250E-005	.	.
	Erreur	,000	0	.[c]		

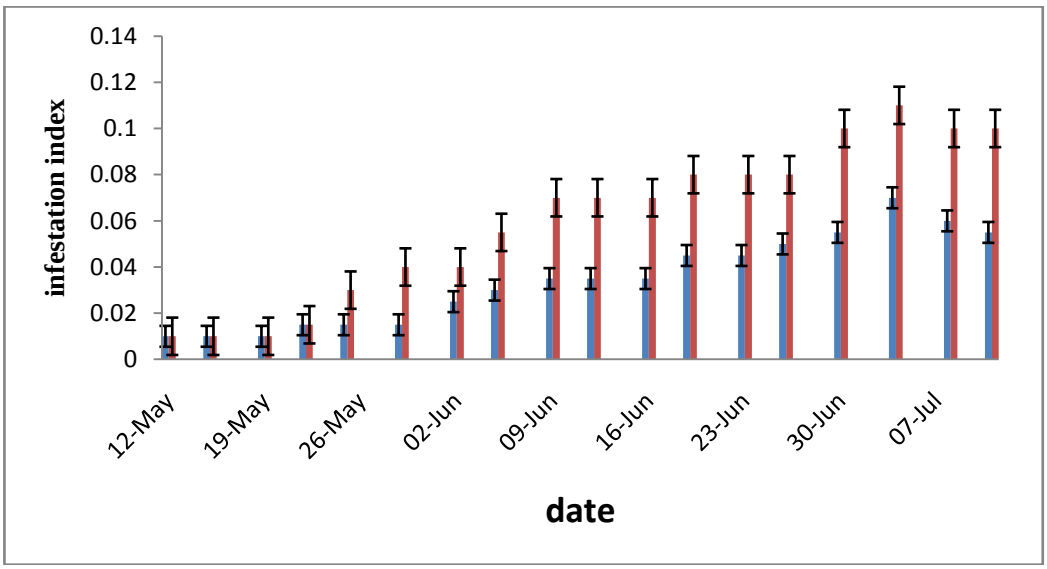


Figure 9: Comparison of the mean infestation index (mean ± standard error) of the populations of Myzus persicae on peach for two systems of fertigation (T1 and T2).

The figure clearly shows the importance of infestations by the aphid to peach at trees subjected to treatment T1 (100%) compared with those assigned to treatment T2 (75%). This difference is due to the amount of azote for each plan.

Effect of water restriction and nitrogen on the fluctuation of aphid populations winged moth.

The first appearance of the adults at the level of stressed trees (T2 regime) began in the first week of May (04/05/2013), while their beginnings in T1 diet (best), the first winged, appeared 16 days after. Near the beginning of the last third of May (exactly 20/05/2013), there was a highly significant effect of water and nitrogen on the development of winged individuals as well; differences are large enough between the two fertigation systems regarding individuals winged green peach aphid that appeared. These differences remained significant until mid-June, again, with a number of adults at the most prominent trees well supplied with water and nitrogen at the level of those under stress of these elements.

As for the disappearance of winged forms, it was relatively early in Q2 compared to Q1 diet regime. Indeed, a full output of aphids T2 system was observed as early as 06/19/2013 while the departure of those T1 regime were held a week later (06/25/2013) (Figure 3).

The general appearance of winged evolution shows that the curvesare similar to the curves of wingless evolution with a few differences. Indeed, the number of adults was more important in the diet at the level of T1 T2, while normally the opposite should happen since the nutrient richness is greater in Q1. However, this finding is quite evident because the development of the adults is not explained only by the provision of food, but also by the high wingless population density at a limited space, Indeed, a linear relationship was established between the index of wingless infestation and the number of adults; this shows that a large proportion of variability (R^2 = 0.509 with P <0.05) is explained by the index of infection and a high coefficient correlation (R = 0.71) links the two variables (Figure 3).

In the first year

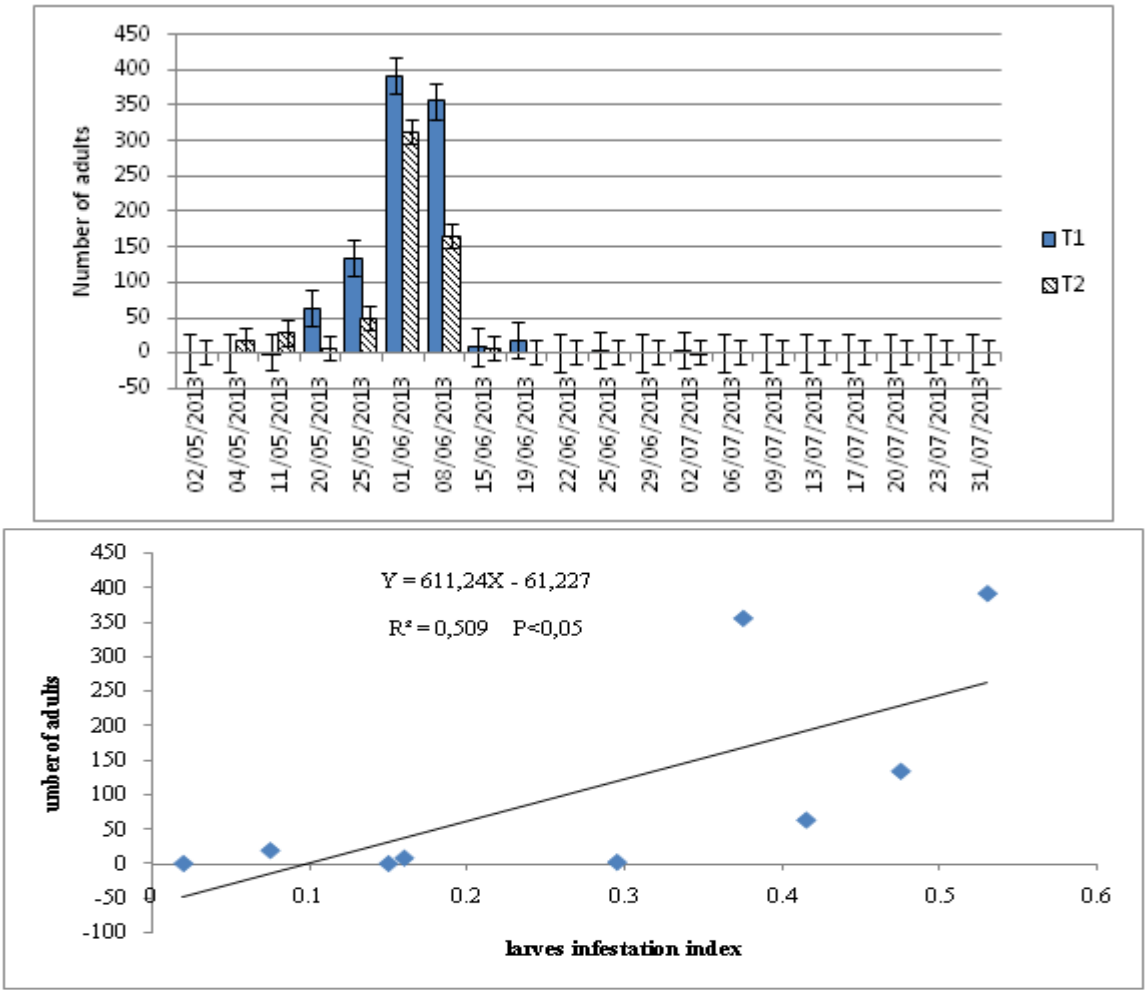


Figure 10: Relationship between the number of winged Myzuspersicae and the index of infestation wingless.

In the second year

For T1

Modèle	R	R-deux	R-deux ajusté	Erreur standard de l'estimation
1	,890[a]	0,792	0,779	0,01927

For T2

Modèle	R	R-deux	R-deux ajusté	Erreur standard de l'estimation
1	,881[a]	0,775	0,762	0,01365

Table 4: Correlation between the number of winged Myzuspersicae and the index of infestation wingless.

Figure 11: Damage caused by *M.persicae* in T1 diet (left) and T2 regime (right)

4. Discussion

Water restriction causes a decrease in the total production of leaves and side branches of young trees peach. However, the results obtained, which corresponds to a restriction of 75%, showed no such effect (Steinberg et al., 2002).

The monitoring of leaf development in both regimes is similar. Indeed no distinction was found between the leaves and shoots of the first order and those developed on the shoots of the second order (Steinberg et al., 2002).

The fruit size is a very important quality of the fruit of nectarine. In this sense, Naor and al. (1999) reported that irrigation is one of the most important components of agricultural practices that influence the size of the fruit, and the response of the peach tree to water stress is a function of the phase of the fruit development. However, the comparison between the physical and chemical parameters of the fruits from the two diet shows no difference.

The green peach aphid is a pest to fear because of the damage caused by its attack and its ability to develop resistant strains against a wide range of active ingredients. The use of means of biological and cultural control becomes an obligation to face the attack of this pest. Among the cultural techniques to be studied is the control of the water system and nitrogen fertilization.
The results obtained for the study of the green peach aphid have shown a significant effect on the availability of water and nitrogen on the level of infestation of the aphid. Indeed, the most favorable fertigation regime (T1 = 100%) proved much more favorable to the development of an eminent aphid populations, while the deficit procedure (T2 = 75%) was able to minimize the infestation of thesepopulations. Alongside this finding, with regard to the growth and production parameters of trees, reducing water and nitrogen intake does not affect their evolution. Although foliar analyzes and analyzes of fruit from both fertigation regimes have shown no significant difference, with regard to their physical and chemical characteristics.

In fact, nitrogen is the mineral element that the plant needs most in quantity and availability which often limits the productivity of the plant (BÉCEL 2010). So the contribution of nitrogen promotes the development and growth of the tree and particularly the young shoots, which are the attractive element of the green peach aphid. Similarly, the water deficiency is one of the most environmental factors affecting the growth and the development of plants (BENIKENet al., 2013). Several studies have reported that when water stresses the plants, the first short-term reaction searching, reduced stomatal conductance to avoidthe loss of water by sweating and medium term by augmentation of root growth to maximize the water absorption. So under these conditions of severe stress, plants develop other tolerance mechanisms as the accumulation of sugars reduces osmotic potential at the cellular level by osmotic adjustments (BENIKENet al., 2013). Similarly, CHENAFI and al. (2013) showed that deficit irrigation (reduction of irrigations from 60 days after full flowering until the fourth week before harvest apple 'Gala'), enabled the economy of 47% of water compared to a comfort irrigation applied at all the season. And that deficit of irrigation (RDI) had no Surle yield and fruit quality of two harvest consecutives impact compared to irrigation comfort and no significant effect was observed on the caliber, coloring, the total sugar content, total acidity, firmness and on Vitamin C content and polyphenols from fruits.

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Appendix

Appendix 1: The procedure of the Kjeldahl method for nitrogen determination and the determination of crude protein.

Material:
1 burette: 250 ml of Erlenmeyer (or conical) flask; 400 ml of beaker; 50 ml of beaker; 50 ml of test tube; 1000 ml of flask for the solution of 0.1N HCl; 1000ml of flask for the solution of boracic acid; 1 wash bottle; 10 ml of pipette; 1 device of nitrogen distillation.

Principle:
Organic compounds containing nitrogen, heated with concentrated sulfuric acid , in the presence of a catalyst, are decomposed; they mineralize nitrogen quantitatively and give it ammonium sulphate.

Ammonium salts so formed are converted to ammonia by passing in an alkaline medium:

The ammonia released is distilled or entrained in the steam and collected in a solution of boric acid. It is then dosed with a solution of sulfuric acid.

Reagents:
- Boric acid solution 4%: 80 g of boric acid + 20 ml of a bromocresol solution, fill to 2 1 with distilled water.
- Bromocresol solution: 0.1 g of bromocresol + 100 ml of ethanol.
- 40% NaOH solution: 400 g of NaOH and adjusted to 1 1 with distilled water.

Procedures (or ways of working or operative mode).
A Mineralization
- Weigh 0.75 to 1.5 g of foodin a filter paper.
- Add 2 g of catalyst (catalyst is composed of 100 parts of K2SO4; 20 parts CuS04 and part of Se).
- Wrap all in the filter paper and insert it into the digestion tube.
- Add 20 ml of H2SO4 (d = 1.84).
- Add a few beads in the glass.
- Stir the mixture neatly.
- Perform a witness under the same conditions; the witness must not contain the product in the analyzer.
- Wear the tubes on plates of digestions .
- First heat gently for about 30 min and then progressively increase the intensity of the heating until the content of the tube is heated to boiling. The ramp temperature must be adjusted to take 25 ml of water to boil 5 minutes.
- When the content of the tube becomes faded, mineralization is complete.
  Stop the supply of the ramp and let cool gradually the content of the tube of mineralization.

B- Distillation :
- Connect the distillation glass directly in the Buchidevice.
- Insert slowly in the distillation glass, along the wall, 70 ml of sodium hydroxide 40%.
- Put 50 ml boric acid 4% and a few drops of the indicator TASHIRO (0.2 g bleu.deméthylène +0.1 g of ethylene red + 100 cc of ethyl alcohol 90 °) the beaker in the recovery apparatus.
- Circulate steam; distillation settled quickly.

The ammonia that is released is driven by the steam generated by the Buchi apparatus. Collect approximately 150 ml of the condensate; then place the beaker on the lower support, continue distillation for 1 min and remove the beaker after having littered the point of the coolant. Stop the distillation; the unit drains.

C- Dosage
The assay (titration) is performed using sulfuric acid known as (0.1 N), the latter is added very slowly to 10 ml of distillate removed from the beaker until the color purple is obtained . At this time, the neutralization of ammonia finished. Carry the result to the total volume of distillate.

- Either "V" the number of ml of H2SO4 used to neutralize the sample ammonia.
- Or "Vo" the number of ml of H2SO4 used to neutralize traces of ammonia in the control (distilled water, chemicals and filter paper).

D Results
- Make three dosages.
- Show that the percentage of nitrogen is expressed by:
- V and Vo; acid volumes used in the assay of the sample and the blank respectively;
- N concentration of the sulfuric acid.
- Calculate% nitrogen in your test sample? The protein content is then given by the formula:% protein =% N x 6.2.

Appendix 2: The procedure of the determination of ash and organic material.

Principle:
An inflamed sample loses its organic material (proteins, carbohydrates, lipids, nucleic acids, etc.) that gasifies (C02) and escapes, leaving the mineral material (MM), also called ash. The organic material (OM) is the part that remains when subtracted MM MS. MM normally do not contain atoms (component of the MO). But, in some situations, it can be found (as carbonate) in the ash. In contrast, some mineral elements can volatilize at high temperatures.

Procedure:
- Weigh with precision ± 2g sample (MF) into a previously dried and weighed crucible (T), preferably cold (after passing through a dryer).
- Calcining in an oven at 550 ° C for at least 2 hours (or 450 ° C pdt 5h).
- Check for black deposit. Otherwise, increase the residence time in the oven
- Weigh, coldly, mineral matter with the tare (MM + T).

Calculation:
% MM = (MM / MS) * 100
%M0 = 100 - %MM

Appendix3: The procedure of assay of monosaccharides by HPLC in dried figs
Reference: HARMONISED OF THE INTERNATIONAL HONEY COMMISSION 2002 (METHOD 7.2)
1) Scope of the study:

All food products
2) Principle of the method:
   Extraction of sugars in aqueous medium is assay byHPLC in reverse phase.
3) Equipment and chromatographic conditions:
   a) Equipment:
      • HPLC chain is composed of:
      - On-line Degazer
      - Auto sampler
      - Pump can deliver an isocraticsheme.
      - Differential refractometer detector.
• Standard laboratory equipment.
   b) Chromatographic conditions:
- Column: NH2 silica graft with 25 * 4.6 * 5 cm * mm * microns
- The composition of the mobile phase is as follows:
- If the column is new: Acetonitrile / Water (75/25). Flow rate: 0.7 ml / min
- When the resolution fructose - glucose begins to degrade, while the mobile phase is a mixture Acetonitrile / Water 80/20. The flow rate: 1 ml / min

4) Reagents
- Water quality Milli Q
- Acetonitrile for HPLC
- Fructose, glucose; sucrose, maliose and minimum 99% purity lactose.

5) The Procedure:
- Preparation of standard solutions:
- Preparing a mixture of fructose, glucose; sucrose, maltose and lactose concentration 4g / l in Milli-Q water.
- Samples preparation:
- Case of solid samples:
- Prepare a homogeneous mass and weigh at milligram a massof M 5 g in a beaker.
  Marinate and extract this mass with water aliquots until a volume of 50 mlis obtained.
  The extract is filtered through fast filter paper and membrane 0.45 microns. Its concentration should approximate that of the working solution.

Case of liquid samples:

The sample liquids filtered on 0.45 pm membrane are either injected directly or diluted (10 times) so as to have a concentration nearby the solution of the work.

6) Expression of results
The sugar concentration is computed by external calibration in the aid of the following formulas:
For liquid samples case:
$\mathbf{C}_{ech} = (A_{ech} / A_{stsuc}) * \mathbf{C}_{stsuc} * D$; with
Cech: sugar concentration in the sample, g / l
Aech: sugar area in the sample u.a
Astsuc: sugar area in the standard solution ua
Cstsuc: sugar concentration in the standard solution in g / l

D dilution factor
Case of solid samples:
$\mathbf{C_{ech} = -(A_{ech} / A_{stsuc}) * C_{stsuc} * (V / Pe)}$; with
Cech: sugar concentration in the sample in g / l
Aech: sugar area in the sample u.a
Astsuc '• area of sugar in the standard solution ua
Cstsuc: sugar concentration in the standard solution in g / l
Pe test sample in g; V: initial volume in ml The calculated values

Appendix 4: the procedure of determination of mineral elements by atomic absorption in the flame. (Eg calcium)

1- Principle:
   The sample is dissolved in hydrochloric acid F after destruction of organic materials. Calcium is determined,

after appropriate dilution, by the spectrometry of atomic absorption in the flame.

2- Equipment:
2-1- Glassware:
2-1-1- Volumetric flasks of class A of 25; 50; 100; 200; 250 and 500 ml.
2-1-2- Bulb pipettes of class Aof 1; 2; 5 and 10 ml.
2-2- Spectrophotometry
2-2-1- Spectrophotometer of atomic absorption in the flame of Varian Spectra A 220
2-2-2- Instrumental parameters:
- Flame air -acétylène (Pair pressure 4 bar, C2H2 pressure: l, 2 bar)
- Wavelength: 422.7 nm
- Slit width: 0.5 nm
- Lamp current: 10 mA
- With non-specific absorption correction.

3- REAGENTS

3.1) Distilled water (electric conductivity <3 ¡Lis / cm)
3.2) Concentrated hydrochloric acid p.a d: 1.19
3.3) super-pure nitric acid 65%
3.4) p.a Hydrogen peroxide (30% v / v)
3.5) lanthanum solution at 50 g / I from the lanthanum oxide or lanthanum chloride
3.6) calcium stock solution at 1 g / l (Titrisol Merck) or equivalent
- Calcium solution 100 mg / 1

Place 5 ml of the solution - mother (3.6) in a 50 ml flask (2.1.1), make up the volume with distilled water (3.1). Calibration range 1; 2 and 4 mg / 1 of calcium: Place successively. 1; 2 and 4 ml of the solution containing 100 mg / 1 (3.6.1 in 100 ml volumetric flasks (2.1.1). Add 1 ml of nitric acid (3.3) and 2 ml of lanthanum solution (3.5) up to volume with distilled water (3.1). Perform a blank without calcium in the same conditions.

1- The preparation of the samples
4-1 Sample containing organic compounds:

According to the content of the element to be determined, and to avoid potential dilution , if not necessary, place a sample test, weighing 0.01 mg in a capsule porcelain or platinum, dry clans an oven at 105 ° C or in a hot plate (mild drying), then place the dish in a muffle furnace. Set the oven to reach 550 ° C gradually and let incinerate for 12 hours (sometimes less, depending on the product). Cool and add 5 to 10 ml of concentrated hydrochloric acid (3.2) and 3-5 drops of oxygenated water (3.4), heated on a hotplate until the ashes apparently dissolve more. Filter and recover in a volumetric flask whose volume V depends on the contentin the research :

- If the content is> 0.1%) V - 250 or 500 ml.
- If the content is <0.1%) V- 25: 50 or 100ml.

Prepare a blank sample under the same conditions

4-2- Sample containing no organic compounds

2- Determination:

Successively present the white and the calibration solutions (3.6.2). white thee is a I% solution of nitric acid (3.3) and 4% (v / v) of the lanthanum solution (3.5). Then present the blank sample and samples added to the 4% lanthanum solution (v / v), perform dilutions if necessary (eg If the sample reading is outside the calibration range). The device software provides the calcium concentration of samples in mg / 1 Calculation: The% calcium content: Music player (Ech) - Music player (B) .V .F

10.000 P.E

Music player (Ech): Sample Playback mg / 1
Music player (B): Reading of blank sample in mg / 1
V: Volume recovery after incineration in ml
P.E: Sample Test portion in grams.