



# Tomato Diseases, Quality, Yield and Pesticide Use

A Field Study in Nicaragua

*Anna Berlin och Sofia Eitrem*



Handledare: Dr Aldo Rojas (Nicaragua)  
Prof Henrik Eckersten  
Prof Jonathan Yuen

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Institutionen för växtproduktionsekologi  
SLU

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## Abstract

Nicaraguan farmers have for a long time struggled with tomato production. The primary problem has been Begomovirus infection, which devastated the tomato production in the country in the 1990's. In the battle against the Begomovirus other problems in the native tomato production have been neglected. The overall goal with these projects was to identify common tomato diseases and to evaluate their effect on yield and quality of the tomato fruits at different nitrogen levels. This report contains two separate theses (chapter 2 and 3). The results of the studies are based on the same field experiments. To increase the understanding about local farming conditions (chapter 1), interviews were made at two locations. During the interviews the farmers were asked questions about the farms, their production and pesticide use. Today, the farmers overuse chemical products to control different pests. The Nicaraguan farmers in general have a strong belief that pesticides are the solution of all their agricultural problems.

The thesis in chapter 2 deals with the effect of nitrogen on some diseases of tomato. The symptoms that appeared in the field experiment indicated that the plants were not infected by the suspected pathogens and lab results showed that symptoms on the plants were caused by two other diseases: bacterial wilt and powdery mildew (causing agents: *Ralstonia solanacearum* and *Leveillula taurica*). The results showed that plants fertilised with double the normal amount of nitrogen were significantly more resistant to powdery mildew. There was also a significant difference between the varieties in severity of powdery mildew. Regarding bacterial wilt the results are ambiguous, one of the field experiments show no difference between the treatments but in the other experiment there was a considerably higher rate of infection in the to highest levels of nitrogen fertilisation. The aim of the study in chapter 3 was to evaluate the effects of nitrogen fertilization on the yield and quality of the tomato fruits and to evaluate the relations between the incidence of diseases and nitrogen fertilization on one hand and fruit quality on the other. The harvest was divided in good (marketable) and bad (not marketable) fractions. To evaluate the fruit quality, taste tests were carried out on the two first harvests and laboratory analyses of acid (% titratable acids) and sugar content (°Brix) were measured on the second harvest. The results showed that the unfertilized plots had significantly lower foliar nitrogen content. The total harvest levels of the experiment were in similar range as the average harvest for the region (12-18 t/ha), but the marketable fraction was only between 39 and 50 % of the total yield. In the taste test, one of the varieties was significantly the most preferred variety, independent of fertilizing level. The high not marketable fraction was probably due to poor pollination and fruit set caused by high temperatures and heavy infections of Begomovirus and other diseases. It is also very important to identify the pathogen to know which diseases that are present in the field and how to treat them properly. A solution to many disease and quality problem could be a break in tomato production some time during the year to create a host free period for diseases and vectors.

## Keywords

Tomato (*Lycopersicon esculentum*, Mill.), Nicaragua, *Leveillula taurica*, *Ralstonia solanacearum*, *Fusarium oxysporum*, *Alternaria solani*, nitrogen fertilization, quality, yield, taste test

## Sammanfattning

Nicaraguanska bönder har länge tvingats kämpa med problem i tomatodlingen. Det största problemet har varit infektion av Begomovirus, vilket fick katastrofala följder för tomatproduktionen på 1990-talet. I kampen mot Begomovirus glömdes andra problem i den inhemska tomatodlingen bort. Det övergripande målet med de här projekten var att identifiera vanliga tomatsjukdomar och utvärdera deras effekt på skörd och kvalitet på tomaterna vid olika kvävegivor. Rapporten innehåller två separata examensarbeten (kapitel 2 och 3). Resultaten från studierna bygger på samma fältexperiment. För att öka förståelsen om den inhemska tomatproduktionen (kapitel 1), intervjuades bönder i två byar. I intervjuerna ställdes frågor om gårdarna, produktionen och bekämpningsmedel. Idag finns en överanvändning av kemiska preparat för att kontrollera olika skadegörare och Nicaraguanska bönder har generellt sett en övertro på att bekämpningsmedel som en lösning på alla problem inom jordbruket.

Examensarbetet i kapitel 2 handlar om effekten av kväve på vissa tomatsjukdomar. Symptomen som uppträdde i fält tydde på att det inte var de förväntade sjukdomarna och laboratorieresultaten visade att plantorna hade infekterats av två andra sjukdomar: bacterial wilt och mjöldagg (orsakad av *Ralstonia solanacearum* och *Leveillulla taurica*). Resultaten visade att plantorna som gödslats med dubbla normalgivan av kväve var mer motståndskraftiga mot mjöldagg, det fanns också en skillnad mellan sorterna när det gäller angrepp av mjöldagg. Angående bacterial wilt var resultaten tvetydiga, ett av fältförsöken visade ingen skillnad mellan behandlingarna, medan i det andra var det högre infektionsgrad i de högsta kvävegivorna. Syftet med studien i kapitel 3 var att utvärdera effekterna av kvävegödsling på skörden och kvalitén på tomaterna samt att utvärdera sambandet mellan förekomsten av sjukdom och mängden kväve å ena sidan och kvalitén å andra sidan. Skörden delades in i en bra (säljbar) och en dålig (icke säljbar) del. För att utvärdera fruktkvalitén gjordes smaktest på två skördar och laboratorieanalyser av syra (% titrerbar syra) och sockerinhåll (°Brix) på den andra skörden. Resultaten visade att ogödslade rutor hade signifikant lägre kväveinhåll i bladen. Den totala skördenivån i fältförsöket var jämförbar med normala skördar i området (12-18 t/ha), men andelen säljbara frukter var endast 39 till 50 % av den totala skörden. Smaktesten visade att den ena sorten föredrogs av panelen oberoende av kvävegiva. Den höga andelen icke säljbara frukter berodde troligen på dålig pollination och fruktbildning orsakad av hög temperatur och kraftiga infektioner av Begomovirus och andra sjukdomar. Det är också mycket viktigt att identifiera patogener för att veta vilka sjukdomar som finns i fält och hur de bäst skall bekämpas. En lösning till många sjukdoms- och kvalitetsproblem skulle kunna vara att införa ett uppehåll i tomatodlingen någon gång under året, för att skapa en värdfri period för sjukdomar och vektorer.

# CONTENT

<b>1 TOMATO DISEASES, QUALITY, YIELD AND PESTICIDE USE – A FIELD STUDY IN NICARAGUA .....</b>	<b>5</b>
1.1 BACKGROUND .....	5
1.2 INTERVIEWS WITH LOCAL FARMERS REGARDING USE OF PESTICIDES IN TOMATO PRODUCTION .....	6
1.3 DISCUSSION OF THE PROJECTS AND FUTURE PROSPECTIVE .....	9
1.4 REFERENSES .....	10
ACKNOWLEDGEMENT .....	11
<b>2 THE EFFECT OF NITROGEN ON SOME DISEASES OF TOMATO .....</b>	<b>12</b>
2.1 INTRODUCTION .....	13
2.2 FUSARIUM WILT .....	14
2.3 EARLY BLIGHT .....	16
2.4 BACTERIAL WILT .....	18
2.5 POWDERY MILDEW .....	20
2.6 LATE BLIGHT .....	23
2.7 MATERIALS AND METHODS .....	24
2.8 RESULTS .....	27
2.9 DISCUSSION .....	32
2.10 REFERENSES .....	35
<b>3 TOMATO YIELD AND QUALITY IN RELATION TO NITROGEN FERTILIZATION AND DISEASES.....</b>	<b>37</b>
3.1 INTRODUCTION .....	38
3.2 MATERIAL AND METHODS .....	43
3.3 RESULTS .....	47
3.7 DISCUSSION .....	53
3.8 CONCLUSIONS .....	55
3.9 REFERENSES .....	56
<b>APPENDIX .....</b>	<b>58</b>

# 1 TOMATO DISEASES, QUALITY, YIELD AND PESTICIDE USE – A FIELD STUDY IN NICARAGUA

Anna Berlin and Sofia Eitrem

## 1.1 Background

Tomato (*Lycopersicon esculentum* Mill.) is a key food and cash crop for many low-income farmers in the tropics (Prior *et al.*, 1994). Tomato is a fresh vegetable with increasing popularity and the global production of tomato is 80 million tons (INTA, 2002).

Nicaraguan farmers have for a long time struggled with tomato production. Efforts were made to build up processing factories for tomatoes in the 1990's. The factories required large-scale intensive production areas with irrigation and tomato production year-round. Soon the tomato crop was heavily affected by various diseases, which led to great reduction of both yield and quality of the tomato fruits.

In Nicaragua the primary problem for the tomato production is Begomovirus infection. These viruses are transmitted by white flies (*Bemisia tabaci* Genn.). The viruses cause curling of the leaves, stunted plants and reduced yield and quality (Hull, 2001). The first observation of the disease in Nicaragua was made in the 1970's (Polston and Anderson, 1997). In less than twenty years the problem had become nation-wide, mostly due to the changes in the cropping system, and the tomato production virtually vanished (Rojas *et al.*, 2000).

Some research has been done to find out which viruses that is present in Nicaragua, but the focus on virus diseases have left a gap of knowledge about other important factors for tomato production (Rojas, 2003, personal communication). Other diseases also cause yield losses. The small yield is often of bad quality and this leads to even lower income for the farmers.

In Nicaragua 2000 hectares are used for the yearly tomato production. Tomatoes are grown on fields and the varieties are determinate or bushy. Approximate yield is 12-18 tons per hectare (INTA, 2002).

The farmers protect their crop by the most acknowledged way, to use pesticides. Tomato production is very pesticide intensive and the farmers usually spray against whitefly every day. The industry does not give accurate information about the pesticides and the farmers often have low education and no knowledge about the cause of disease and effect of the pesticides. This is an environmental and social problem in most developing countries, not only Nicaragua (Rojas, A., 2003, personal communication). An example of the pesticide problem is that the tomato sauce produced in the factories could not be exported because of high levels of pesticide residues (Ingvarsson, 2003).

### 1.1.1 Climate

Nicaragua is the largest country in Central America and has both a tropical and a subtropical climate. The year is divided in two seasons, a wet season between May and November and a dry season between December and April. The climate is also strongly

affected by altitude. The lowlands have considerably higher temperatures compared to the mountain regions (Andersson, 2002). Tomatoes are grown all over the country, but in the drier lowland plains irrigation must be used during the dry season. In the tropics tomatoes are grown at the altitude of 400 to 2000 meters above sea-level (INTA, 2002).

### 1.1.2 Aim of the projects

The overall goal of these projects was to identify common tomato diseases and evaluate their effect on yield and quality of the tomato fruits at different nitrogen levels.

## **1.2 Interviews with local farmers regarding use of pesticides in tomato production**

### 1.2.1 Introduction

As a complement to the field experiment we interviewed farmers with questions regarding pesticide use and storage. The interviews were held in two villages, Tisma and Santa Lucia. The common knowledge of the cause of the diseases and the cure with chemicals is very low. Pesticides are a problem in the Nicaraguan society due to residues in primarily fruits and vegetables and there have been high contents of pesticides in export products (Ingvarsson, 2003).

According to INTA (2002), tomato farmers in Nicaragua are usually smallholders. The average farmer grows tomatoes on 0.3 to 2 hectare. They sell their harvest at the farm or to a dealer who transports the tomatoes to markets in the neighbourhood, in the capital or abroad.

### 1.2.2 Interview sites, Tisma and Santa Lucia

Tisma is a county in the lowland region south of the capital Managua. The farmers who grow tomato in the dry season depend on irrigation and the water is taken from a small lake in the area. In Tisma we had the opportunity to attend a group counselling with our local supervisor, Aldo Rojas, and local farmers. We interviewed 15 farmers, of which 10 grew tomato.

Santa Lucia, on the other hand, is a village in the mountains. The climate in this region is more favourable for tomato growing since it rains all year around. It is also considerably colder in this region. Three tomato farmers were interviewed in Santa Lucia and we visited one farmer's tomato field.

### 1.2.3 Questions

During the interview the farmers were asked questions about the farms, their production and pesticide use and safety. The aim with the questions asked was to get knowledge about the use and storage of pesticides used in tomato production by smallholders in Nicaragua. The questions are listed in appendix 1.

#### 1.2.4 Interview answers and reflections about the pesticide situation

##### *The farmers and the farms*

Of the eighteen interviewed farmers, the mean age was 42 years with a range from 18 to 64 years. All farmers had a total production area of 0.5 to 3 hectares, except for one farmer in Santa Lucia who had 40 hectares. We interviewed 13 tomato producers and 5 pumpkin producers. Beside the main crop (tomato or pumpkin) the most common crops were maize (*Zea mays* L.), cushaw (*Cucurbita maxima*, large white fruit, related to pumpkin), peppers (*Capsicum annuum*) and beans. In Tisma some farmers also grew different types of melon. Most of the farmers have animals (cows, pigs, sheep and/or chicken) for subsistence and a few had dairy cows for commercial milk production.

##### *Tomato production*

Of the tomato farmers, all used 0.3 to 1.5 hectares for tomato production. The number of pesticide applications during a tomato season varies between 5 and 40 sprayings. All farmers agreed on that pests and diseases are major problems in tomato production. Some years when the weather is favourable to insects and pathogens the harvest may be lost completely. In Tisma, virus diseases are a big problem and tomato production in the lowland region demands great chemical input. Infection of Begomovirus is a problem also in Santa Lucia, but since the climate is colder the vector is less abundant and the disease spreads more slowly. Instead, farmers in this region struggle with late blight.

##### *Pesticide use, storage, protection and application*

All the interviewed farmers used pesticides to control pests and diseases. The pesticides are used both as a prevention of disease and when symptoms appear in the field. To achieve the desired effect it is essential to use the chemicals properly, i.e. in the right proportion, right time and right crop and pathogen. The farmers in this region are often unaware of these facts.

Information about how to use and store the pesticides is supplied by the chemical companies, stores where the pesticides are sold and other farmers. If the store or company inform about use and application there could be a conflict between the economy of the farmer and the economy of the company.

The farmer himself, a worker or a family member applies the pesticides in the field. Without exception a knapsack sprayer is used for pesticide application. Traditional spraying equipment is shown in Figure 1.1.



**Figure 1.1.** Nicaraguan farmer with spraying equipment

The most common health problem related to chemical use was itching after spraying. Thirteen of the farmers had experiences of itching skin. More rare was dizziness, vomiting and headache. Five of the farmers had never experienced any problems. If the skin is exposed to the chemicals they wash with water and soap and a few of the farmers visit a doctor. The problems mentioned in the interview are only short term. The long-term effects such as sterility and cancer are far more serious.

Even though most farmers had experienced health problems after using agricultural chemicals, ten of the farmers state that they don't use any protection or only boots when they apply pesticides. Protections used by other farmers are gloves, mask, eye protection and/or plastic clothing. The main reasons why the farmers don't use protection are limited financial resources and lack of habit.

Most of the interviewed farmers seem to guard their pesticides well; they use a separate room in or near the house or a shelter in the field to store the chemicals. The empty containers, though, are either left in the field, burned, buried or just thrown in the streets. This is an attitude to garbage that seems to be generally accepted in the region. The problem with empty chemical containers is that the remains of the pesticides may spread in nature and affect soil, plants, animals and humans. If the chemicals reach lakes or streams the water may become poisoned.



### **1.3 Discussion of the projects and future prospective**

So far we have only listed the cons in Nicaraguan tomato production. The question is why the farmers still grows tomatoes when the losses are great and the yield uncertain. The answer is simple: tomato production has a high potential profit compared with almost all other crops grown in the region.

To manage the problems in the Nicaraguan tomato production, it needs to be evaluated on many different levels and from different perspectives. A possible method is to develop a regional strategy where the farmers co-operate to create host free periods for the pathogens. This would lower the pathogens ability to infect and destroy the crop. This method demands education of farmers and an interest to collaborate. To be successful with this method, all the farmers in the region need to agree on the same strategy.

Today, the farmers overuse chemical products to control different pests. The Nicaraguan farmers in general have a strong belief that pesticide is the solution of all their agricultural problems. They also have a very little knowledge about the mechanism and target of a specific pesticide. The amount of pesticides could therefore be reduced dramatically in the Nicaraguan tomato production, if the correct information about which pesticides or other suitable control methods, was available. Again, the need of education is crucial for optimal pest management.

There is a lot of knowledge about the pathogens and their behaviour in Nicaragua. There is a similar situation with fertilisation and optimal managing for maximum economic profit. The problem is that this knowledge is isolated to a few persons, often researchers who do not work in practical agriculture and have little contact with producers in the countryside. It is very important with education at all levels; researchers, students and farmers.

## 1.4 Referenses

### 1.4.1 Books and articles

- Andersson, S., 2002, *Länder i fickformat nr 704 Nicaragua*, Utrikespolitiska institutet, Stockholm
- Ingvarsson, A. 2003, Gift skapar ond cirkel, *Teknik och vetenskap*, Nr 5
- Instituto Nicaragüense de Tecnología Agropecuaria (INTA). 2002. *El Cultivo del Tomate, Guía Tecnología 22*
- Polston, J.E. and Anderson, P.K., 1997, The emergence of white-fly transmitted Gemini virus in tomato in the western hemisphere, *Plant Disease*, 81:1358-1369
- Prior, P., Grimault, V., Schmith, J., 1994, Resistance to Bacterial Wilt (*Pseudomonas solanacearum*) in tomato: Present status and prospects. In: Edt. Hayward, A. C., Hartman, G. L. *Bacterial Wilt. The Diseases and its Causative Agent, Pseudomonas solanacearum*. CAB International
- Rojas. A., Kvarnheden, A. and Valkonen, J.P.T., 2000. Geminiviruses infecting tomato crops in Nicaragua, *Plant Disease*, Vol 84 no 8 pg:843-846

### 1.4.2 Personal communication

- Rojas, A., 10<sup>th</sup> of September 2003, Agr PhD student UNA-SLU and local supervisor of the projects. Uppsala, Sweden.

The fieldwork of this project was carried out in Nicaragua from January through April 2004. The project was conducted in cooperation between Universidad Nacional Agraria (UNA) in Nicaragua and the Swedish University of Agricultural sciences (SLU). Ing. Agr., Dr Aldo Rojas was our local supervisor in Nicaragua. In Sweden Professor Jonathan Yuen is the supervisor of Sofia Eitrem and Professor Henrik Eckersten is the supervisor of Anna Berlin.

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## 2 THE EFFECT OF NITROGEN ON SOME DISEASES OF TOMATO

Sofia Eitrem

### Abstract

Tomato production all over the world struggles with the threat of diseases. Many of the pathogens are spread world wide and cause enormous yield losses every year. The fieldwork of this thesis was carried out during the dry season in Nicaragua. The essence of the study was to find out if there exists a correlation between two common tomato diseases (Fusarium wilt and early blight) and nitrogen fertilisation. These diseases were believed to cause great losses in Nicaragua during the dry season as well as the wet season. Two field experiments were made to study the diseases in natural conditions during the growing season. Tomatoes of two varieties were planted and the fertilised with five different levels of nitrogen. Weekly records were taken of the symptoms that appeared in the experiment and the results were tested in a statistical analysis.

The symptoms that appeared in the field experiment indicated that the plants were not infected by the suspected pathogens and lab results showed that symptoms on the plants were caused by two other diseases: bacterial wilt and powdery mildew (causal agents: *Ralstonia solanacearum* and *Leveillula taurica*). In some ways the symptoms of these diseases are similar. Both bacterial wilt and Fusarium wilt are typical wilting diseases with the ability to kill off entire fields in a short period of time. The pathogens infect through the roots and the plants lose their ability to transport water, which is what causes the wilting symptoms. Powdery mildew and early blight are caused by fungi and infect through the leaves. A plant with symptoms shows yellow leaf spots that may grow rapidly. A significant difference is that *L. taurica* forms irregular spots and *Alternaria solani* gives characteristic round spots with concentric rings. The results showed that plants fertilised with double normal amount of nitrogen were more resistant to powdery mildew. There was also a difference between the varieties in severity of powdery mildew. Regarding bacterial wilt the results are ambiguous, one of the field experiments show no difference between the treatments but in the other experiment there was a considerably higher rate of infection in the to highest levels of nitrogen fertilisation.

The most important conclusion of this thesis is that you always have to identify the pathogen to know which disease you are dealing with!

### Key words

Tomato diseases, *Leveillula taurica*, *Ralstonia solanacearum*, *Fusarium oxysporum*, *Alternaria solani*, Nicaragua

## 2.1 Introduction

In total, there are more than 200 pathogens that infect the tomato crop and diseases are often the limiting factor in tomato production (Jones *et al.*, 1997). The epidemics of a disease depend on complex interactions between host, pathogen and environment. The disease development may also be influenced by cultural practices such as fertilisation and irrigation (Aust and v. Hoyningen-Huene, 1986). Plant pathogens have different strategies for surviving and spreading to new hosts. Most pathogens have a life cycle that includes both plants and soil, but they usually need to infect a specific host to increase the population (Agrios, 1997).

According to Martinez (2002) all the countries in Central America and the Caribbean are struggling with enormous virus problems. Other important diseases on the tomato crop in this region are: bacterial wilt, early blight and late blight. This information does not completely agree with information from INTA, the national agricultural institute in Nicaragua, which considers early and late blight to be the two most destructive diseases on tomato in the country.

The climate in Nicaragua is favourable to pathogens that spread in hot and dry weather (dry season) and temperate and moist weather (rainy season). In general, tropical countries have more problems with diseases because there is no natural break between the growing periods and the climate is favourable to most pathogens. In Nicaragua the altitude often decides which diseases that are considered important. In the highlands, the temperatures are considerably cooler and water is not a critical factor even in the dry season, but in the lowlands water is the limiting factor and the temperatures are higher. Producers in the highlands have problems with late blight and cracks on the fruits (INTA, 2002).

The original aim of this study was to investigate if there is a relation between Fusarium wilt, early blight and nitrogen fertilisation. According to our local supervisor these two diseases were the most common and destructive non-virus diseases on tomato in Nicaragua. Results were to be used as advice for farmers in the terms of optimum nitrogen fertilisation and possible control of the diseases with pesticide use or agricultural practice.

In the field experiment tomatoes were grown in five levels of nitrogen fertilisation to test the susceptibility to natural infection of early blight and Fusarium wilt. The hypothesis was that infection of early blight and Fusarium wilt would decrease with a higher nitrogen level.

In the literature study I try to clarify differences and similarities between the diseases thought to be important, Fusarium wilt and early blight, and the diseases that actually appeared in the field experiment, bacterial wilt and powdery mildew. I also give a short review about late blight, a disease we came across during the interviews in Santa Lucia.

## 2.2 Fusarium wilt

Species of *Fusarium* are found all over the world, even in the arctic and in deserts. These fungi are saprophytes or pathogens, living on plants or animals. Most *Fusarium* spp. are soil-inhabiting fungi. Symptoms of *Fusarium* diseases are rots, leaf spots, blights or wilts (Mace *et al.*, 1981). The pathogen is spread worldwide and once the soil is infested it is difficult to eliminate the pathogen since it survives in the soil for long periods (Agrios, 1997; Watterson, 1986).

### 2.2.1 The pathogen: *Fusarium oxysporum* f. sp. *lycopersici*

*Fusarium oxysporum* belong to the Deuteromycetes, imperfect fungi, and is a soilborne pathogen with a high level of host specificity. There are more than 120 described formae speciales and races within the species. Together they cause diseases on a wide range of agricultural crops (Correll, 1991; Agrios, 1997). The causal agent of Fusarium wilt on tomato is *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hans (Chambers, 1963).

*F. oxysporum* produces three kinds of spores: microconidia, macroconidia and chlamydospores. Microconidia are small, 5-12 x 2-3 µm, and produced abundantly at the end of mycelium branches in all conditions. Macroconidia have three to five cells and pointed ends. The size varies from 27 to 60 x 3-5 µm. The macroconidia are produced on the surface of dead plant tissue (Agrios, 1997; Jones *et al.*, 1997). Chlamydospores are round spores with thick walls that can survive in the soil for a long time. They are usually produced at the end of old mycelia in decaying plants (Nelson *et al.*, 1981).

### 2.2.2 Development of disease and life cycle

Fusarium wilt occurs both in the field and in greenhouses. The disease develops quickly in warm weather and on sandy soils and the fungus is favoured when the soil is low in nitrogen and phosphorous and high in potassium (Jones *et al.*, 1997). Growth and spreading of *Fusarium oxysporum* f. sp. *lycopersici* is reduced if the soil is low in micronutrients such as copper, iron, manganese, molybdenum and zinc. The micronutrients are more available at a low pH and this is a reason why a high pH (6.5-7.5) is preferable to reduce infection. The source of nitrogen also affects the pathogen, when the nitrogen source is ammonium the pathogen is more virulent (Nelson *et al.*, 1981).

The optimum temperature for growth of *F. oxysporum* is 28-29°C. There are no symptoms of infection if the soil temperature is below 20°C or above 30°C (Holliday, 1980). Growth and survival of *F. oxysporum* is favoured by dry soil, but after infection the spread of the pathogen inside the plant is favoured by moist soil and a high transpiration (Nelson *et al.*, 1981). The fungus spreads between fields with contaminated soil on plants or farm equipment, with seeds or water (Atherthon, 1986).

In the periods between tomato production the fungus survives as mycelia, micro- or macroconidia on plant debris or as dormant chlamydospores in the soil. When new plants grow in the contaminated soil the spores are stimulated to germinate. A germinating chlamydospore forms conidia or new hyphae (Mace *et al.*, 1981). Germ

tubes or mycelia penetrate the plant root directly or through wounds in the cuticle. Inside the plant the fungus moves towards the vascular tissue (Agrios, 1997).

The fungus excretes metabolites and auxin, which are transported upwards in the stem, ahead of the mycelia. When the diseased plant reacts to the high auxin levels, the cells surrounding the vascular tissue start to expand. The pressure on the vascular tissue increases and eventually the vessels collapse (Chambers, 1963). *F. oxysporum* also excretes pectolytic and cellolytic enzymes which cause collapse of the vascular tissue (Holliday, 1980).

Mycelia grow and produce microconidia inside the vessels. Spores are transported upwards in the xylem and germinate when the upper wall in the vessel stops them. Mycelia penetrate the walls and spread upwards in the plant, but also to the adjacent vessels (Agrios, 1997). Damaged vessels are filled with mycelia and spores and they lose their capacity to transport water to the upper parts of the plant (Chambers, 1963). When the water potential decreases the stomata close and the leaves wilt and die. The vessels of diseased plants are smaller, fewer and deformed and the ability to transport water is reduced to 6 % of the capacity of a healthy plant (Mace *et al.*, 1981). The fungus sporulates on the surface of dead plant tissue. The spores spread to new plants by wind or water (Agrios, 1997).

### 2.2.3 Symptoms

When seedlings are infected by *F. oxysporum* the growth is reduced, the leaves curve downward and wilt. Young plants usually die when they are infected at an early stage (Jones *et al.*, 1997). The first visual symptom of Fusarium wilt in the field is yellowing of the lower leaves, often only on one side of the plant. As the lower leaves gradually wilt the disease move upward in the plant and new leaves are infected. Eventually the entire plant may die (Mace *et al.*, 1981). Other visual symptoms on older plants are marginal necrosis, defoliation and stunted plants. If the fruits are infected they rot and fall off the plant (Agrios, 1997). When the fungus enters the plant, *F. oxysporum* grows inside the vascular tissue that becomes dark and damaged. The discoloration is present in the roots as well as in the stem and branches (Jones *et al.*, 1997).

### 2.2.4 Control

Use resistant cultivars, if they are available, to control the disease. The fungus cannot be controlled with fungicides. Since *F. oxysporum* survives in the soil for a long time, even a crop rotation of 5-7 years is not enough to control the pathogen but it may reduce infection. Use healthy seed. If it is possible chose nitrate nitrogen and not ammoniac nitrogen. Lime to raise the soil pH to 6.5-7. Prevent spreading of the pathogen to disease-free areas by using clean tools and equipment (Agrios, 1997; Jones *et al.*, 1997).

### 2.2.5 Economic importance

Fusarium wilt is considered as one of the most destructive diseases to tomato in temperate regions (Agrios, 1997). The disease is described as a serious pathogen on the tomato crop in El Salvador (Pérez *et al.*, 2003) but is not mentioned in the INTA guide for tomato production in Nicaragua (INTA, 2002).

## 2.3 Early blight

Early blight is common on field tomatoes in humid and semiarid climates worldwide (Jones *et al.*, 1997). This disease also infects relatives in the family *Solanaceae*, for example potato, pepper and eggplant. It affects leaves, stems, flowers and fruits (Agrios, 1997) and may occur at any time during the growing season, but tomato plants are more susceptible when they are small (Thomas, 1948).

### 2.3.1 The pathogen: *Alternaria solani*

The causing agent of early blight on tomato is *Alternaria solani* (Ellis & Martin) Jones & Grout. The same fungus also causes collar rot on tomato. Early blight is considered the more serious of the two diseases (Maiero *et al.*, 1991).

The mycelium of *Alternaria* spp. is dark, septate and branched. Reproductive structures, conidiophores, are formed on old infected tissue. The conidiophores are simple and erect and the conidia are produced from the terminal cell of the conidiophores. Conidia are multicellular, pear shaped and large (12-20 X 120-296 µm) and they are loosely attached to the conidiophore as single spores or in chains of two (Jones *et al.*, 1997).

As many species of *Alternaria*, *A. solani* produces toxins. In culture the fungus synthesises two phytotoxins, alternaric acid and zinniol. Alternaric acid is considered to be host specific and causes leaf lesions that are surrounded by a yellow zone. Zinniol affect a wide range of hosts and causes stem wilting and leaf necrosis. The phytotoxins are transported in the vascular tissue of the diseased plant (Maiero *et al.*, 1991).

### 2.3.2 Development of disease and life cycle

*Alternaria solani* is a weak pathogen and the plants that are infected are usually weakened or stressed; therefore the disease develops quickly in fields with early senescence due to poor fertilisation or lack of water/irrigation. Adequate or high nitrogen fertilisation reduces infection of early blight (MacKenzie, 1981). The fungus is favoured by a humid climate and daily dew. Infection of *A. solani* also increases with rising temperature. The optimum temperature is 28-30°C, but the spores are able to germinate at temperatures between 6 and 34°C (Jones *et al.*, 1997; Moore, 1942).

Between hosts *A. solani* survives in the field as mycelium or spores on plant debris, volunteer plants, seed from the host plant or on weeds within the host range. When new tomatoes are planted in a field where the pathogen is present, spores in the soil cause the primary infection. The first infection occurs during warm and rainy or humid weather (Watterson, 1986; Jones *et al.*, 1997). The conidia germinate in water and penetrate plant tissue through the cuticle or via a wound in the plant tissue (Agrios, 1997). If the conditions are favourable the first leaf spots are visible 2-3 days after infection. When the spots reach the size of 3 mm the fungus starts to produce conidia. The conidia are wind spread but can also spread between plants in the field through splashing water (Agrios, 1997; Jones *et al.*, 1997).

### 2.3.3 Symptoms

Leaves on infected plants develop dark brown spots with concentric rings. A yellow area surrounds the spots and leaves with many spots become completely yellow. The



infected tissue can occur anywhere on the leaf. The spots enlarge rapidly and finally the whole leaf dies. Usually the infection starts at the lower leaves, which turn yellow or brown and then droop or fall off. If the climate is favourable, the disease develops rapidly, the spots spread over the leaves and the plant becomes defoliated (Jones *et al.*, 1997; Watterson, 1986).

The visual symptoms on stems and branches are oval and dark brown lesions. In the lesions the stem is sunken and has a centre of dead, grey tissue. The spots are small, often around 2 mm. The spots enlarge from the centre and in large spots you can see concentric rings. On small plants the lesions may develop into a canker at the bottom of the plant, causing the plant's death. This is called collar rot (Agrios, 1997; Glasscock, 1944).

Spots on the fruit first occur at the top of the fruit as small sunken areas with concentric rings. From this area the fungus spreads and eventually the rot covers the top half of the fruit. The leathery surface of infected fruit tissue is black and covered with spores of *A. solani*. Fruit infection occurs when the fruit is green or ripe (Glasscock, 1944; Jones *et al.*, 1997).

#### 2.3.4 Control

There are resistant varieties to both early blight and collar rot and to use resistant or tolerant varieties is the primary control of early blight (Maiero *et al.*, 1991; Jones *et al.* 1997). Use pathogen free seeds and healthy seedlings and add adequate nitrogen fertiliser to reduce infection. *Alternaria solani* can also be controlled by spraying regularly with chemical fungicides (Agrios, 1997). Inoculum of *A. solani* can be reduced by practising long crop rotation, burning plant debris, removing weed hosts and avoid planting tomatoes close to a field with potatoes (Glasscock, 1944).

INTA's recommendations for control of early blight are to use healthy seed and crop rotation. Pesticides should be applied when you find more than five spots on ten plants in five places in the field. Examples of fungicides are Clorotalonil, Maneb and Mancozeb (INTA, 2002).

#### 2.3.5 Economic importance

The yield is reduced when the plants lose their leaves and because the plants fail to set fruit (Glasscock, 1944). Infected fruit fall to the ground and this causes losses of 30-50 % of the harvest (Jones *et al.*, 1997).

Early blight is mentioned in INTA's guide for cultivation of tomato as one of the most destructive diseases in the area. The climate conditions during the rainy season and in the highlands are favourable for the pathogen and the disease is known from all over the country (INTA, 2002).

## 2.4 Bacterial wilt

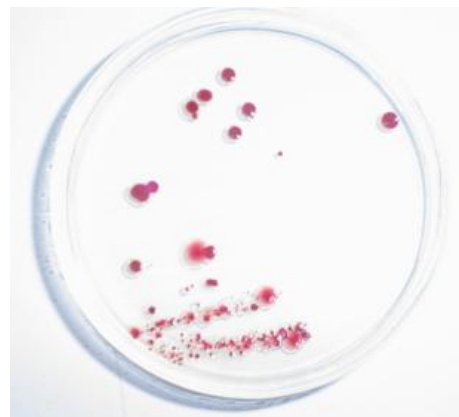
Tomato is an important and valuable crop for low-income farmers in the tropics and the production is often limited by the bacterial wilt. Bacterial wilt is caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (syn. *Pseudomonas solanacearum* (Smith) Smith, *Burkholderia solanacearum* (Smith), Yabuuchi *et al.*, 1995). This pathogen has a wide range of hosts and infects over 200 species in 33 families, among both mono- and dicotyledons. Important agricultural crops with great yield losses are banana, plantain, groundnut, ginger, eggplant, tobacco, potato and tomato (Jones *et al.*, 1997; Hartman & Elphinstone, 1994).

### 2.4.1 The pathogen: *Ralstonia solanacearum*

In 1896 E. F. Smith was the first to describe what we know today as *Ralstonia solanacearum* as the causing agent of wilting of plants in the family Solanaceae, but before that farmers in Japan had then known the disease for at least 200 years (Kelman *et al.*, 1994). *Pseudomonas* was always a heterogeneous genus and over the years species has been moved to other genera (Yabuuchi *et al.*, 1992). In 1995 the new genus *Ralstonia* was created based on DNA studies. Among the species in this genus is *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995).

There are five known races of *R. solanacearum*. Of the five races, one attacks only plants in the banana family, one causes infection only in potato and tobacco, one (called race 1) has a wide host range, including crops in the family *Solanaceae* and the last two are of little economic importance (Agrios, 1997). Race 1 is believed to originate from lower altitudes in warm and humid areas in the Americas and Asia (Hartman & Elphinstone, 1994).

*R. solanacearum* is an aerobic, rod shaped non motile and gram negative bacterium. The size of the bacteria are 0,5-0,7 x 1,5-2,0  $\mu\text{m}$ . The optimum temperature is 35-37°C (Jones *et al.*, 1997; Yabuuchi *et al.*, 1992). When grown on tetrazolium medium the pathogen forms dark red colour colonies with a white border, see figure 2.1. When *R. solanacearum* is kept in culture on a medium such as TZC the bacteria loses its virulence. To maintain virulence the bacteria should be kept in mineral oil (Kelman, 1954).



**Figure 2.1** Colonies of *Ralstonia solanacearum* grown on TZC

### 2.4.2 Development of disease and life cycle

*R. solanacearum* enters the host through wounds or cracks in the roots. Careless treatment of seedlings at transplantation, cutting of roots when earthing up the plants and insect or nematode wounds increases infection (Proir *et al.*, 1994). The pathogen multiplies in the vascular tissue, which becomes brown and filled with slime from dead

cells and bacteria. The water transport is disrupted and affected plants usually wilt and die (Agrios, 1997; Watterson, 1986).

Dissemination of *R. solanacearum* with infected plants and through soil management practices are a major problem because when the pathogen reaches new fields it is very hard to get rid of it. Within the field *R. solanacearum* infects new plants when a root reaches a bacterium in the soil. The pathogen survives on alternate hosts or weeds between the growing seasons. In intensive growing systems the pathogen multiplies rapidly, the losses become greater over the years and the farmer is forced to move the tomato production to a new area (Kelman *et al.*, 1994).

The bacterium is favoured by high temperatures and is limited to areas with warm temperate or tropical climate where the environment is stressful for plant production. Strains of *R. solanacearum* that are more tolerant to cool weather have been found and could be a future threat to many potato producing areas in the northern hemisphere (Kelman *et al.*, 1994).

Researchers all over the world try to breed for resistance, but so far with limited success. The bacterial strains are variable and bacterial wilt resistance is genetically complex. Often resistant cultivars are only resistant in very specific environments and they are of no global use (Young & Danesh, 1994).

#### 2.4.3 Symptoms

The first symptom of bacterial wilt is drooping leaves during the hottest part of the day and this usually occurs 2-5 days after infection. The leaves lose turgor, wilt and become brown. Figure 2.2 shows a diseased plant. Under good conditions for the pathogen (high temperature and moisture) the disease spreads rapidly and the plant may wilt entirely in a few days. Some plants may form adventitious roots on the stem to compensate for the damaged vascular system. Other symptoms of bacterial wilt are stunted plants and necrosis of the leaf marginal (Agrios, 1997; Hartman & Elphinstone, 1994).



**Figure 2.2** Plant infected by *R. solanacearum*

The pathogen survives on debris or on wild host plants between the growing periods. Bacteria from dead plants spread in the soil water and can survive in the soil for some time (Jones *et al.*, 1997).

The vascular system of infected plants is water-soaked, brown or hollow. If the stem is cut and placed in water, a white stream of bacteria floats from the stem (Agrios, 1997).

#### 2.4.4 Control

Since there are no effective chemicals on the market to control Bacterial wilt, all means of control must be done with cultural control. Since *R. solanacearum* has a wide range of hosts it is difficult to control bacterial wilt with crop rotation. The best control the disease is to avoid fields where *R. solanacearum* is present (Jones *et al.*, 1997).

However, some results show that crop rotation with at least two years of rice or maize between tomatoes reduces the infection and losses dramatically. A longer rotation than two years is preferable to minimise the risk of great losses (Hartman & Elphinstone, 1994).

Green house experiments show a reduction of *R. solanacearum* when the bacteria are exposed to competition from avirulent strains of the bacteria or from bacteriocin-producing species (Hartman & Elphinstone, 1994).

The INTA guide for tomato production does not mention this disease, but the advice from the national centre of agricultural technology (CENTA) in El Salvador is, apart from cultural practices, to treat the disease with the antibiotic chemical Agrimycin (Pérez *et al.*, 2003). There are two major problems with this chemical. Spraying on the soil is an ineffective way to treat a pathogen and the bacteria easily develop resistance that may spread to pathogens infecting animals and humans.

#### 2.4.5 Economic importance

Bacterial wilt has a world wide economic importance since the disease is devastating to a large number of important crops. The disease causes great losses in tomato.

Depending on infection pressure and environmental circumstances the loss may vary between 15 and 95 % of the total yield (Hartman & Elphinstone, 1994).

### 2.5 Powdery mildew

Powdery mildew is a common disease that affects many plant species. The disease has a great affect in crops such as cereals, cucurbits, sugar beets and strawberries. Although powdery mildew appears in all climates, the disease is more severe in warm and dry areas. Spores of the fungi spread and germinate in dry weather and in most species the mycelium grows on the surface of the plant. The host is usually not killed by powdery mildew but since the fungus steals nutrition and light from the plant an infection will lead to reduced growth and yield (Agrios, 1997). Many different fungi in the family *Erysiphaceae* cause powdery mildew. The fungi are all obligate plant parasites and the perfect state of these fungi is rarely found in the tropics (Holliday, 1980).

There are at least three species causing powdery mildew and economic damage on tomato. Two of them, *Oidium lycopersici* and *O. neolycopersici*, cause similar symptoms but are present in different parts of the world. The symptoms of powdery mildew caused by *Oidium* spp. are powdery white lesions on all aerial plant parts except for the fruit. In severe outbreaks the lesions coalesce and the disease is debilitating the plants ([www.bspp.org.uk](http://www.bspp.org.uk)). *O. neolycopersici* has non-catenate conidia and is widespread in Europe, Africa, Asia, North and South America while *O. lycopersici* has catenate conidia and is endemic in Australia. Until recently these two species were considered as one, but studies show differences in structure and morphology (Kiss *et*

*al.*, 2001). The sexual stage or cleistothecia have not been observed in either species (Whipps *et al.*, 1998)

The third species of powdery mildew fungus on tomato is *Leveillula taurica*, known to be the causing agent in warm and dry regions (Kiss *et al.*, 2000 and Whipps *et al.*, 1998). Symptoms that appeared in the experiment and laboratory examination of the pathogen led to the conclusion that *L. taurica* was the causal agent of powdery mildew in this case.

#### 2.5.1 The pathogen: *Leveillula taurica*

The fungus causing powdery mildew on tomato in the field experiment was *Leveillula taurica* (Lév.) Arn. (= *Oidiopsis taurica* (Lév.) Salmon) (Correll *et al.*, 1988). Most of the fungi causing powdery mildew only infect one or a few hosts but this is not the case with *L. taurica*. This pathogen is able to infect more than 700 species, both crops and weeds. The fungus is spread worldwide and the economically most important crops are cotton, onion, eggplant, pepper and tomato (Correll *et al.*, 1987). *L. taurica* originates from areas with warm and dry climate but is adapting to regions with more humid conditions. The pathogen has been reported from Mexico and Peru (Dixon, 1978).

*L. taurica* has endophytic mycelium, long and often branched conidiophores with conidia borne singly or in chains of 2-6 spores. The size of conidia, conidiophores, cleistothecia and other morphological structures of *L. taurica* vary with host species and the status of the host plant. Conidia may be pyriform or cylindrical and the size varies; 71-45  $\mu\text{m}$  x 24- 16  $\mu\text{m}$ . The length of conidiophores is 147-236  $\mu\text{m}$  (Correll *et al.*, 1987; Dixon, 1978).

#### 2.5.2 Development of disease and life cycle

The development of a plant disease is influenced by the microclimate in the crop. Powdery mildew is favoured by a dry climate, moderate temperatures, low humidity and reduced light. Rain or sprinkler irrigation reduces inoculum since the spores that have not yet germinated are flushed off the surface of the leaves and the air is cleaned from spores. Heavy raindrops also destroy the spore producing conidiophores. Experiments from the 1960's showed that the disease was 30-40% more severe in furrow irrigated fields than in sprinkler irrigated (Aust and v. Hoyningen-Huene, 1986; Dixon, 1978).

The air-spread conidia germinate on the leaf surface. The spore develops a germ tube and an appressorium, which makes it possible for the fungus to enter the plant through the cuticle between epidermal cells. *L. taurica* grows inside the plant and this is unusual among the fungi causing powdery mildew. Branched conidiophores emerge through stomata and the spores are released into the air. Sporulation is more common on the older leaves. In the absence of tomato *L. taurica* may survive on a number of weed hosts of (Correll *et al.*, 1987 and Holliday, 1980).

The optimal conditions for disease progress are a temperature of 20-25°C and a relative humidity of 50-70%. In these conditions the first conidiophores are produced 9-10 days after the infection. Spore germination is favoured by high relative humidity and is unlikely above 30°C in natural conditions (Guzman-Plazola *et al.*, 2003).

When the tomato canopy is disturbed a large number of spores leave the conidiophores and are able to infect new leaves. In tomato production the plants are disturbed when different cultural practices, such as earthing up and harvesting, take place. These practices could result in a spread of the disease (Correll *et al.*, 1988).

Powdery mildew is known to be more severe on mature plants, but in field experiments in 1984 and 1985 Correll (1988) showed that the infection depends on available inoculum in the area and not the physiological age of the plant. Usually the inoculum builds up during the season and this is the reason that the damage is more obvious in mature plants, but if new plants are planted next to a field with infected plants, the new plants will be infected at an earlier stage of development.

### 2.5.3 Symptoms

The symptoms of powdery mildew when *L. taurica* is the causing agent vary depending on host and plant organ. The most common symptoms are yellow and necrotic spots on the upper leaf surface or powdery white spots on the lower side of the leaves (Holliday, 1980). On tomato the first symptoms are small yellow spots on the leaves, usually visible 9-14 days after infection depending on environmental conditions (Guzman-Plazola *et al.*, 2003). The spots grow and form irregular lesions; sometimes the entire leaf becomes yellow and necrotic. Symptom of powdery mildew is shown in figure 2.3. Powdery mildew on tomato increases defoliation. The symptoms are usually first visible on the lower leaves and then the disease moves upward in the canopy (Correll *et al.*, 1988).



**Figure 2.3** Symptom of powdery mildew caused by *Leveillula taurica*

### 2.5.4 Control

Try to reduce inoculum and possibly avoid or delay infection by reassuring that there are no fields with infected crops (tomato, eggplant or pepper) near by (Correll *et al.*, 1988). Use sprinkler irrigation instead of furrow irrigation since *L. taurica* is favored by low humidity (Holliday, 1980). There are effective chemical sprays and properly timed foliage treatment can protect the crop from powdery mildew (Jones *et al.*, 1987).

### 2.5.5 Economic importance

According to Agrios (1997) the powdery mildews may reduce the yields to 60-80% of the normal harvest. Field experiments from various sites in the United States shows a

yield reduction between 0 and 40%. In general processing tomatoes are harvested much later and the harvest of processing tomatoes is more affected by powdery mildew. Fresh tomatoes are harvested earlier and it is possible to have high levels of infection in the field but no yield reduction (Correll *et al.*, 1988; Jones *et al.*, 1987).

A secondary problem with powdery mildew is defoliation. When the leaves fall off the plant the fruit is exposed to sun radiation. Sunburned fruit is a quality defect, which leads to reduced prices for the farmers (Correll *et al.*, 1988).

## **2.6 Late blight**

Late blight on tomato and potato is a major problem in the highlands of Nicaragua, where the climate is optimal for disease development and both potato and tomato are produced in the same area. The disease is very aggressive and may destroy large production areas in a short time (INTA, 2002). The disease is caused by *Phytophthora infestans* (Mont.) de Bary and the pathogen is believed to originate from Central America. The disease develops quickly and is very destructive in moist weather with cool nights and warm days. The disease can infect plants any time during the growing season and entire fields may be infected and destroyed in only one week (Jones *et al.*, 1997; Agrios, 1997).

Spores are produced on sporangiophores emerging from stomata of diseased leaves at high relative humidity and the fungus has an optimum temperature at 18-22°C. Spores from *P. infestans* are lemon shaped and 21-38 by 12-23 µm. The spores spread to new tissue by wind or rain. The pathogen survives on plant material, volunteer plants or as oospores in the soil (Jones *et al.*, 1997).

Symptoms of late blight are water-soaked spots that later develop into brown blighted areas and have an indefinite margin. The bottom surface the leaf is covered with a grey mold, a cover of spores. If the fruit is attacked it develops soft grey spots on green tomatoes and eventually the complete fruit rots (MacNab *et al.*, 1983; Agrios, 1997).

INTA recommendations are to use healthy seed and resistant varieties, to destroy harvest residues and plant seedlings with great distance between plants and rows to reduce humidity. Fungicides should be used as prevention; examples of effective fungicides in Nicaragua are Mancozeb, Clorotalonil and Maneb. There are also systemic fungicides such as Metalaxyl (INTA, 2002). The same control methods and chemicals are mentioned in Agrios, 1997. *Phytophthora infestans* has been known to develop resistance to Metalaxyl in other parts of the world and the effect of this chemical can't be guaranteed.

## 2.7 Materials and methods

### 2.7.1 Field experiment

The field experiments were carried out during the dry season at two locations at experimental sites belonging to the Nicaraguan agricultural institute, INTA.

#### *Campos Azules*

Campos Azules is an experimental station placed on a mid high plain, about 600 to 800 meters above sea level. This is a forest region where coffee, citrus, banana and other perennial crops are common. The climate is cooler and more humid than in Sébaco.

There were no fields with tomato close to the experiment, but the last crop in the field had also been tomatoes. During the experiment cassava was planted next to the tomatoes. A sprinkler irrigation system was used to water the crop.

Tomato seedlings were transplanted in Campos Azules the 27<sup>th</sup> of January, day 0. The experiment in Campos Azules was not maintained very well; the soil was badly prepared and the sprinkler irrigation was not sufficient. After a couple of weeks we realised we had to give up the experiment. By then the plants were small, affected by water deficiency and there seemed to be no difference between the treatments. Records of bacterial wilt were taken from Campos Azules on three occasions, the first one month after transplanting the seedlings.

#### *Sébaco*

Sébaco is a valley with intensive agriculture practice in the lowlands, 470 meters above sea-level. Traditional crops in this area are rice and horticultural crops such as onion, pepper and tomato.

Workers at the station prepared the experimental field. Onion was the last crop before our tomatoes and pepper was already growing in the adjacent field. Furrow irrigation is used at this experimental station.

In Sébaco the seedlings were transplanted on the 21<sup>st</sup> of January, day 0. The plants were watered regularly with furrow irrigation and developed at normal speed. All three diseases, bacterial wilt, powdery mildew and Begomovirus infection, were found on plants in the field experiment. Records of the diseases were taken from Sébaco on seven occasions, the first one month after transplanting the seedlings.

#### *The tomato varieties*

The two varieties used in the project were TY4 and TY13. They both descend from an imported Israeli variety. Since 1992 plant breeders in Sébaco have developed new varieties from the imported seeds by selecting plants with high yield and acceptable virus tolerance.



### Design and fertilisation

Each experiment consisted of four blocks, each with five treatments consisting of different amounts of nitrogen fertilisation and two tomato varieties. The design of the experiments is shown in figure 2.4.

The standard fertilisation is 600 pounds NPK (12-30-10) and 400 pounds Urea (46% nitrogen) per manzana (Mz). One Mz equals 7026 m<sup>2</sup> and one kg is equal to 2.2 pounds. The standard fertilisation then equals 160 kg N, 50 kg P and 30 kg K per hectare. In the experiment we chose to use the standard fertilisation, 50, 150 and 200% of this amount and no fertilisation (Table 2.1). The fertilisation was split in two and given twice during the season.

**Table 2.1** Fertilisation (N, P, K) applied in each treatment. The standard fertilisation is 160 kg N, 50 kg P and 30 kg K per hectare.

Treatment	Nitrogen (kg/ha)	Phosphorous (kg/ha)	Potassium (kg/ha)
0 %	0	0	15
50 %	80	50	30
100 %	160	50	30
150 %	240	50	30
200 %	320	50	30

In each plot both varieties, TY4 and TY13, were planted in two rows with 16 plants in each row. This equals a total number of 640 plants of each variety, a total of 1280 plants.

<b>200%</b> TY13   TY4	<b>50%</b> TY4   TY13	<b>150%</b> TY4   TY13	<b>0%</b> TY4   TY13	<b>100%</b> TY13   TY4
<b>200%</b> TY13   TY4	<b>100%</b> TY4   TY13	<b>0%</b> TY13   TY4	<b>50%</b> TY4   TY13	<b>150%</b> TY4   TY13
<b>50%</b> TY4   TY13	<b>200%</b> TY13   TY4	<b>100%</b> TY4   TY13	<b>150%</b> TY4   TY13	<b>0%</b> TY4   TY13
<b>200%</b> TY4   TY13	<b>100%</b> TY13   TY4	<b>150%</b> TY4   TY13	<b>0%</b> TY4   TY13	<b>50%</b> TY13   TY4

**Figure 2.4** Design of the field experiment with five treatments (% of standard nitrogen fertilisation) and placement of rows of TY4 and TY13 in the plots

The disease that appeared in the field was caused by natural infection. Records were taken once a week for six weeks. The records taken were:

- number of leaves with spots. Records taken from 12 plants of each variety, in the centre of the rows, in Sébaco on five occasions.
- % of surface covered by spots. Records taken from 12 plants of each variety, in the centre of the rows, in Sébaco on five occasions. To estimate the percentage of leaf area covered by spots a key for late blight of potatoes was used (James, 1971).

- number of wilted and dead plants. Counting of the total number of all plants, records were taken in Sébaco on five occasions and in Campos Azules on three occasions.
- virus symptoms on a 5-level scale. Records taken from 12 plants of each variety, in the centre of the rows, in Sébaco on three occasions.

#### 2.7.2 Lab procedures

Parts of tomato plants (leaf and stem) were analysed to isolate and identify the pathogen causing the infection. Diseased plants and leaves with symptoms were brought to the lab at UNA. To establish if *Ralstonia solanacearum* was present in the field, soil samples were also brought to the lab.

#### Materials:

Potato dextrose agar (PDA), Tetrazolium medium (TZC), Agar plates, Alcohol, 70%

#### Methods:

Fragments from stems of wilted plants were cut and (after sterilisation in 70% alcohol) put on TZC and PDA for 2-3 days. The cut stem was put in water for 10-15 minutes and a small amount of the water was applied on TZC.

Parts from leaves with spots were cut out, sterilised in alcohol and put on PDA and in moist chambers for 2-3 days and then examined under the microscope.

The soil samples were dried in room temperature. 10 g of soil was mixed in 100 ml of distilled water and agitated for 20 minutes. Dilutions were made by adding 1 ml of the sample to 9 ml of distilled water. From each dilution ( $10^{-1}$  and  $10^{-2}$ ) 0,1 ml was cultured on TZC for 2-3 days.

#### 2.7.3 Statistical analysis

The statistical analysis of the results was made as an analysis of variance (ANOVA) in SAS.

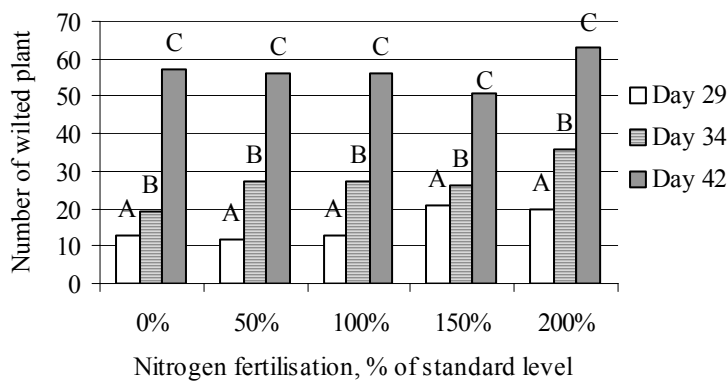
## 2.8 Results

### 2.8.1 Field experiments

Three diseases occurred in the field experiments: bacterial wilt caused by *R. solanacearum*, powdery mildew caused by *L. taurica* and infection of whitefly transmitted Begomoviruses.

#### *Bacterial wilt*

The result from the Campos Azules experiment is presented in figure 2.5. The ANOVA-test shows no significant differences between varieties or nitrogen fertilisation levels, but the number of plants infected by *Ralstonia solanacearum* increases on each occasion.



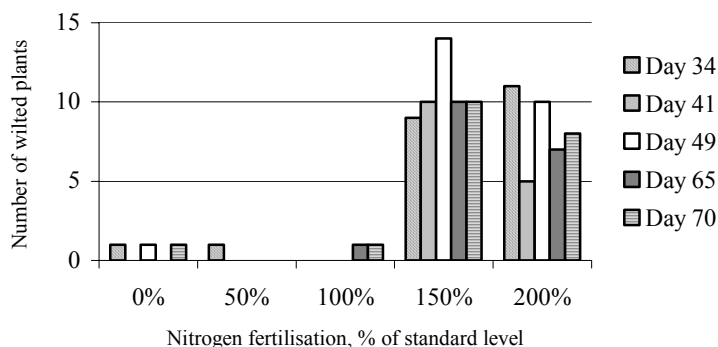
**Figure 2.5** Development over time of bacterial wilt in Campos Azules. The statistics show significant differences between the dates, shown by A, B and C in the figure. There was no significant difference between the treatments.

The first symptoms of bacterial wilt in Sébaco occurred one month after transplanting the seedlings. Until infected, the plants were growing normally but after infection they wilted and died, see photo in figure 2.6.



**Figure 2.6** Wilted plant in Sébaco

In Sébaco there were significantly fewer infected plants in the plots with lower fertilisation levels than in those with 150 and 200% (figure 2.7).



**Figure 2.7.** Number of wilted plants in the Sébaco experiment. There are significantly more infected plants in 150 and 200%,  $p < 0,0001$

Differences in the number of wilted plants between the sampling dates may be due to that the records were not always taken by the same person and that the plants that were taken to the lab for analysis are not counted for in the figure.

#### *Powdery mildew*

Symptoms of powdery mildew were observed in the experiment in Sébaco on day 41 (2<sup>nd</sup> of March) after transplanting.

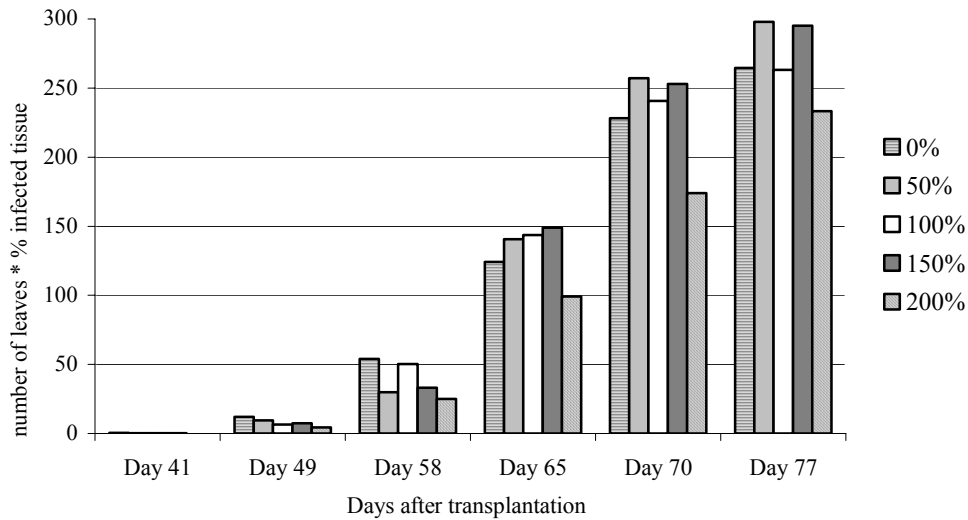
The symptoms started as small yellow spots on the lower leaves and developed into large irregular lesions. As the spots grew larger necrosis developed in the infected tissue, see picture in figure 2.8.

Later in the season the spots spread upwards in the canopy and white mildew lesions were also observed.



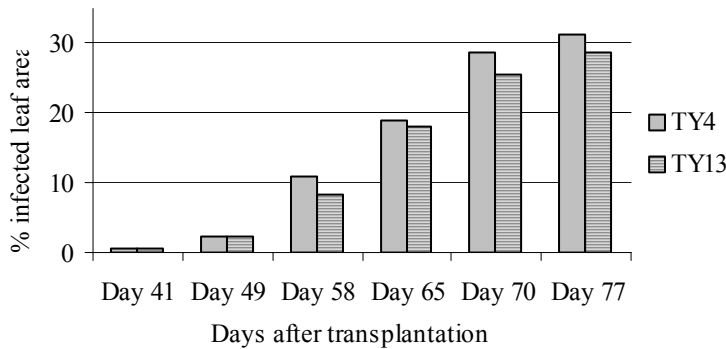
**Figure 2.8** Tomato plant with symptoms of powdery mildew

The disease developed quickly in the field, infecting both new leaves and plants. There were visible differences between each occasion. The severity of powdery mildew, measured in % infected leaf area, also increased over time. Records of number of infected leaves and disease severity were taken from twelve randomly chosen plants of each variety in every plot. Both disease severity and number of infected leaves were lower in the plots with 200% nitrogen fertilisation (figure 2.9).



**Figure 2.9** Infection of powdery mildew in different nitrogen fertilisation levels. Both the number of leaves with spots and percent of the leaves covered with spots increases over time. There is significantly lower infection rate in the highest nitrogen level (200%).

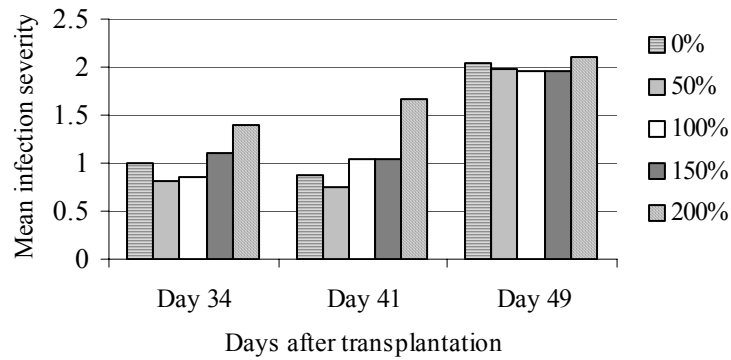
There seems to be a difference between the varieties regarding severity of powdery mildew. According to the ANOVA-test, the infection is more severe in TY4 than in TY13. The results are presented in figure 2.10.



**Figure 2.10** Development of powdery mildew, a comparison between TY4 and TY13. The infection is more severe in TY4 ( $p < 0,05$ )

### *Begomovirus*

Plants showed very clear symptoms of virus infection and there were white flies present in the field. The most probable source of virus inoculum was the pepper field next to the experiment, but tomato production is common in the vicinity and the vector, whiteflies, could have come from other fields in the valley of Sébaco. The first symptoms of virus infection were observed three weeks after replanting the seedlings. Figure 2.11 shows that there was a development of the disease over time ( $p < 0,0001$ ) and there were more infected plants and the infection was more severe in plots with 200% nitrogen ( $p < 0,05$ ). There was no difference in virus infection between the varieties.



**Figure 2.11** The number of plants with virus symptoms increases over time. Plants in plots with 200% nitrogen show significantly more severe symptoms.

Even though virus infection is not a part of this study, records were taken because so many of the plants were infected. In some plots as many as 80% of the plants showed symptoms of virus infection, shown in figure 2.12. These results will not be discussed in the conclusions. A possible reason for the differences in the field experiment could be that plants with high nitrogen content are more attractive to the vector and this could lead to more infection.



**Figure 2.12** A stunted tomato plant with severe symptoms of Begomovirus infection

## 2.8.2 Lab results

### *Bacterial wilt*

Soil samples: *Ralstonia solanacearum* was identified in all the soil samples collected from both Campos Azules and Sébaco, which show that the pathogen was present in the fields.

Plant samples: Both stem material from plants with symptoms and liquid extracted from stems were positive for *R. solanacearum* when grown on TZC agar, see figure 2.13.



**Figure 2.13** Colonies of *Ralstonia solanacearum* grown on TZC agar. Left: liquid extracted from stem. Right: sterilised stem parts on agar.

### *Powdery mildew*

*Leveillula taurica* is an obligate parasite and will not grow on PDA. Under examination with a microscope after incubation in a moist chamber, shown in figure 2.14, both conidia and cleistothecia from *L. taurica* were found. This proves that *L. taurica* was the causing agent of the powdery mildew that appeared in Sébaco.



**Figure 2.14** Tomato leaf with symptom of powdery mildew in moist chamber

### **Begomovirus**

Even though plants in the experiment showed severe and specific symptoms of Begomovirus infection, the virus was not identified in the lab. This was probably due to inactive enzymes used in the PCR.

## 2.9 Discussion

### *Bacterial wilt*

*Ralstonia solanacearum* is a major problem in the tropics. The bacteria may survive in the soil for a long time and every year with a host crop the pathogen accumulates in the field. Soil samples from both Campos Azules and Sébaco showed that the pathogen was present in the field.

In Campo Azules it was easy to see the disease progress and how more and more plants became infected over time. Last year's crop was also tomato and this is a possible reason for a large pathogen population. The plants were weakened from water deficiency and probably malnutrition since the soil was so dry and hard that the fertiliser granules didn't dissolve and the plants could not utilise the nutrients. The weak plants were very susceptible to bacterial wilt.

In Sébaco it seems as if all plants were infected at the same time and after that there was no increase of the disease. Agricultural practises taken place in the field, i.e. fertilisation and earthing up, causing root damage and create an opportunity for the pathogen to enter the host. The second fertilisation was done the 18<sup>th</sup> of February and soon after that the first plants showed wilting symptoms. Knowing the fact that the first symptoms normally appear 2-5 days after infection it seems likely that infection took place soon after this action. All the plants were fertilised in the same way: a small hole was made near the plant; the nutrient granules were poured in and covered. There is no reason to suspect that roots on plants in the plots with higher fertilisation levels were more damaged than other plants.

The results indicate that plants with more nitrogen are more susceptible to *R. solanacearum*. According to Watterson, 1986, infection of bacterial wilt increases with high nitrogen fertilisation, but later reports show opposite results. Experiments by Michel *et al* (1997) with soil amendments show a reduction of the pathogen if a mix of calcium oxide and urea is added to the soil a month prior to transplanting the tomatoes.

Another possible explanation to the differences in Sébaco is that the excessive use of urea caused toxic levels of ammonia which may have damaged the plants and made them more susceptible to infection by pathogens. This would support the hypothesis of contemporaneous fertilisation and infection by *R. solanacearum*.

There are some similarities between Fusarium wilt and bacterial wilt. Both diseases may infect the crop and kill off entire fields soon after the seedlings are planted in the field. Both cause rapid and fatal wilting also on more mature plants. The differences are that *Fusarium oxysporum* often infects one side of the plant first and yellowing proceeds wilting. *R. solanacearum*, on the other hand, causes wilting of the entire plant at once and there is no yellowing. Both pathogens survive for a long time in the soil and can not be treated with chemicals. Bacterial wilt is generally considered as the bigger problem in tropical regions. It is possible that Fusarium wilt also causes losses in Nicaragua. Another possibility is that there is a confusion about the diseases and that most of the wilting really is caused by *R. solanacearum* but is thought to be *F. oxysporum*.



### *Powdery mildew*

The symptoms caused by *Leveillula taurica* do not resemble other powdery mildew symptoms. *L. taurica* is not a very well known pathogen in Nicaragua and there were some problems to find and identify the fungus. When the first symptoms, small yellow spots, appeared in the field *Alternaria solani* was thought to be the cause, but as the disease developed and the characteristic concentric rings did not appear the doubts about which the pathogen was arose. After incubating infected leaf tissue in a moist chamber I was, with the help of a plant pathology professor at UNA, able to identify spores and cleistothecia from *L. taurica*.

There are some similarities between *A. solani* and *L. taurica*, they both cause leaf spots, yellow discoloration and necrosis. The spots from *L. taurica* are irregular. *L. taurica* does not affect the fruit and the damage is indirect with increased sunburn and insect damage. *A. solani* cause circular spots with concentric rings. This pathogen also develops spots with concentric rings on the fruit and causes great losses. Probably both pathogens are present in Nicaragua. Early blight is thought to be a bigger problem in the rainy season and in fields with sprinkler irrigation since spores from *A. solani* depend on high humidity and daily dew to germinate and the pathogen is more easily spread with rain. Powdery mildew on the other hand is limited by rain or sprinkler irrigation; the water destroys conidiophores and flushes spores on the leaf surface and in the air to the ground.

*L. taurica* is favoured by furrow irrigation and this system was used in the Sébaco experiment. When furrow irrigation is used, the relative humidity in the canopy is lower than with sprinkler systems and *L. taurica* is a fungus that spread in dry conditions. Experiments, Guzman-Plazola *et al.* (2003), have shown that spore germination is unlikely above 30°C in natural conditions and this may be a limiting factor for the disease in Nicaragua since the daytime temperature in the dry season often rises above 35-40°C.

Although maize was planted as a barrier around the tomato field it seems likely that the source of powdery mildew was a pepper field next to the tomatoes. Another possibility is that the pathogen survived on plant residues from the onions grown in the field previous to the tomatoes. Both peppers and onion are known hosts of *L. taurica* and according to Dixon (1978), *Leveillula taurica* has no host specificity and cross inoculation often appears in natural conditions.

*L. taurica* may appear at any time during the season, but powdery mildew is generally considered as a serious disease on mature plants and this agrees with our results. When the first symptoms appeared, the plants were already mature and had fruits. The symptoms spread quickly to new leaves and the yellow spots enlarged and necrosis developed. The results clearly show the progress of powdery mildew.

The fact that the new variety TY4 is more susceptible to powdery mildew could be of interest to farmers in Nicaragua. The results also show significantly lower infection in plots with 200% nitrogen fertilisation. A possible reason could be that these plants are stronger and therefore able to resist infection. *L. taurica* is known to be a weak pathogen that mainly infects stressed or weak plants. There is probably no practical use of this information since the slightly higher yield from these plants could never compensate for the extra cost of fertilisation.

### *General discussion*

There is no simple solution of the complex situation with diseases infection tomatoes. Research teams all over the world try to develop resistant tomato varieties. This is one way of trying to control the disease or at least reduce the losses. Plant breeding is very expensive and in a poor country like Nicaragua it is more realistic to find other ways to control diseases. One example is to make change in the cropping system so that epidemics are avoided or stopped before the cause great damages.

Today there is a big gap between the knowledge of farmers, advisors and researchers. It seems as if symptoms in the field are often misinterpreted and this may lead the farmers to take the wrong actions in the battle against diseases. Farmers tend to put all their trust into chemicals and when they are not used properly the farmers end up losing money and not getting rid of the problem. In some cases, like bacterial wilt and Fusarium wilt, the best way to protect the crop is to avoid fields with known presence of pathogen or use long crop rotation. Other diseases such as early blight and powdery mildew may be treated successfully with well-timed pesticide sprayings.

The most important conclusion of this thesis is that there is a great need for an inventory of diseases that are current in Nicaragua and in which season and region they are most common. It is not enough to identify the disease by plant symptoms; it is absolutely necessary to make a profound lab analysis to identify the pathogen and know the disease!

## 2.10 Referenses

### 2.10.1 Books and articles

- Agrios, G. N. 1997. *Plant Pathology* p. 246-247, 252, 274-278, 300-302, 343-346, 430-433. Academic press, California.
- Aust, H. J. and v. Hoyningen-Huene, J. 1986. Microclimate in relation to epidemics of powdery mildew. *Annual Review of Phytopathology*. 24:491-510
- Chambers, L. and Corden, M. E. 1963. Semiography of *Fusarium* wilt of tomato. *Phytopathology* 53, 1006-1010
- Correll, J. C., Gordon, T. R. and Elliot, V. J. 1987. Host Range, Specificity and Biometrical Measurements of *Leveillula taurica* in California. *Plant Disease* 71:248-251
- Correll, J. C., Gordon, T. R. and Elliott, V. J. 1988. Powdery mildew of tomato: The effect of planting date and triadimefon on disease onset, progress, incidence and severity. *Phytopathology* 78:512-519
- Correll, J. C. 1991. The relationship between formae speciales, races and vegetative groups in *Fusarium oxysporum*. *Phytopathology* 81, 1061-1063
- Dixon, G. R. 1978. Vegetable and allied crops. In: Spencer, D. M. *The powdery mildews*. Academic press, London
- Glasscock, H. H. and Ware, W. M. 1944. Alternaria blight of tomatoes. *Agriculture* 51, 417-420
- Guzman-Plazola, R. A., Davis, R. M. and Marois, J. J. 2003. Effects of relative humidity and high temperature on spore germination of tomato powdery mildew (*Leveillula taurica*). *Crop protection* 22:1157-1168
- Hartman, G. L. and Elphinstone, J. G. 1994. Advances in the control of *Pseudomonas solanacearum* Race 1 in major food crops. In: Hayward, A. C. and Hartman, G. L. *Bacterial Wilt The Disease and its Causative Agent, Pseudomonas solanacearum*. CAB International, Wallingford
- Holliday, P. 1980. *Fungus diseases of tropical crops* p. 21-23, 175-178, 252-253. Cambridge university press, Cambridge.
- Instituto Nicaragüense de Tecnología Agropecuaria (INTA). 2002. *El Cultivo del Tomate, Guía Tecnología 22*
- James, W. C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. *Can. Plant. Dis. Surv.* Vol. 51, no 2.
- Jones, J. B., Jones, J. P., Stall, R. E. and Zitter, T. A. 1997. *Compendium of Tomato Diseases* p. 1-8, 13-15, 28-29. APS press.
- Jones, W. B. and Thomson, S. V. 1987. Source of inoculum, yield and quality of tomato as affected by *Leveillula taurica*. *Plant disease* 71:266-268
- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony apperance on tetrazolium medium. *Phytopathology* 44, 393-395
- Kelman, A., Hartman, G. L. and Hayward, A. C. 1994. Introduction. In: Hayward, A. C. and Hartman, G. L. *Bacterial Wilt The Disease and its Causative Agent, Pseudomonas solanacearum*. CAB International, Wallingford
- Kiss, L., Cook, R. T. A., Saenz, G. S., Cunnington, J. H., Takamatsu, S., Pascoe, I., Bardin, M., Nicot, P. C., Sato, Y. and Rossman, A. Y. 2001. Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. nov. and *O. lycopersici*, infecting tomato in different parts of the world. *Mycol. Res.* 105: 684-697
- Mace, M. E., Bell, A. A. and Beckman, C. H. 1981. *Fungal Wilt Diseases of Plants*. Academic press, London.
- MacKenzie, D. R. 1981. Association of potato early blight, nitrogen fertilizer and potato yield. *Plant Disease* 65, 757-577.

- MacNab, A. A., Sherf, A. F. and Springer, J. K. 1983. *Identifying diseases of vegetables*. The Pennsylvania State University Press, University Park.
- Maiero, M., Bean, G. A. and Ng, T. J. 1991. Toxin production by *Alternaria solani* and its related phytotoxicity to tomato breeding lines. *Phytopathology* 81, 1030-1033.
- Martinez, J. 2002. Tomato market, Central America and Caribbean. Tomato Breeders' Round Table 2001, *Horticultural and Crop Science Series* no. 724.
- Michel, V. V., Wang, J. F., Midmore, D. J and Hartman, G. L. 1997. Effects of intercropping and soil amendment with urea and calcium oxide on the incidence of bacterial wilt of tomato and survival of soil-borne *Pseudomonas solanacearum* in Taiwan. *Plant Pathology* 46:600-610
- Moore, W. D. 1942. Some factors affecting the infection of tomato seedlings by *Alternaria solani*. *Phytopathology* 32, 399-403.
- Nelson, P. E., Toussoun, T. A. and Cook, R. J. 1981. *Fusarium: Diseases, Biology and Taxonomy*. The Pennsylvania State University Press, University Park.
- Pérez, J., Hurtado, G., Aparicio, V., Argueta, Q. and Larín, M. A. 2003. *Guía Técnica: Cultivo de tomate* p.33-35. Centro Nacional de Tecnología Agropecuaria y Forestal (CENTA), San Salvador
- Prior, P., Grimault, V., Schmith, J., 1994, Resistance to Bacterial Wilt (*Pseudomonas solanacearum*) in tomato: Present status and prospects. In: Edt. Hayward, A. C., Hartman, G. L. *Bacterial Wilt. The Diseases and its Causative Agent, Pseudomonas solanacearum*. Cab International
- Rojas, A., Kvarnheden, A. and Valkonen J. P. T. 2000. Geminiviruses infecting tomato crops in Nicaragua. *Plant Disease* 84:843-846
- Thomas, H. R. 1948. Effect of nitrogen, phosphorus and potassium on susceptibility of tomatoes to *Alternaria solani*. *Journal of Agricultural Research* 76, 289-306.
- Watterson, J. C. 1986. Diseases. In: Atherton, J. G. *The Tomato Crop* p. 443-445, 453, 457-460.
- Whipps, J. M., Budge, S. P and Fenlon, J. S. 1998. Characteristics and host range of tomato powdery mildew. *Plant Pathology* 47:36-48
- Yabuuchi, E., Kasako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T. and Arakawa, M. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiology and immunology* 36:1251-1275
- Yabuuchi, E., Yoshimasa, K., Yano, I., Hotta, H. and Nishiuchi, Y. 1996. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology and immunology* 39:897-904
- Young, N. D and Danesh, D. 1994. Understanding bacterial wilt resistance in tomato through the use of DNA genetic markers. In: Hayward, A. C. and Hartman, G. L. *Bacterial Wilt The Disease and its Causative Agent, Pseudomonas solanacearum*. Cab international, Wallingford

## 2.10.2 Internet

The British Society for Plant Pathology: <http://www.bspp.org.uk/>

### **3 TOMATO YIELD AND QUALITY IN RELATION TO NITROGEN FERTILIZATION AND DISEASES**

Anna Berlin

#### **Abstract**

During the last decade, Nicaraguan farmers have struggled with their tomato (*Lycopersicon esculentum* Mill.) production due to heavy infections of Begomovirus, and other management methods have been set aside. The aim of this study was to evaluate the effects of nitrogen fertilization on the yield and quality of the tomato fruits and to evaluate the relations between the incidence of diseases and nitrogen fertilization on one hand and fruit quality on the other. A field experiment was carried out in the Sébaco valley, with five fertilizing levels (0, 50, 100, 150, and 200 % of standard nitrogen fertilization in the area) and two varieties (TY4 & TY13). During the vegetative growth, the nitrogen content and biomass were measured. The field was harvested at five occasions, dividing the harvest in good (marketable) and bad (not marketable) fractions. To evaluate the fruit quality, taste tests were carried out on the two first harvests and laboratory analyses of acid (% titratable acids) and sugar content (°Brix) were measured on the second harvest. The 0 % nitrogen fertilizing level differed significantly in foliar nitrogen content from the other (50, 100, 150 and 200 % of standard nitrogen fertilization). The difference was probably due to both lack of nitrogen and potassium. The total harvest levels of the experiment were in similar range as the average harvest for the region (12-18 t/ha), but the marketable fraction was only between 39 and 50 % of the total yield. The high not marketable fraction was probably due to poor pollination and fruit set caused by high temperatures and heavy infections of Begomovirus and other diseases. In the taste test, TY13 was the most preferred variety, independent of fertilizing level. It is suggested to stop growing tomatoes in the Sébaco valley during the hot and dry period. That would lower the risk for physiological deficiencies and break the vector chain of Begomovirus and the fungal diseases and increase the fraction of marketable yield in the region

#### **Keywords**

Tomato, *Lycopersicon esculentum* Mill., nitrogen fertilization, quality, yield, taste test, Nicaragua

### 3.1 Introduction

During the last decade, Nicaraguan farmers have struggled with their tomato production due to heavy infections of Begomovirus (Rojas, 2003 personal communication). Other management methods, such as control of fungal diseases, fertilization and quality management of the tomato production have been set aside. The objects of investigation in this study were to study the yield and quality of the yield at different nitrogen fertilization levels.

#### 3.1.2 Quality of tomatoes

##### *What is quality?*

The consumers define quality (Auerswald *et al.*, 1999). Therefore, to be able to produce a product with high quality, it is not only important to know the quantitative and qualitative sensory changes of a product but also the consumers' acceptance of it (Auerswald *et al.*, 1999), and especially the relations between the two. For tomatoes, the most important quality factors for consumers' acceptance are that they look and taste good, are firm and have a good nutrient value (Grierson and Kader, 1986). Important quality factors for tomatoes in Nicaragua are size, color and taste (Rojas, 2003 personal communication).

The external appearance of tomato fruits strongly affects the consumers' first impression. The color and firmness of the tomatoes are the most important factors when the consumers purchase tomatoes (Batu, 2004). In a consumers test made by Batu (2004), the most accepted tomatoes were the slightly soft to very firm tomatoes.

##### *Taste*

The taste of the tomato fruits depends on the variety, state of maturity at harvest, amount of nutrient during growth, environmental stress and water management (Hobson, 1988; Baldwin *et al.*, 1991). Field grown tomatoes normally have more flavor than tomatoes grown in glasshouses (Hobson, 1988). The degree of maturity at harvest is very important for the fruit quality and the composition of the tomato. Tomatoes ripened on the plant have an overall better quality and taste than room-ripened tomatoes, because tomatoes accumulate acids and sugars during ripening on the vine (Grierson and Kader, 1986). The more intensive the tomato-like flavor and aftertaste and the less bitter the fruits, the higher is the acceptance of the consumers (Auerswald *et al.*, 1999; Baldwin *et al.*, 1991).

The most significant chemical compounds for good taste are high levels of organic acids (malic and citric acid in the gel around the seeds) and sugars (fructose and glucose in the fruit walls) in the tomato (Grierson and Kader, 1986). More than 400 volatile compounds (Baldwin *et al.*, 1991) are responsible for the typical tomato flavor and their interaction with the sugars and acids are also very important for the fruit quality (Malundo, 1995). The amount of acid affects the taste more than the amount of sugar (Löfkvist, 2000). Sugars and organic acids comprise most of the dry matter content in tomatoes, and the amount of reduced sugars generally correlates with soluble solids content (Malundo, 1995; Jones and Scott, 1984). The sugars account for 75 % of the water-soluble solids in a ripe tomato fruit (Ho, 1996).

An important external factor that determines the taste is light. The carbohydrate content, which is the source to sugar in the fruit, increases with the amount of sunlight. And the taste quality increases with the sugar content (Hobson, 1988). The citric acid dominates over the malic acid (Malundo, 1995) and the acid concentration is strongly affected by the potassium

fertilization and accumulation in the fruit (Kinet and Peet, 1997; Ho, 1996). Generally, an increase of both acid and sugar content will increase the overall flavor intensity, but it does not have to improve the flavor quality (Malundo, 1995; Jones and Scott, 1984).

The tomato skin does not have any stomata or lenticels, therefore the volatile compounds can not be lost to the atmosphere (Hobson, 1988). The size affects the taste indirectly, small fruits have more walls that contain more sugar and will be sweeter than larger fruits and vice versa (Grierson and Kader, 1986).

### 3.1.3 Tomato growth and quality establishment

#### *Growth*

The growth and development of the tomato plant and fruit depends on many different factors. The most important are sun irradiance, nutrient and water supply. The total uptake of nutrients varies with temperature, light intensity, health of the plant, especially the root system, aeration and humidity (Adams, 1986). Nitrogen (N), phosphorus (P) and potassium (K) are in quantitative terms the most important minerals for the tomato fruit as they account for more than 90 % of the mineral content (Kinet and Peet, 1997).

The vegetative growth is often very short, since floral transition is initiated when the third leaf is expanding. After 6-11 leaves the plant has developed its first cluster of flowers (Kinet and Peet, 1997). Normally in determinate plants used in field production, only two or three clusters of flowers separated by one or more leaves are developed during the growth (Kinet and Peet, 1997).

A massive cell expansion occurs during growth of the tomato fruit until it reaches its green mature stage. Then the degradation of cell walls starts and it results in a soft and juicy texture. 2-3 days after the maturity of the green fruit, the color starts to change from green to yellow, orange and finally red. The red color of a mature fruit is due to the destruction of chlorophyll and the accumulation of the carotenoids  $\beta$ -carotene and lycopene when the chloroplasts are transformed to chromoplasts (Grierson and Kader, 1986). About 10 days after the start of the color change, a thin layer is developed between the calyx and the fruit which cuts the assimilate transport to the fruit. (Kinet and Peet, 1997) The size of the fruit depends on the number of carpel's in the ovary, the number of seeds, the position of the fruit and environmental conditions (Kinet and Peet, 1997).

#### *Temperature*

The optimum temperature for vegetative growth is 18°C to 25°C during the day and 10°C to 13°C during the night (Ho, 1996). The most heat sensitive stages for the tomato plant are flowering and fruit set, although the impact of heat stress is not limited to only these processes (Song, 1999). High temperatures at optimal water conditions can increase the assimilation of carbon, which increases the fruit growth. However, high temperatures also increase the canopy transpiration and can induce water stress, resulting in lower fruit volume growth (Ho, 1996). The long-term adaptive responses to high temperature are reduced duration of fruit growth and reduced final fruit size (Ho, 1996). This means that the beneficial effect of high temperatures will only occur when the assimilate supply is unlimited and water stress is prevented (Ho, 1996).

High temperature can also affect the tomato yield negatively by inhibiting the pollination process. The tomato flower is self-pollinated and the flowers open in the morning and the stigmata are receptive from 16 to 18 h before up to 6 days after anthesis (Kinet and Peet, 1997; Kaul, 1991). Extreme temperatures above 37°C or below 5°C limit the pollen grain germination and inhibit the tube growth of the pollen grain. Periods of four hours with temperatures above 40°C, two to three days after pollination, have been showed to damage the pro-embryo and cause degradation of the endosperm (Kinet and Peet, 1997). High temperature will result in fruit abnormalities such as green locules, probably due to failure of viable seed formation (Song *et al.*, 1999).

### *Nitrogen*

In most crops, the nitrogen concentration decreases with increasing plant mass (Lemaire and Gastal, 1997; Greenwood *et al.*, 1990). The plant nitrogen concentration varies according to the proportion of the metabolic and structural compartments. The metabolic compartments are physiologically active in growth such as leaves and fine roots and the structural compartments are holding the foliage and storage tissues such as stems. The metabolic parts are higher in nitrogen content than the structural parts (Tei *et al.*, 2002).

The growth is directly associated with the relative leaf area and to some extent to the metabolic component of the plant (Tei *et al.*, 2002). There is a close interaction between nitrogen availability, leaf development, light interception, growth and nitrogen uptake (Tei *et al.*, 2002). Nitrogen supply slightly affects the average weight of red fruits but largely increases the number of fruits per plant (Tei *et al.*, 2002; Colla *et al.*, 2001). A high nitrogen level promotes leaf area expansion and increase leaf area index (LAI), but also initiate the leaf senescence that lowers the green area (Tei *et al.*, 2002). A high LAI increases the light interception and the photosynthetic activity by larger leaves, which increases the crop growth (Tei *et al.*, 2002). But a high LAI also causes self-shading, which in turn limits the beneficial effect on growth of increased leaf area (Dumas *et al.*, 2003). A low level of nitrogen and calcium reduces the vegetative growth, and lack of phosphorus reduces the reproductive growth (Ho, 1996).

The fruits become the main sink and the allocation to stems and leaves progressively drops 40 days (indeterminate variety) after transplanting (Tei *et al.*, 2002). This means that the first part of plant development is crucial for the final yield, since it affects the development of the photosynthetic parts of the plant during fruit growth and ripening (Tei *et al.*, 2002; Ho, 1996). The relative growth rate of nitrogen-limited plants is linearly related to the nitrogen concentration within the plant (Tei *et al.*, 2002; Greenwood *et al.*, 1991). The nitrogen also affects the ripening of the fruit; more fruits will be unevenly ripe at low nitrogen concentrations than at high (Grierson and Kader, 1986).

### *Ripening*

During ripening of the fruit the composition, color and texture of the tomato changes. The sugar and acids levels also change, as does the composition of the volatile compounds. The color development rate of tomatoes increases with increasing maturation (Batu, 2004). Ripening is mainly influenced by genotype, growing conditions, diseases and post-harvest treatments (Grierson and Kader, 1986).



Ripening depends on a wide range of separate degradative and synthetic reactions that are highly coordinated and regulated by plant hormones (Grierson and Kader, 1986) and is triggered by the rise in endogenous ethylene production. Ethylene regulates several ripening-related genes in tomato, but other growth regulators are also involved. Ripening is not influenced much by light, however not optimal temperatures will lower the ripening rate (Kinet and Peet, 1997).

### *Yield*

Tomato productivity is limited by temperature, light, nutrient and water supply and lack of protection from weeds and other pests. The yield depends on both the number and the weight of individual fruits, which is due to fruit set and the development of the fruit (Kinet and Peet, 1997). The nutritional need of determinate tomato plants reaches a maximum during the flowering, fruit setting and early fruit growth period (Fisher *et al.*, 2002; Dumas, 1990) and the fruit yield peaks over a short period of time in determinate field grown tomato varieties. The overall and most difficult factor to get high yields and good quality of the tomatoes is the water management (Kinet and Peet, 1997).

High temperature, high nitrogen and low light intensity are factors that encourage vegetative growth more than reproductive growth, especially in combination (Kinet and Peet, 1997). Also poor pollination, which leads to flower abscission often gives yield and quality problems due to the physiological disorders (Kinet and Peet, 1997). There is a negative correlation between fruit yield and quality of the fruit (Grierson and Kader, 1986).

#### 3.1.4 Diseases affecting the fruit quality

There are several diseases that will lower the quality of the fruit and the acceptance by consumers (see figure 3.1). The symptoms are often similar for different diseases and it can be difficult to distinguish what causes the damage. The disease symptoms can be parasitic diseases such as fungi, bacteria, viruses or nematodes or non-parasitic diseases caused by extremes in heat, light, soil moisture, nutrient imbalances, herbicide or pesticide damages (Jones *et al.*, 1997). For example, unevenly ripening with yellow



**Figure 3.1** Tomatoes with different disease symptoms and physiological disorders

patches on the mature fruit are symptoms for either virus infection, direct sunlight exposure of the green fruit, or nutritional deficiency.

In this study, the tomato crop was affected by Begomoviruses, Powdery mildew of tomato and Southern Bacterial Wilt of tomato (Eitrem, 2005; in this issue). Also the physical disorder Blossom-end rot was present in the field.

### *Begomoviruses*

The symptoms for Begomoviruses differ with environmental conditions, virus and strain, variety and plant age at the time of infection (Polston and Anderson, 1997). If the plants are

infected in early stages, they will be bushy and dwarfed without any fruit. Later infections results in reduced new growth and fruit set and the fruits will ripe unevenly (Hull, 2001). Islam *et al.* (2003) wrote that the total nitrogen and protein content are higher in infected fruits, and sugar content is lower, compared to non-infected fruits. Their investigations also indicated decrease in fruit weight and number of fruits per plant of infected plants, which causes an overall yield reduction.

*Powdery mildew of tomato, Leveillula taurica (Lév.) Arn.*

*L. taurica* is one of three fungi that cause mildew of tomato (Whipps, 1998). Powdery mildew increases defoliation that exposes the fruits to direct sunlight and insect pest, which may lower the quality of the fruit (Correl *et al.*, 1988).

*Southern Bacterial Wilt of tomato, Ralstonia solanacearum*

*R. solanacearum* is a bacterial disease. It appears as suddenly wilting, and the tomato plants finally wilt permanently and die (Agrios, 1997). The fruits of an infected plant are not affected, but the total yield is reduced due to the shorter period of growth.

*Blossom-end rot, BER*

Blossom-end rot is a physiological disorder caused by local calcium deficiency. It first appears as areas of white or brown tissue around the blossom scar of the green tomato fruit. When the spot grows bigger, the affected tissue dries out and turns light to dark brown and becomes a sunken, well-defined leathery spot (Kinet and Peet, 1997). There are two main factors causing BER: inadequate supply of calcium related to reduced xylem transport and accelerated cell enlargement in the distal reproduction tissues (Ho, 1996). BER does not affect the amount of yield, but the leathery spot lowers the quality of the fruit and the yield will be un-marketable.

### 3.1.4 Objectives

During the last decade, Nicaraguan agricultural research has been focused on the effects of Begomovirus on tomato production. No research has been published in the region about the effects of nitrogen fertilization, nor the quality and yield of the tomato fruits (Rojas, 2004 personal communication).

The objectives of this study were to quantify the effects of nitrogen fertilization on yield and quality of field grown tomatoes in Nicaragua. Previous studies provide us a broad knowledge about tomato growth, quality and yield (Grieson and Kader, 1984; Ho, 1996 and more). This study aims to evaluate the conditions for the Nicaraguan tomato production.

*Objectives of the project*

1. Evaluate the effects of nitrogen fertilization on yield and quality of tomato fruits (taste, acid and sugar content).
2. Evaluate the relations between the incidence of fungal and virus diseases, nitrogen fertilization and the fruit quality.

### 3.2 Material and Methods

#### 3.2.1 Experimental design

One field experiment was carried out during the dry season at Sébaco experimental station in Nicaragua. Sébaco is a valley with intensive agriculture practice in the lowlands, 470 meters above sea-level. The traditional crops in the area are rice, onion, pepper and tomato. The experimental field was prepared by workers at the experimental station. Furrow irrigation was used and the last crop before the tomatoes was onion. The field experiment was completely randomized in a split-plot design (figure 3.2). All plots were treated several times with insecticide during the experimental time to prevent virus infection by white flies.

200 % TY13   TY 4	50 % TY4   TY13	150 % TY4   TY13	0 % TY4   TY13	100 % TY13   TY4
200% TY13   TY4	100 % TY4   TY13	0 % TY13   TY4	50 % TY4   TY13	150 % TY4   TY13
50 % TY4   TY13	200 % TY13   TY4	100 % TY4   TY13	150 % TY4   TY13	0 % TY4   TY13
200 % TY4   TY13	100 % TY13   TY4	150 % TY4   TY13	0 % TY4   TY13	50 % TY13   TY4

**Figure 3.2** Design of filed experiment. 100 % correspond to the standard amount of nitrogen fertilizer used in tomato fields. 0 % is no nitrogen, 50 % is half of the standard amount, 150 % one and a half standard amount and 200 % doubled the standard amount of nitrogen. The two varieties were placed randomly in each fertilizing plot, as shown in the figure.

The plant material used in the field experiment consisted of two varieties of tomato; TY4 and TY13. The varieties origin from the variety TY that was imported from Israel to Nicaragua in 1992. The new varieties TY4 and TY13 are the result of a breeding program at the experimental station in Sébaco (Rojas, 2004 personal communication).

The tomato plants were sown the 23<sup>rd</sup> of December 2003 in screen houses at Sébaco experimental station and replanted into the field 29 days after sowing (21<sup>st</sup> of January 2004).

The plants were fertilized at two occasions, 2 (23<sup>rd</sup> of January) and 28 (18<sup>th</sup> of February) days after transplantation. The standard amount of fertilizer applied in tomato fields in Nicaragua is 400 kg NPK (12-30-10) per hectare and 260 kg urea (46% N) per hectare, and is fertilized at two occasions (Rojas, 2004 personal communication). 0 %, 50 %, 100 %, 150 % and 200 % of the standard fertilizing amount of nitrogen were added and the amount of phosphorus and potassium were the same in all levels except for the 0 %. The recalculated in amount available nutrients applied is shown in table 3.1. Only 15 kg potassium per hectare and no phosphorus were added to the 0 % nitrogen fertilizing level.

**Table 3.1** Amount of applied nutrients at the different fertilizing levels in kg N, P and K per hectare, respectively

<b>Fertilization</b>	<b>N</b>	<b>P</b>	<b>K</b>
<b>0 %</b>	0	0	15
<b>50 %</b>	80	50	30
<b>100 %</b>	160	50	30
<b>150 %</b>	240	50	30
<b>200 %</b>	320	50	30

The climatic condition during the growth period is shown in table 3.2. The data is from 2003, but the annual difference is very small and for that reason this data are similar for 2004. The night temperature during the period was 22 - 26°C (Rojas, 2004 personal communication).

**Table 3.2** Climate conditions at Sébaco experimental station 2003, monthly average

<b>Month</b>	<b>Temp. (°C)</b>	<b>Relative humidity (%)</b>	<b>Precipitation (mm)</b>	<b>Evaporation (mm)</b>	<b>Wind velocity (m/s)</b>
<b>January</b>	25.0	69	0.0	7.3	6.0
<b>February</b>	25.6	68	0.0	7.9	4.5
<b>March</b>	26.4	67	0.6	8.7	4.3
<b>April</b>	27.6	66	0.0	8.9	3.5
<b>May</b>	27.7	69	4.2	5.8	3.2

### 3.2.2 Nitrogen content

An N-tester (Yara Ltd.) was used to estimate the amount of nitrogen in the plants during growth. The N-tester measures the transmission of emitted radiation through a leaf. The value (KS-value) is interpreted as being proportional to the chlorophyll content and the unit is not expressed explicitly. One KS-value is a mean of thirty records. A high KS-value indicates high nitrogen concentration in the plant tissue (Yara homepage, 2005). Each record was made on the tip of the third fully developed leaf on healthy looking plants in each plot. One KS-value was taken for each plot.

KS-value data were taken weekly for six weeks during the vegetative growth. At the first two occasions, measurements were taken in plots fertilized with 50 %, 100 % and 150 % nitrogen. The following weeks, data from all levels of nitrogen fertilizations were collected. Measurements were only taken in the variety TY13.

### 3.2.3 Dry matter

Plant samples were taken for measurement of dry matter at two occasions, 34 (24<sup>th</sup> of February) and 65 (26<sup>th</sup> of March) days after transplantations. The plant material was dried at 60°C for 72 hours and weighed. One plant from each plot was collected at the first occasion. At the second occasion, only plants from two blocks were dried. Measurements were only taken in the variety TY13.

### 3.2.4 Disease and incidence of disease

The analyses and data of which diseases were present in the field were taken from project A (Eitrem, 2005; in this issue). The incidences of disease (severity and incidence of virus and fungal diseases and wilted plants) were collected in field and statistically analyzed by analysis

of variance (SAS version V8). 70 days after transplantation, all fruits on 13 plants per plot were examined to evaluate the on-plant fruit quality. The fruits were categorized in total number of fruits, fruits with Blossom-end rot, number of deformed fruits and number of healthy fruits. The deformed fruits were both deformed fruits due to poor pollination and small fruits due to the virus infection.

### 3.2.5 Harvest

Tomato fruits from all plants in all plots were harvested once a week for five weeks. All light to dark red fruits were harvested. The fruits were split in two fractions, one good (marketable) and one bad (not marketable) and weighed. The healthy looking fruits, without deformations or pests were sorted into the marketable fraction, and all fruits with defects were sorted into the not marketable fraction.

Fruits from the first harvest at day 65 after transplantation and second harvest at day 70 were sorted. The tomatoes were collected just before the last stage of ripening for taste test and laboratory analyses. The second harvest was analyzed for acid and sugar content. The size and weight of the fruits were measured and visual controls of the fruits were made.

Two students from Universidad Nacional Agraria, Managua (UNA) carried out the third to fifth harvests. The harvests were sorted into one marketable and one not marketable fraction and weighed.

### 3.2.6 Taste test

The taste test was carried out at two occasions, using the first and second harvest. The first harvest was very small and many fruits showed virus symptoms. Therefore all fruits of one variety, with or without virus symptom were collected. The panel was given four samples for the first taste test, both varieties with respectively without virus symptoms. For the second taste test, only healthy looking fruits were tested. For this test, five different samples were given to the panel. The tomatoes for the first harvest were stored for three days and the second harvest was stored over night, both at room temperature (30°C). In the taste test, teachers, employees and students of the Universidad Nacional Agraria, Managua were used as a panel. At the first occasion 38 persons, and at the second, 33 persons tasted the tomatoes.

The panelists were given one evaluation sheet (appendix 2), a pencil and a glass of water. One or two fruits from each group of tomato samples were exposed on white plates. First, they answered three questions about the outer quality. A six-point scale was used: the visual



**Figure 3.3** For the taste tests, the tomatoes were placed on a numbered plate and one plate was given to each panellist

quality for color (6 even red – 1 mixed red and green), the shape (6 regular – 1 irregular) and the firmness (6 unripe – 1 overripe) of the fruits.

After evaluating the outer quality of the tomato, they were given plates with four or five pieces of tomato samples (figure 3.3). The samples were randomly numbered within the two tests, presented on identical white plates and served at room temperature. Each taste test sample was one or two segments of a tomato. The panelist evaluated the taste of the tomato on a six-point scale. The attributes and scales used were: mouth-feel (6 soft and juicy – 1 hard and dry), sweetness (6 very sweet – 1 not sweet), acidity (6 very acidic – 1 not acidic), intensity (6 ideal flavor for tomatoes – 1 not characteristic for tomatoes), lasting (6 long – 1 short) and over all acceptance (6 like it very much – 1 dislike it strongly).

### 3.2.7 Sugar and acid analysis

On the second harvest, chemical analyses were carried out on at least three fruits from each variety and fertilizing level. The tomatoes were crushed into a liquid solution for about 1 minute with a mixer and filtered through two Melitta filters placed in a funnel over an E-flask. The clear solution was used for chemical analyses.

The total titratable acidity was measured by diluting 5 ml of the tomato solution in 20 ml distilled water and titrated to pH 8.1 with 0.1M NaOH. The pH of the solution was measured and the amount of acid used was recorded and the total acidity (in % citric acid) was calculated.

The volume of NaOH titrated (V) was multiplied by the normality of NaOH (N) and the milliequivalents of acid (mEqwt = 0,064 for citric acid). This number was divided with the volume in ml or weight in grams of sample (Y) and everything was multiplied with 100 to get % acid in sample (Gould, 1983):

$$\% \text{ acid in sample} = \frac{V * N * mEqwt}{Y} * 100$$

The sugar content (Brix value) was measured with a hand held refractometer (type N- $\alpha$ 1, Atago Co., Ltd) with 0.5 % accuracy, using the same solution as above. The room temperature was measured for each measurement and the corrections of the Brix values were calculated.

All results were statistically analyzed by analysis of variance (Proc GLM function in SAS V8.2; Bonferroni t test at  $\alpha = 0.5$ ).

### 3.3 Results

The fungal diseases (*L. taurica* and *R. solanacearum*) that infected the tomato plants in Sébaco were not the expected ones, and they have not been reported in the area before (Eitrem, 2005; in this issue; Rojas, 2004, personal communication). The tomato plants were also severely infected with Begomovirus. Up to 80 % of the plants showed symptoms (Eitrem, 2005; in this issue).

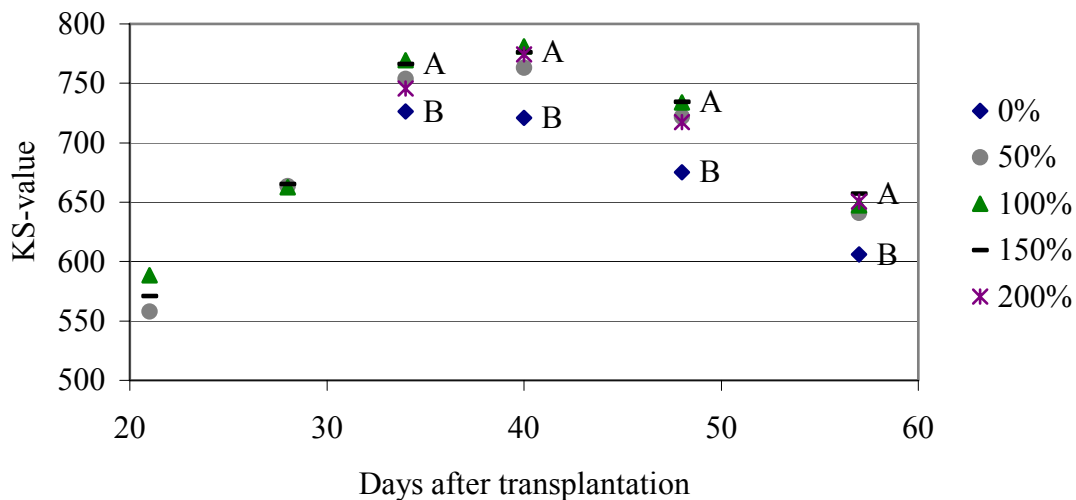
#### 3.6.1 Dry matter and nitrogen content

The tomato plants developed normally during the growing period (table 3.3).

**Table 3.3** Development stages of the tomatoes after 21, 28, 34, 41, 49 and 58 days after transplantation.

Day after transplantation	Development stage
21	7-8 leaves, bud forming, > 5 % flowering
28	10-12 leaves, most flowering, start of fruit set
34	All flowering and branching, about 50 % fruit set
41	50 cm high plants
49	60 cm high plants, 100 % fruits set
58	60-70 cm high plants, fruits starting to mature

KS-value data for 0 % and 200 % were not taken at the first two occasions. Only the KS-value from 0 % is significantly lower than the other treatments (figure 3.4). The fruits become the main sink after 40 days.



**Figure 3.4** KS-value at different occasions during the vegetative growth and fertilizing levels. 0 % (B) is have a significantly KS-value than the other fertilizing levels (A)

Data from dry matter measurement are shown in table 3.4. There is no significance of the dry matter between the different fertilizing levels. The high value for the 100 % level 65 days after transplantation is uncertain due to lack of replicates.

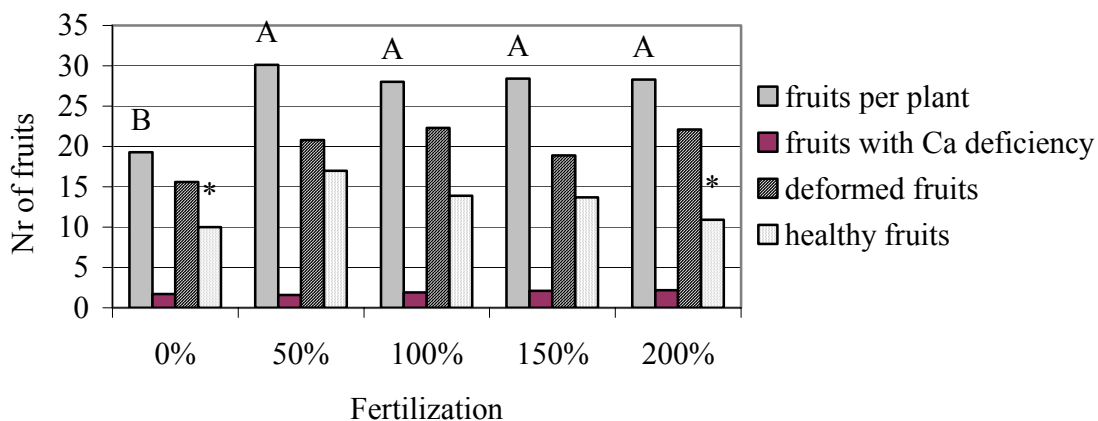
**Table 3.4** Dry matter in average gram per plant for the two sampling dates

<b>Fertilization</b>	<b>34 days after transplantation</b>	<b>65 days after transplantation</b>
<b>0%</b>	13.5	99.4
<b>50 %</b>	17.1	106.5
<b>100 %</b>	18.8	160.4
<b>150 %</b>	29.0	105.1
<b>200 %</b>	25.8	91.9

### 3.6.2 Disease and severity of disease

The use of NPK 12-30-10, with the high amount of phosphorus was believed to impede the development of the Begomovirus infection (Rojas, personal communication 2003). Even though the field was fertilized with a lot of phosphorus and tolerant varieties were used, most of the tomato plants became infected with Begomovirus. In this experiment, the number of fruits on plants infected with Begomovirus increased, and the size of the fruits decreased (data not shown).

Severity of fruits affected of infection was expressed in numbers of non-healthy fruits on the plant before harvest, 70 days after transplantation. The 0 % treatment had a significantly lower number of total fruits per plant than all other fertilizing levels. 0 % and 200 % nitrogen fertilization levels resulted in significantly lower number of healthy fruits per plant than the other levels of fertilizer (figure 3.5). Most of the deformed fruits were very small and affected by virus, and some were bigger and probably deformed because of poor pollination. There were also fruits affected by larvae (not characterized).



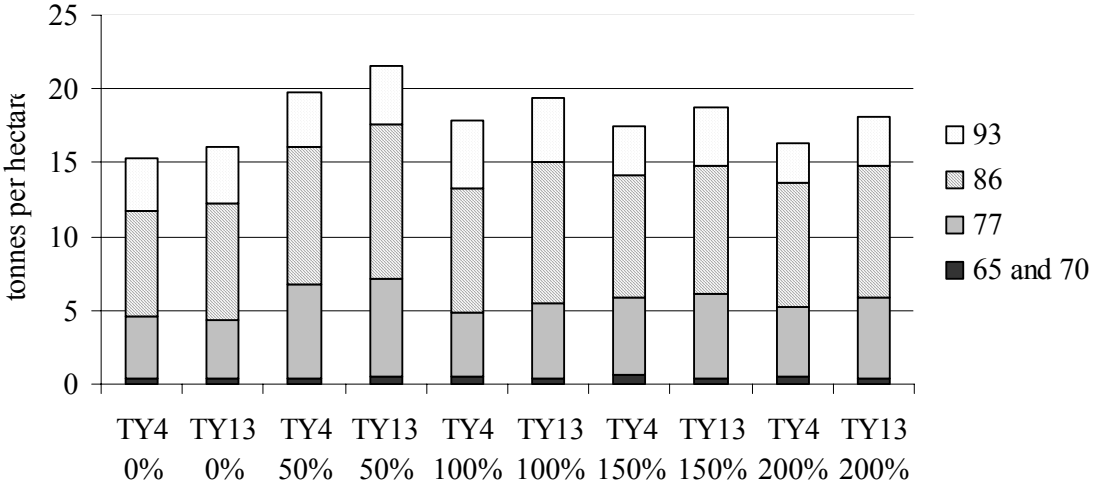
**Figure 3.5** Total fruits per plant and affected and healthy fruits before harvest at 0 %, 50 %, 100 %, 150 % and 200 % of the standard nitrogen fertilization level 70 days after transplantation. Some fruits are both deformed and have calcium deficiency expressed as Blossom-end rot. There was a significantly lower number of healthy fruits in 0 % and 200 % (\*) and significantly lower number of total fruits at 0 % nitrogen fertilization (B).

### 3.6.3 Harvest

The first and second harvests were very small, never higher average than 300 kg per hectare (0.03 kg per m<sup>2</sup>) and the third to fifth harvests were bigger. The accumulated harvests are shown in figure 3.6. The high harvest the 86<sup>th</sup> day after transplantation is probably due to the longer interval between the harvests. TY13 has a higher average harvest than TY4 in all



fertilization levels. This might be due to the fact that TY4 had a significantly higher degree of the fungal disease *L. taurica* than TY13.

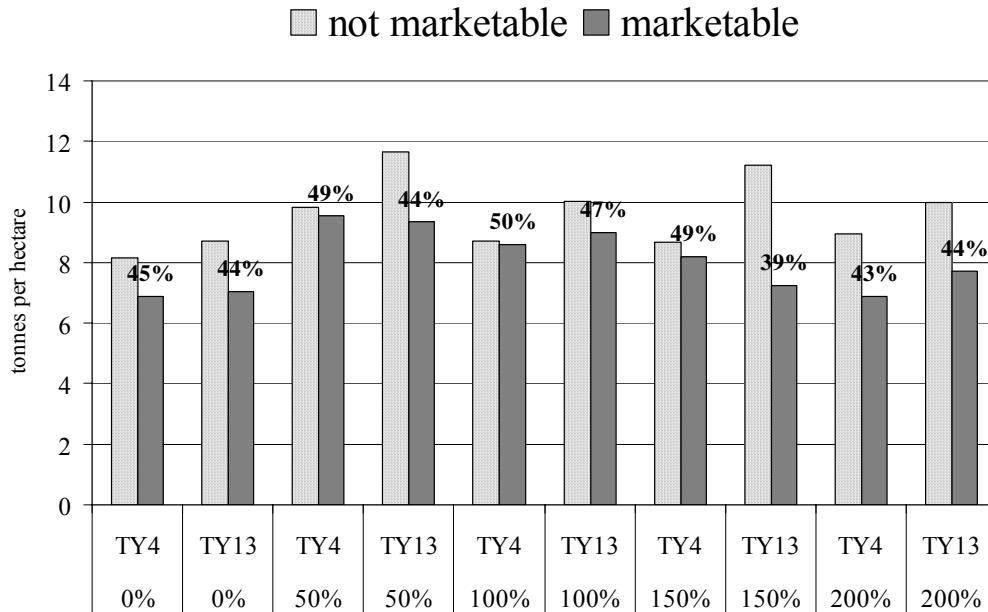


**Figure 3.6** Accumulation of total harvest for treatments with 0 %, 50 %, 100 %, 150 % and 200 % of the standard nitrogen fertilization level for TY4 and TY13 in tones per hectare for 65 and 70, 77, 86 and 93 days after transplantation.

There is no significant difference between the two varieties and the marketable and not marketable fractions. There is a tendency that the 0 % and 200 % have a lower percentage of not marketable fruits compared to the other fertilization levels. The marketable fraction is in all cases smaller than the not marketable fraction. TY13 has a higher percentage of marketable fruits in the two highest and TY4 in the three lowest fertilization levels.

The normal yield for field grown tomatoes in Nicaragua is 12 – 18 tonnes per hectare (INTA, 2002). In this study, the total yield corresponds well with the normal yield for field grown tomatoes in Nicaragua. The marketable yield is between 39 % and 50 % of the total yield. The 0 % nitrogen fertilization level had a slightly lower total harvest than the other fertilization levels (figure 3.6)

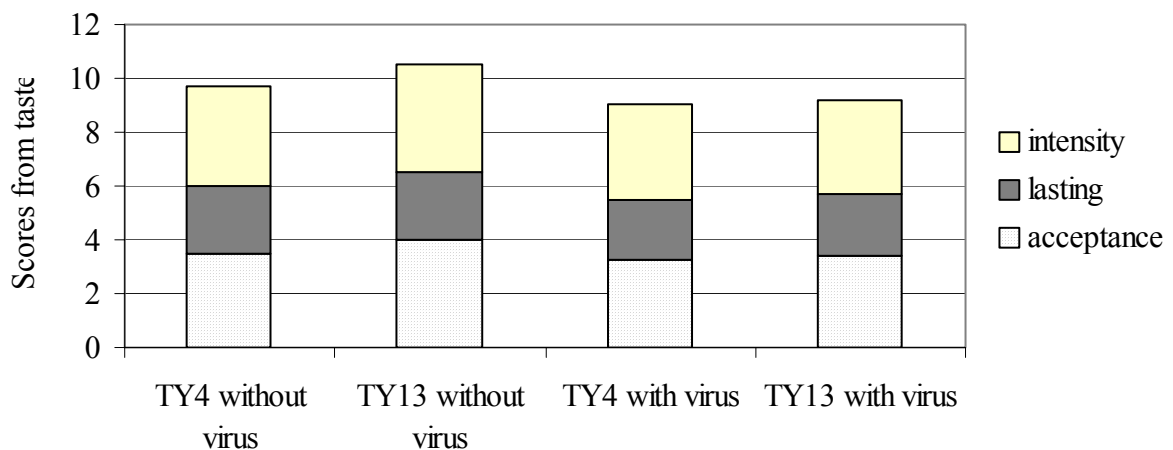
The high percentage of not marketable fruits is probably due to Blossom-end rot, poor pollination and larvae (figure 3.7). Since the most infected virus plants only formed very small fruits, the harvest from virus-infected plants did not contribute much to the total harvest and should not play a big role when the harvest is measured in tonnes.



**Figure 3.7** Harvest of not marketable and marketable tomatoes of the two varieties TY4 and TY13, mean of total yield in tonnes per hectare. The numbers describe % marketable tomatoes of the total yield.

### 3.6.4 Taste test, sugar and acid analysis

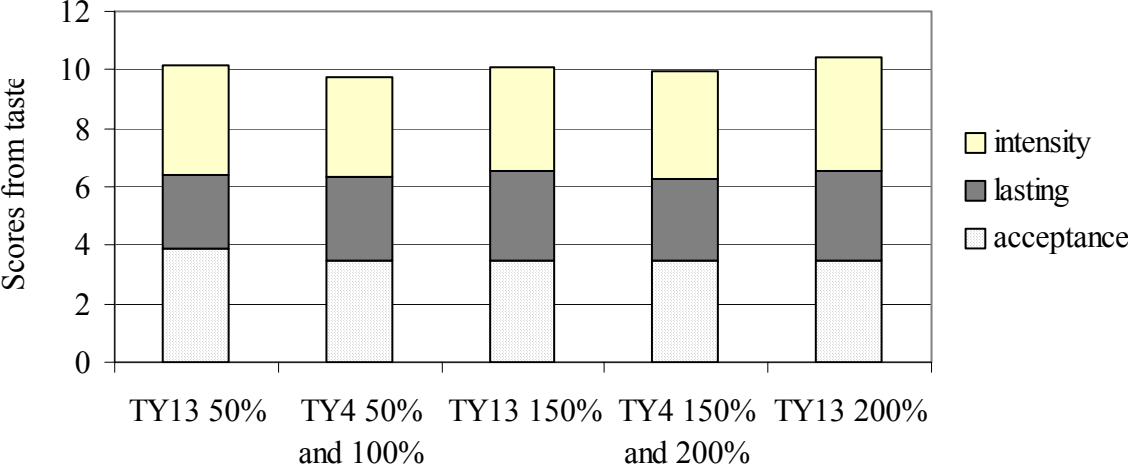
The first harvest was very small and many fruits showed virus symptoms. Therefore all fruits of one variety, with or without virus symptom were collected, each in one group (figure 3.8) for the first taste test. Fruits with any type of disease symptom were assumed to be not marketable. The not marketable fruits were therefore not exposed to the panelist, and not evaluated for quality. No chemical analyses were done of these samplings. The panelists preferred the TY13 without virus symptoms, however, no differences were statistically significant.



**Figure 3.8** Scores from the first taste test for intensity, lasting and acceptance of the tomato taste

At the second harvest occasion, the sample was large enough to separate into different varieties. Fertilizing levels could be divided into three groups for TY13 and two groups for TY4 (figure 3.9). The differences between the different samples were smaller than in the first

taste test. TY13 scored higher than TY4 in all fertilization levels, but the differences were not statistically significant.



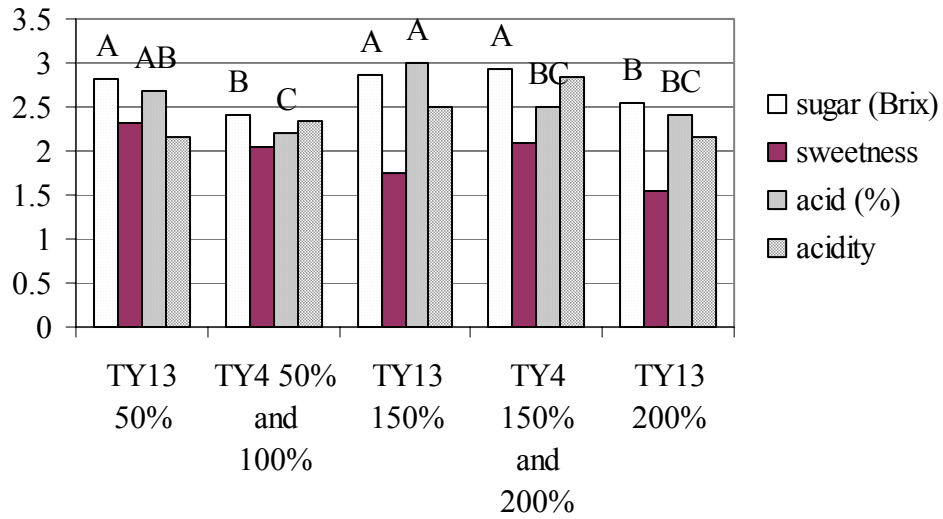
**Figure 3.9** Scores from the second taste test for intensity, lasting and acceptance of the tomato taste

The laboratory analyses of acid and sugar content were made on the second harvest. Results are shown for both varieties and all fertilization levels in table 3.5. The sugar values were higher than acid for all fertilization levels except TY13 100 % and TY13 150 %. For TY4 there is a tendency that 50 % and 100 % is lower for both acid and sugar than the other fertilization levels. For TY13, the tendency is opposite; where 0 % and 200 % have lower values than the other.

**Table 3.5** Laboratory analyses of acid (%) and sugar (Brix) content for TY4 and TY13 at all fertilizing levels.

Variety	Sugar (Brix)	Acid (% acid)
<b>TY4 0 %</b>	2.9	2.6
<b>TY13 0 %</b>	3.0	2.4
<b>TY4 50 %</b>	2.3	2.0
<b>TY13 50 %</b>	2.9	2.8
<b>TY4 100 %</b>	2.4	2.2
<b>TY13 100 %</b>	2.9	3.0
<b>TY4 150 %</b>	3.0	2.6
<b>TY13 150 %</b>	2.9	3.1
<b>TY4 200 %</b>	2.6	2.5
<b>TY13 200 %</b>	2.5	2.4

Comparisons between the laboratory analyses and taste test for acid and sugar content is shown in figure 3.10. There was no clear relation between the result of the second taste test and the laboratory analyses of the % acid and sugar (Brix) content. According to the taste test, all TY13 have a tendency to taste better than TY4. According to the laboratory analyses, TY13 200 % have significantly lower sugar contents than the other samples but scored best in the taste test.



**Figure 3.10** Laboratory analyses of the acid and sugar content in the tomatoes from the second taste test and scores for sweetness and acidity from the same taste test. Different letters show statistical significances for the laboratory analyses between the different fertilizing levels for the sugar and acid content.

### 3.7 Discussion

#### 3.7.1 Dry matter and nitrogen content

The dry matter of the tomato plants did not differ significantly between the different fertilization levels, which indicate that growth was not limited by lack of nitrogen in any of the fertilization levels (Ho, 1996). Dry matter is also affected by the amount available water (Fisher *et al.*, 2002), which is known to be a growth-limiting factor. However, no visible symptoms on water deficiency were visible in the field experiment.

The fruits are known to generally become the main sink after 40 days (Tei *et al.*, 2002). This is in agreement with the results of the KS-measures, where there is a decreasing trend of decreased chlorophyll content in leaves, starting 40 days after transplantation. In accordance to previous studies (see Tei *et al.*, 2002), the KS-value is highest before the fruits become the main sink of the nutrient allocation (compare table 3.3).

Ho (1996) writes that the terminate tomatoes demand for potassium is above the potassium uptake capacity. To cover the demand potassium is remobilized from the leaves to the fruits, which then might cause foliar potassium deficiency. The N-tester measure how green the leaves are. Both potassium and nitrogen deficiencies decrease the greenness of the leaves. Hence, although often regarding the N-tester to give an indirect measure of the nitrogen concentration, low values could also be due to lack of potassium in the leaves (see Fischer *et al.*, 2002). Potassium is also extremely movable in the plant, which also might contribute to the significant differences between 0 % and the other fertilizing levels.

#### 3.7.2 Disease and incidence of disease

Tomatoes are grown in all seasons in the Sébaco valley, which contributes to the presence of Begomovirus the year around. All known measures were taken against Begomovirus infection in the field experiment. However they were not very successful and 80 % of the tomato plants got visual virus symptoms (Eitrem, 2005; in this issue). In this experiment, the number of fruits on plants infected with Begomovirus increased and the size of the fruits decreased (data not shown). Islam *et al.* (2003) found that both fruit weight and number of fruits decreased when tomato plants were infected with Begomovirus. In this experiment, the Begomovirus infected plants had a high number of fruits. This might be due to pre-maturation of all fruits at once on the sick plants compared to the healthy looking plants.

High temperatures are known to inhibit the floral development and the pollen germination and causes physiological deformations on the tomato fruit. The optimum temperature for growing tomato is 18°C to 25°C during the day (Ho, 1996). The temperatures during this field experiment were more than 10°C higher than the optimum. Thus high temperature is probably one explanation factor for the high number of deformed fruits in the field experiment.

There was some incidence of Blossom-end rot in all fertilizing levels in the current field experiment. There were no significant differences between the fertilizing levels and Blossom-end rot causes an over all yield quality reduction. The cause of Blossom-end rot is not known, it could be both inadequate supply of calcium from the soil or poor development of the xylem. The first effect might be a consequence of the second effect causing water deficiency and decreased transpiration, which might lead to reduced uptake of calcium. The nitrogen fertilization does not seem to affect the occurrence of Blossom-end rot.

### 3.7.3 Harvest

Compared to the normal Nicaraguan tomato fruit yield 12 – 18 tonnes per hectare (INTA, 2002), the total yield of the current field experiment is in similar range with yields between 15 and 21 tonne per hectare. But the marketable fruit fractions were only between 39 and 50 %. The low marketable yield fraction might be due to heavy virus infections, high temperatures, larvae or fungal diseases. Lopez *et al.* reports marketable yields between 88.2 and 92.6 %, which proves that there is a possibility to raise the sellable fraction significantly.

In this experiment, the marketable fruit yield did not increase with nitrogen level. The highest total harvest was in the 50 % fertilization treatment. The results are supported by findings by Fischer *et al.* (2002) that the fruit yields do not increase with the amount of nutrient added, but the larger nutrient fertilization increases the vegetative growth. They suggest that high temperature during fruit set may have lowered the sink strength of the fruit. This explains why the yield may be lower in the higher nutrient levels because lack of sink strength.

The TY4 had a significantly higher incidence of the fungal disease *L. taurica* than TY13, which probably lowered the total yield for TY4. At the same time, TY4 had higher percentage of marketable yield than TY13. The reason why TY4 has a lower total yield but higher marketable fraction could be due to variety differences.

### 3.7.4 Taste test, sugar and acid analysis

It is very difficult for an untrained panel to evaluate the taste differences objectively. Although not intended, close to half of the testing persons spoke with each other during the evaluation at both occasions, causing a dependency between observations. The panelists were encouraged to taste the whole sample at once, both the gel and the wall of the tomato. But at the first taste test, not every testing person did so. They were instructed to rinse their mouths with water between the different tomato samples (Malundo, 1995), but at both occasions less than 10 % did so.

The tomatoes used for the taste test were placed in the same order on the plate for each occasion. This contributes to the fact that the panelists could discuss and compare the same samples with each other, which was not desirable. There were no significant differences between the taste test samples, and it is impossible to say how the conversations affected the result. These factors make the taste test not very reliable, but it does give an indication of which type of tomatoes that are preferred of the Nicaraguans.

The fertilization level does not seem to affect the taste as much as the variety. TY 13 was the most preferred variety according to the taste test. The laboratory analyses do not reveal which fruits that have superior taste compared to the other fruits like the taste test does.

In the first taste test, the panelists did not like the virus fruits. One reason could be that these fruits were not as mature as the fruits without virus symptoms. Fruits in the later stages of maturation could, by mistake, have been mixed with virus-infected fruits. Islam *et al.* (2003) found that sugar content in fruits from infected plants was significantly lower than in healthy fruits, which also explains the inferior taste of virus infected fruits.

TY13 was the most preferred variety at both occasions. In the second taste test, TY13 200 % fertilization level got the highest score (the panelist liked the TY13 fruits most), but according

to the laboratory analyses TY13 200 % had significant lower sugar (Brix) and acid (%) content than the other samples. The laboratory analyzes did not correlate with the results from the taste test. The relationship between the sugar (Brix) and acid (%) content might be more important for the taste than the total amount of each of them. The results confirm opinions that the Nicaraguans like more acidic tomatoes (Rojas, 2004, personal communication).

### **3.8 Conclusions**

The most interesting question for the Nicaraguan tomato producers is how to grow tomatoes with high yield and quality. The highest total yield was achieved with 50 % nitrogen fertilization, and the highest marketable fractions were 49 % for nitrogen level 50 % for variety TY13 (49 % was marketable) and for fertilization level 100 % for TY4 (50 %). The results suggest that the normal nitrogen fertilization might better be reduced to half of the today recommended.

The low marketable yield might be due to the high temperature and disease pressure. It demands a lot of management to grow tomatoes during the dry and hot period since they need water every day. Not only have the tomatoes in this experiment suffered from heavily virus infections, all of the field grown tomatoes in the area did so. If tomatoes were not grown during the hot and dry season, the vector chain of the diseases would be cut off, which would decrease the disease attacks.

The objectives of this study were to evaluate the effects of nitrogen fertilization on yield and quality of field grown tomatoes in Nicaragua. The differences in yield and quality could not only be explained with the nitrogen fertilizing effect. This because the 0 % fertilizing level were not fertilized with any potassium and only half of the standard amount of phosphorus fertilizing level. This and the fact that there were no significances between the other fertilizing levels make it impossible to explain the differences only as a nitrogen effects.

### 3.9 References

#### 3.9.1 Books and articles

- Adams, P., 1986, Mineral nutrition, pg 281-324 in: Atherton J.G. and Rudich, J. (eds.) *The Tomato Crop*, Chapman and Hall Ltd.
- Agrios, G.N., 1997, *Plant Pathology*, 4<sup>th</sup> edition, Academic Press
- Auerswald, H., Schwarz, D., Kornelson, C., Krumbein, A., Brückner, B., 1999, Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution, *Scientia horticulturae* 82: 227-242
- Baldwin, E.A., Nisperos-Carriedo, M.O., Moshonas, M.G., 1991, quantitative analysis of flavour and for certain constituents of two tomato cultivars during ripening, *Journal of the American Society for horticultural science* 116(2):265-269
- Batu, A., 2004, Determination of acceptable firmness and color values of tomatoes, *Journal of food engineering*, pg 471-475
- Colla, G., Battistelli, A., Moscatello, S., Proietti, S., Casa, R., Lo Cascio, B. and Leoni, C., 2001, Effects of reduced irrigation and Nitrogen fertigation rate on yield, carbohydrate accumulation, and quality of processing tomatoes, In: Harts, T.K. (eds.) Proc. 7<sup>th</sup> Int. Symp. on Processing Tomato, *Acta Hort.* 542
- Correll, J. C., Gordon, T. R., Elliott, V. J., 1988, Powdery mildew of tomato: The effect of planting date and triadimefon on disease onset, progress, incidence, and severity, *Phytopathology*, 78:512-519
- Dumas, Y., Dadomo, M., Di Lucca, G. and Grolier, P., 2003, Review Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes, *Journal of the Science of Food and Agriculture*, 83:369-382
- Dumas, Y., 1990, Tomatoes for processing in 90's: Nutrition and crop fertilization, *Acta Horticulturae* 277
- Eitrem, S., 2005 The effect of nitrogen on natural infection of diseases on tomato, In: Eitrem, S. and Berlin, A., *Tomato diseases, quality, yield and pesticide use - a field study in Nicaragua*, Examensarbete inom agronomprogrammet, Sveriges Lantbruksuniversitet, this issue
- Fischer, K.J., Johnstone, P.R., Michols, M.A., 2002, Nutrition of processing tomatoes, pg 45-59 In: Booij, R. Neeteson, J. (eds.) Proceedings of the ISHS workshop towards an ecologically sound fertilisation in field vegetable production, *Acta Hort.* 571
- Gould, 1983, *Tomato production, processing and quality evaluation*, 2<sup>nd</sup> edition
- Greenwood, D.J., Lemaire, G., Gosse, G., Cruz, P., Draycott, A., 1990, Decline in percentage N of C3 and C4 crops with increasing plant mass, *Annals of Botany* 66:425-436
- Grierson, D. and Kader, A. A., 1986, Fruit ripening and quality, pg 241-280 in: Atherton J.G. and Rudich, J. (eds.) *The Tomato Crop*, Chapman and Hall Ltd.
- Ho, L. C., 1996, Tomato, In: Zamaski, E and Schaffer, A.A. (eds.) *Photoassimilate distribution in plants and crops. Source-sink relationships*. Marcel Dekker Inc, pg 709-728
- Hobson, M., 1988, How the tomato lost its taste, *New Scientist*, 29 Sept 1988, pg 46-50
- Hull, R., 2001, *Matthew's plant virology*, 4<sup>th</sup> edition, Academic Press, San Diego, USA
- Islam, M.T, Hossain, T., Ahmend, J.U., Karim, S., 2003, Biochemical changes in tomato fruits caused by tomato yellow leaf curl virus, *Bangladesh Journal of botany*, 32(2):31-84, only abstract available
- Instituto Nicaragüense de Tecnología Agropecuaria (INTA), 2002, El Cultivo del Tomate, *Guía Tecnología* 22
- Jones, J. B., Stall, R. E, Zitter, T. A., 1997, *Compendium of Tomato Diseases*, The American Phytopathological Society



- Jones, R.A., Scott, S.J., 1984, Genetic potential to improve tomato flavor in commercial F<sub>1</sub> hybrids, *Journal of the American society for horticultural science* 109(3): 318-321
- Kaul, M.L.H., 1991, Reproductive biology in tomato, pg 39-50 in: Kalloo, G. (eds.) *Genetic improvement of tomato. Monographs on theoretical and applied genetics*. Vol 14, Spriger-Verlag, Berlin
- Kinet, J. M. and Peet, M. M., 1997, Tomato, pg 208-258 in: Wien, H. C. (eds.), *The Physiology of Vegetable Crops*, CAB International, Cambridge
- Krumbein, A., Auerswald, H., 1998, Characterization of aroma volatiles in tomatoes by sensory analyses. *Nahrung* 42:395-399
- Lemaire, G. and Gastal, F., 1997, N uptake and distribution in plant canopies. In: Lemaire G. (eds.) *Diagnosis of Nitrogen status in crops*, Springer-Verlag, Berlin
- Lopez, J., Tremblay, N., Voogt, W., Dubé, S. and Gosselin, A., 1996, Effects of varying sulphate concentrations on growth, physiology and yield of the greenhouse tomato, *Scientia Horticulturae*, 67:207-217
- Löfkvist, K., 2000, Smak och hållbarhet hos åtta tomatsorter: Sensory quality and shelf-life of tomatoes from eight different cultivars, *Examensarbete inom Hortonomprogrammet*, 2000:13, Sveriges Lantbruksuniversitet
- Malundo, T.M.M., Shewfelt, R.L., Scott, J.W., 1995, Flavour quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels, *Postharvest biology and technology* 6:103-110, USA
- Polston, J.E. and Anderson, P.K., 1997, The emergence of white-fly transmitted Gemini virus in tomato in the western hemisphere, *Plant Disease*, 81:1358-1369
- Rudich, J. and Luchinsky, U., 1986, Water economy, pg 335-368 in: Atherton J.G. and Rudich, J. (eds.) *The Tomato Crop*, Chapman and Hall Ltd.
- Sangketkit, C, Savage, G.P., Martin, R.J, Searle, B.P, Mason, S.L, 2000, Sensory evaluation of new lines of oca (*Oxalis tuberosa*) grown in New Zealand, *Food Quality and Preferences* 11; 189-199
- Song, J., Nada, K. and Tachibana, S., 1999, Ameliorative effect of polyamines on the high temperature inhibition of in vitro pollen germination in tomato (*Lycopersicon esculentum* Mill.) *Scientia Horticulturae* 80; 203-212
- Tei, F., Benincasa, P., Guiduci, M., 2002. Critical Nitrogen concentration in processing tomato. *European Journal of Agronomy* 18; 45-55
- Tei, F., Benincasa, P., Guiduci, M., 2002. Effect of N availability on growth, N uptake, light interception and photosynthetic activity in processing tomato, In: Booij, R. And Neeteson, J. (eds.), Proc Workshop Eco. Fertilization Veg. *Acta Horticulturae* 571
- Whipps, J.M., Budge, S.P., Fenlon, J.S., 1998, Characteristics and host range of tomato powdery mildew, *Plant Pathology* 47; 36-48

### 3.9.2 Personal communication

Rojas, A., 10<sup>th</sup> of September 2003, Agr PhD student UNA-SLU and local supervisor of the projects. Uppsala, Sweden.

Rojas, A., 7<sup>th</sup> of June 2004, Agr PhD student UNA-SLU and local supervisor of the projects. Uppsala, Sweden.

### 3.9.3 Internet

Yara, N-tester

[http://fert.yara.se/se/crop\\_fertilization/tools\\_and\\_services/n-tester/n-tester.html](http://fert.yara.se/se/crop_fertilization/tools_and_services/n-tester/n-tester.html) 2005-04-19. 14:20

Interview questions:

1. How big area do you use for crop production?
2. Which crops do you grow?
3. Do you have animals?
4. Which are the major problems with tomato growing?
5. How do you control pests and diseases?
6. What kind of pesticides do you use?
7. When do you use pesticides? (How do the plants look, do they have any symptoms?)
8. Are the pesticides effective?
9. How do you apply the pesticides?
10. How many times during the growing season do you use pesticide on tomato?
11. Who does the spraying?
12. Do you use any protective equipment?
13. What do you do if your skin is exposed to the pesticide?
14. Have you experienced any illness after you have sprayed?
15. Where do you store the pesticides?
16. What do you do with the empty containers?
17. Where did you get information about how to use and store pesticides?

Taste test of tomatoes

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Date: \_\_\_\_\_

Sex:	Male ♂	Female ♀	
Age:	< 20	21-30	31-40
	41-50	> 50	

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1. Looking at the tomatoes, what is your first impression of the appearance?

Color

	Even red					Mixed red and green
1.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	Shape Regular					Irregular
1.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	Firmness Unripe					Overripe
1.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

2. How do the tomatoes feel in your mouth when you chew them?

	Soft and juicy					Hard and dry
1.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

3. How sweet does the tomatoes taste?

	Very sweet				Not sweet	
1.	☺	☺	☺	☺	☺	☺
2.	☺	☺	☺	☺	☺	☺
3.	☺	☺	☺	☺	☺	☺
4.	☺	☺	☺	☺	☺	☺
5.	☺	☺	☺	☺	☺	☺

4. How acidic dose the tomatoes taste?

	Very acidic				Not acidic	
1.	☹	☹	☹	☹	☹	☹
2.	☹	☹	☹	☹	☹	☹
3.	☹	☹	☹	☹	☹	☹
4.	☹	☹	☹	☹	☹	☹
5.	☹	☹	☹	☹	☹	☹

5. How do you judge the tomatoes intensity of flavour?

	Ideal flavour for tomatoes				Not characteristic for tomatoes	
1.	☺	☺	☺	☺	☺	☺
2.	☺	☺	☺	☺	☺	☺
3.	☺	☺	☺	☺	☺	☺
4.	☺	☺	☺	☺	☺	☺
5.	☺	☺	☺	☺	☺	☺

6. How long do you feel the tomato flavour?

	Long				Short	
1.	☺	☺	☺	☺	☺	☺
2.	☺	☺	☺	☺	☺	☺
3.	☺	☺	☺	☺	☺	☺
4.	☺	☺	☺	☺	☺	☺
5.	☺	☺	☺	☺	☺	☺

7. Overall acceptance of the tomatoes?

	Like it very much				Dislike it strongly	
1.	☺	☺	☺	☺	☺	☺
2.	☺	☺	☺	☺	☺	☺
3.	☺	☺	☺	☺	☺	☺
4.	☺	☺	☺	☺	☺	☺
5.	☺	☺	☺	☺	☺	☺

Thank you very much!