CRANFIELD UNIVERSITY

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PSEUDOPERONOSPORA CUBENSIS DEVELOPMENT UNDER DIFFERENTIATED NITROGEN AND POTASSIUM FERTILIZATION OF *CUCUMIS SATIVUS*

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PSEUDOPERONOSPORA CUBENSIS DEVELOPMENT UNDER DIFFERENTIATED NITROGEN AND POTASSIUM FERTILIZATION OF *CUCUMIS SATIVUS*

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ABSTRACT

Cucumber (*Cucumis sativus*) is one the most economically important vegetable crops worldwide and downy mildew caused by *Pseudoperonospora cubensis* is considered among the most destructive diseases that attack this plant. Although the effect of mineral nutrition on plant diseases for various crops has been studied, there is practically none on cucumbers. The overall objectives of this study were (a) to investigate the effect of increasing inoculum concentrations of *P. cubensis* on downy mildew emergence on different aged cucumber leaves (b) to examine the effect of various N and K concentrations in the fertilization solution on the disease development and the leaf surface (c) to study the potential interaction of these two essential nutrients in relation with the application of a fungicide used for downy mildew control (d) to determine the disease progress with time (e) to investigate the nutritional status of cucumber tissues and substrate. A range of greenhouse experiments were conducted.

Cucumbers were grown under six K (200, 300, 400, 500, 600, 700 ppm) and six N (100, 200, 300, 400, 500 and 600 ppm) fertilization regimes, in two Randomized block experiments respectively. Two factorial designs, one with three levels of spore concentrations and two leaf ages and the other with two N and three K levels plus two fungicide treatments were also established. Cucumber plants (*Cucumis sativus* L. cv. Knossou) susceptible to downy mildew were artificially inoculated with *P. cubensis*. Disease was digitally assessed.

Statistical analysis of the obtained data indicated that the pathogen attacked leaves regardless of age. The lesion area was significantly increased with increasing spore content at low disease pressure. N concentration of 300 ppm had a positive effect on the leaf area when compared with the lower N rates. A concentration of 400 ppm K resulted in a marked increase in leaf area in relation to the other treatments although the differences were not statistically significant (P=0.05). There were indications that downy mildew was significantly decreased on the plants grown in 300 ppm N, 300 and 400 ppm K. However, N and K interaction was observed for leaf and lesion area. Thus, inhibition of infection was

recorded with increasing K levels only at low N rates. The best combination for disease limitation was 200 ppm N and 400 ppm K, which was found to be comparable with the fungicide used. The disease progress for downy mildew followed a cubic curve in all cases (N and K fertilization treatments, fungicide application). The importance of leaf and soil nutrient status on infection and leaf area index was also noted.

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NOTATION

%	per cent
<	less than
=	equals
>	greater than
°C	degrees Celsius
AAS	Atomic Absorption Spectroscopy
ANCOVA	analysis of covariance
ANOVA	analysis of variance
Ca	calcium
$CaCO_3$	calcium carbonate
$CaCO_3$	calcium carbonate
cal	calorie
CH_3COONH_4	ammonium acetate
$cm/cm^2/cm^3$	centimetre/square centimetre /cubic centimetre
Cu	copper
cv.	cultivar
d.f.	degrees of freedom
DF	dilution factor
DM	dry matter

e.g.	for example
EC	electrical conductivity
et al.	and others
etc.	etcetera
FAO	Food and Agriculture Organization of the United Nations
Fe	iron
g/mg	gram/milligram
h / hrs	hour / s
H_2SO_4	sulphuric acid
H_3BO_3	boric acid
H_3PO_4	orthophosphoric acid
HCl	hydrogen chloride
$HClO_4$	perchloric acid
HNO_3	nitric acid
i.e.	that is to say; in other words
K	potassium
K_2SO_4	potassium sulfate
KCl	potassium chloride
KNO_3	potassium nitrate
L	litre
M	molarity (moles/L)

m.s.	mean square
Mg	magnesium
min	minute
ml/mls	millilitre/s
mm	millimeter
Mn	manganese
mS	milliSiemens
N	Nitrogen
N	normality (greqs/ml)
NH_4-N	ammonium nitrogen
NH_4NO_3	ammonium nitrate
$(NH_4)_2SO_4$	ammonium sulfate
nm	nanometer
NO_3-N	nitrate nitrogen
P	significance
P	phosphorus
P. cubensis	*Pseudoperonospora cubensis*
pH	power of hydrogen (measure of the acidity or basicity of a solution)
ppm	parts per million
RH	relative humidity

s.g.	specific gravity
s.s.	sum of squares
Sig.	significance
SL	sandy loam
TEI	Technological Educational Institute
Zn	zinc
µl/µls	microlitre/s

CHAPTER ONE
GENERAL INTRODUCTION

1.1 BACKGROUND

Cucumber (*Cucumis sativus* L.) is an important crop that is cultivated worldwide. Especially in Greece, where economy is based on agriculture to a large degree, cucumber is essential among vegetables and of a great economic significance. However cucumber is susceptible to many diseases. Downy mildew caused by *Pseudoperonospora cubensis* is one of the most destructive diseases of cucumber and causes major yield losses. Climatic conditions favour disease incidence and spread and make the cost to producers excessive. Disease control is generally achieved by the use of fungicidal chemicals. Nevertheless, in periods of epidemics even fungicides cannot limit downy mildew development and thus, serious problems in cucumber production are certain to occur. Furthermore, fungicides are unwelcome due to environmental pollution and negative effects on human health. Although in the past possible alternative control methods and management have been examined, such as the effect of nutrition disease spread on various crops, very little research has been done on the effect of nutrients on cucumber downy mildew. Therefore alternative methods for disease control are imperative as part of an integrated and sustainable management system for cucumber.

1.2 AIM AND OBJECTIVES

- **Aim**

This PhD project aimed at examining a possible alternative method for controlling cucumber downy mildew by studying the influence of nutrient status on disease development.

- **Objectives**

- To study the downy mildew disease emergence in relation to the inoculum load. This included estimation of the effect of different zoospore concentrations used for inoculation, on downy mildew development.

● To examine the effect of extreme doses of nitrogen when applied to cucumber cultivation in order to evaluate concentrations which might favour or inhibit downy mildew development.

● To determine the effect of potassium as a fertilizer on the incidence of downy mildew disease in cucumber by applying excessive doses of this nutrient.

● To assess the impact of nutritional factors on micro and macro nutrient content of plant tissues (leaves) and growth matrix (soil) to help understand the conditions which are conducive to infection.

● To evaluate the influence of fungicide use and interactions with inorganic fertilization on cucumber downy mildew control.

1.3 THESIS STRUCTURE

This thesis is arranged in eight chapters. Chapter 1 covers the background, the aims and the objectives of this study as well as the thesis structure. Chapter 2 presents a literature review on the subject, primary, botanical characteristics of the cucumber plant and its cultivation and cmprehensive description of the fungus *P. cubensis*. The effect of nutrition on downy mildew development is then analysed. The chapter concludes by reporting the current control methods of this pathogen in Crete and the need for alternative methods.

Chapter 3 details the general materials and methods used, including experimental design, cultivation and inoculation methods. Analytical methods used and measurements for disease assessment and nutrient determination followed by the statistical analysis of the data.

The experiments that were conducted during this work are described in chapters 4 to 7. Each chapter refers to a specific target objective. Their structure is as for individual papers and thus includes: an Introduction, Materials and Methods, Results, Discussion and Conclusions. The effect of three different zoospore concentrations on the development of cucumber downy mildew is reported in

Chapter 4. Old and young cucumber leaves were artificially inoculated with the fungus in a factorial design in order to study the main effects of inoculum concentrations and leaf developmental stage as well as to ascertain whether there are interactions between them.

In order to obtain successful infection the concentration that gave the best results from the first experiment was used in all the subsequent experiments. Chapter 5 details how N fertilization influences the spread of downy mildew. This was assessed by calculating the lesion area caused by the fungus on the upper surface of leaves. To study the nutritional factors involved in this relationship of cucumber nutrition – downy mildew, the nutrient status of soil and host were determined. An abstract concerning this research has already been submitted to the European Journal of Plant Pathology (Appendix H).

The cucumber-downy mildew pathosystem in greenhouse conditions in relation to inorganic nutrition has not been comprehensively investigated previously. After the above experiments, given that (a) K is an essential plant nutrient and (b) N and K are antagonistic nutrients a third study was conducted, where the effect of K as a fertilizer on disease development was studied (Chapter 6).

This was further built on by carrying out a factorial experiment with two levels of N and three levels of K which were based on the results of the previous randomized block experiments, (Chapter 7). The effect of these nutrients on cucumber downy mildew in relation to the effect of fungicides used for control of this disease gives important information for an integrated management approach. Therefore the interaction of these nutrient levels in relation to the effect of two fungicides treatments on disease is also reported here.

Chapter 8 is a general unifying discussion and conclusions, based on an integration of the results obtained from all the studies. This chapter also makes recommendations for future research and examines the implications of the results in terms of their practical value for cucumber growers. This is followed by the Reference list cited followed by the Appendices. Figure 1.1 summarises the general plan of these studies.

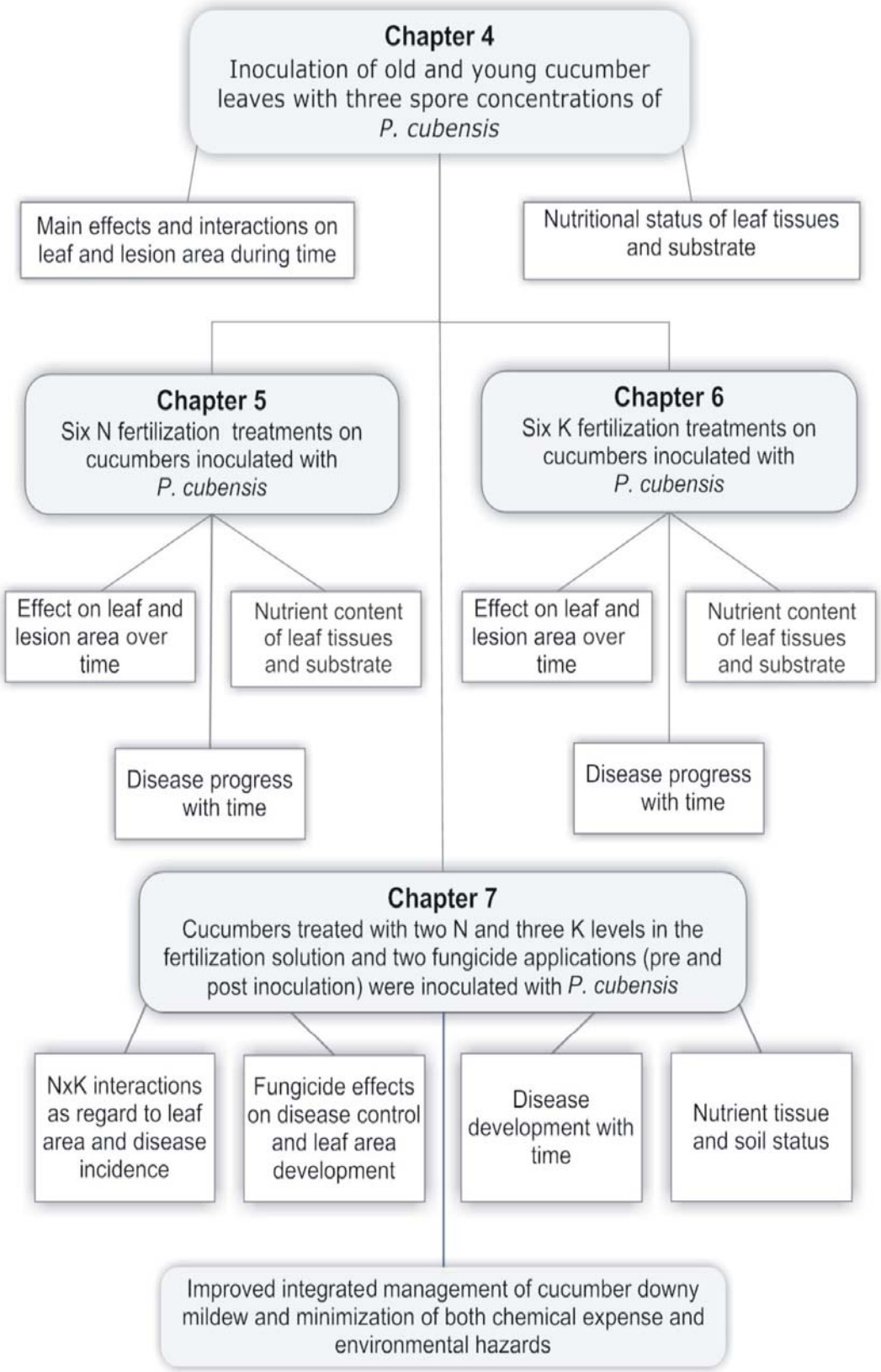

Figure 1.1. Diagram of the experimental work carried out in this thesis.

CHAPTER TWO
LITERATURE REVIEW

2.1 *CUCUMIS SATIVUS* L.

2.1.1 Classification and plant characteristics

The cucumber originated in India, in an area between the bay of Bengal and the Himalaya mountains (Peirce, 1987; Smith, 1977). It is one of the oldest crops; it was cultivated as long as 3000 years ago. Cucumber was probably domesticated in Asia and then introduced into Europe, where the ancient Romans adopted it into their cuisine and then all Europe followed. The classification of cucumber according to the United States Department of Agriculture (USDA) is shown in Table 2.1. Cucumber is classified as a warm season, annual, vining plant. Its edible part is the fruit and its rooting depth is moderately deep 90-120 cm. Its classification based on sensitivity to pH is that it is moderately tolerant (pH 6.8-5.5). Very low pH (5.5) can reduce fruit set (Peirce, 1987).

The stems of cucumber grow like a vine. They are four angled and produce stiff hair on them. They also possess laterals and tendrils. The vining begins as soon as 2-3 leaves form. So the plants in greenhouses can be trained on trellises to save space and improve yield and fruit quality (Mills, 2001). The leaves are also covered with bristly hairs. They are simple, alternate, lobed, triangular and located at the base of the main axils. The cucumber flower can display several sex types: Perfect flower which is the flower with both male (stamens) and female (pistil) organs (rare in cucumbers) (Mills, 2001), male (staminate) which is a flower lacking a pistil and female (pistilate) is a flower lacking the stamens (Smith, 1977).

The cucumber fruit is considered a ‘pepo’ and varies in size, shape and color depending on the cultivar (Smith, 1977). Greenhouse varieties have elongate, cylindrical, thin skinned fruit which tend to loose moisture quickly after picking and must be wrapped in plastic (Smith, 1977; Peirce, 1987). The fruits of European types are seedless, have a slightly wrinkled surface, are uniformly green, non-bitter and usually have a short neck on the stem end. Peeling is not required before eating

(Wittwer and Honma, 1979). The cucumber fruits have high water content and provide some vitamin A and C (Wehner, 1996). According to Peirce (1987), the nutritional constituents of cucumber are as follows. Water content 95%, energy 15 cal, protein 0.9 g, fat 0.1 g, carbohydrate 3.4 g, vitamin E (ascorbic acid) 11 mg, Ca 25 mg, P 27 mg, Fe 1.1 mg, Na 6 mg, K 160 mg (data expressed per 100 g fresh weight). Its water content is the highest of the major vine crops.

Table 2.1. Cucumber classification according to the United States Department of Agriculture.

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae –
Order	Violales –
Family	Cucurbitaceae – Cucumber family
Genus	*Cucumis* L. – melon
Species	*Cucumis sativus* L. – garden cucumber

Cucumber plants grow very fast and are normally monoecious (separate staminate and pistillate flowers on the same plants) (Wehner, 1996). Most of the current modern cultivars are gynoecious which have mostly female flowers (only about 5% are male) (Mills, 2001). Production of male flowers is promoted by long days and high temperatures. Male flowers are also produced when the plant is stressed or by applying gibberellin which alters the plant's endogenous auxin (hormones that control cell elongation) level. High auxin levels are associated with femaleness, low levels with maleness. Since the mid-1960s the European cucumber has replaced the standard greenhouse varieties (Peirce, 1987). The new varieties are now called 'long cucumbers' but have also been called 'European cucumbers',

'English cucumbers', 'seedless cucumbers'. They are used for greenhouse production and they produce higher yields. They are gynoecious (producing mostly female flowers) and parthenocarpic (set fruit without pollination) and therefore require no bee activity for pollination (Lamb *et al*. 2001). Pollination in these types should be avoided, since it causes seed development and the fruits become clubbed at the blossom end and develop a bitter taste (Wittwer and Honma, 1979). Recommended varieties include Toska 70, Sandra and Fidelio (Marr, 1995). In Greece Sandra, Diana, Monica and Herta are cultivated extensively in greenhouses (Zarboutis and Gakni, 1992).

2.1.2 Crop establishment and maintenance

Cucurbits require well drained, neutral or slightly alkaline soil for maximum growth and fruit set. For this reason the ideal pH is between 6.0-6.8 (Peirce, 1987). Soil types that are sandy, silty or contain clay should be improved by adding organic matter (Lerner and Dana, 2001; Mills, 2001). So, heavy or poorly drained soils are not suitable for cucumber production, since the root systems of these plants are very susceptible to oxygen deficiency and do not perform well under anaerobic conditions (Peirce, 1987). As Marr (1995) stated, well drained, light sandy loam soils are preferable.

Temperature is one of the factors that play a significant role in the plant development. The best temperature for seed germination is 27-29 ^{o}C (Zarboutis and Gakni, 1992). Since the cucumber is a warm season plant it grows rapidly when the temperatures are between 23-30 ^{o}C with plenty of sunlight (Marr, 1995; Wittwer and Honma, 1979). For greenhouse cucumbers the minimum temperatures should be no lower than 18 ^{o}C during daytime and no higher than 30 ^{o}C (Marr, 1995).

Good quality fruits and high production can be achieved as long as the cucumber plants have established strong root systems and adequate soil moisture is available (Zarboutis and Gakni, 1992). It is thus critical in greenhouses to manage

the irrigation to control humidity which may become conducive to diseases of cucumbers.

The cucumber requires a proper balance of nutrients to produce a good crop. Greenhouse cucumbers have a high fertilizer requirement and thus they should never lack nutrients (Marr, 1995). Before planting, well decomposed manure or compost should be applied into the soil and then tillage and sterilization is necessary (Lerner and Dana, 2001). If the soil is low in nitrogen, phosphorus and potassium, fertilizers such as ammonium nitrate, potassium nitrate or super phosphate should be added after sterilization and tilled into the soil prior to planting (Wittwer and Honma, 1979). During crop growth, nutrients are injected in the irrigation water. These are commonly nitrogen and potassium by using soluble fertilizer materials such as potassium nitrate (13 percent N, 46 percent K) and ammonium nitrate (33 percent N). If magnesium deficiency should appear in the crop, the deficiency should be corrected by spraying magnesium sulfate (Wittwer and Honma, 1979; Zarboutis and Gakni, 1992).

Soil moisture is important and the crop must be supplied with adequate water for vigorous plant establishment and growth. It is necessary to maintain an adequate supply of water to plant roots, after transplantation. The requirements are dependent on the soil type, the air temperature and the radiation intensity (Zarboutis and Gakni, 1992). Watering should be limited in the morning or early afternoon so the foliage dries by the evening. This prevents the spread of leaf diseases (Lerner and Dana, 2001). The water used must not be cold because it can chill the roots and lead to slow plant growth with direct impacts on yield (Wittwer and Honma, 1979).

2.1.3 Diseases and insect pests

All crops are susceptible to insect damage. Whitefly is a very common insect that attacks vegetables in greenhouses. Its scientific name is *Trialeurodes vaporariorum* and not only feeds on plants but it also produces honeydew, which

detracts from the plants' appearance and attracts other insects and sooty mould. Whiteflies can also transmit plant viruses. They appear both on the upper and lower leaf surfaces. Off-colour or stunted plants are signs of a whitefly infestation (Greer, 2000). Thrips (*Frankliniella occidentalis)* are also a significant enemy to cucumbers that puncture the leaves, flowers, or stems with their mouthparts and suck up the exuding sap. Generally thrip injury on foliage causes a characteristic silvery appearance, eventually browning and dying with small black specks on the undersides of leaves (Mau and Kessing, 1993). A well known enemy to cucumbers is the twospotted spider mite (*Tetranychus urticae).* It feeds underneath the leaves and causes a stippled-bleached effect and later the leaves turn yellow, gray or bronze (Fasulo and Denmark, 2000). Aphids are also a common insect to cucumbers that can kill young plants and carry mosaic disease. They attack plants in late spring to early summer and spread rapidly (Peirce, 1987). Other pests include beetles, cutworms and nematodes.

The greenhouse environment with high temperature and humidity is ideal for diseases to develop. Downy mildew is a serious, destructive disease and causes a great deal of damage to greenhouse cucumbers. It is caused by *Pseudoperonospora cubensis* and is characterized by yellow-brown spots on the upper leaf surface with a purplish mould on the underside. Within a short time the whole leaf may die and the fruit becomes dwarfed (Horst, 1979). Powdery mildew is also a foliar disease caused by *Erysiphe chicoracearum*. The disease first appears as a white powdery spot on leaves with many spots eventually coalescing to cause infected leaves to wither and die. Powdery mildew can cause serious reduction in productivity in very warm growing areas and in greenhouses (Peirce, 1987). *Botrytis cinerea* is an important fungus that attacks greenhouse crops and causes gray mould disease. The symptoms are usually brown, soft lesions covered with grey-brown powdery masses of spores (Jarvis, 1992). Other known diseases are *Alternaria* leaf spot, *Fusarium* wilt, cucumber mosaic virus, white rot and *Verticillium* wilt (Panagopoulos, 1995).

2.2 THE PATHOGEN

2.2.1 *Pseudoperonospora cubensis*

The fungal pathogen which causes downy mildew diseases of plants belongs to the family Peronosporaceae (Thakur and Mathur, 2002) and in cucumbers this is caused by the fungus *Pseudoperonospora cubensis* (Berk. and M.A. Curtis) Rostovzev (Plate 2.1). It occurs mostly on cultivated members of the cucumber family (Cucurbitaceae) squash, muskmelons, and pumpkins and less frequently on watermelons (Sherf and Macnab, 1986). Its position in the classification hierarchy according to the Dictionary of the Fungi (9th ed.) is the following: Kingdom: Chromista, Phylum: Oomycota, Class: Oomycetes, Order: Peronosporales, Family: Peronosporaceae, Genus: *Pseudoperonospora*, Species: *Pseudoperonospora cubensis* (Berk. and M.A. Curtis) Rostovzev. Fungi belonging to the class of Oomycetes possess branching strands without cross-walls, aseptate mycelium (Snowdon, 1990). It was found in Cuba during 1868 and 20 years later in Japan. Since then the disease has been reported from every area of the world where moisture is adequate for infection and where the temperature is moderately high. (Sherf and Macnab, 1986; Panagopoulos, 1995).

The fungus causes a great deal of damage to all cucurbit crops but the disease is most severe on cucumber and cantaloupe. There are specific cucurbits crops that are more severely affected than others. However, different crops appear to be most severely affected in different areas. These differences could be due to the presence of different physiologic races of pathogen (Sherf and Macnab, 1986). The pathogen is an obligate parasite (Panstruga 2003; Latijnhouwers *et al*., 2003) so it does not survive from season to season where its hosts cannot over winter (Keinath *et al.*, 2007). It may exist on crop plants, volunteer crop plants, or wild cucurbits year-round in tropical and subtropical regions.

2.2.2 Symptoms of downy mildew

Symptoms of downy mildew vary with the host and environment. The first signs of lesions margins on most cucumbers are usually the appearance of indistinct pale green areas on the upper leaf surface. At this stage the symptoms on leaves have a mosaic mottling appearance (Zitter *et al*. 1996). Soon the pale green areas change to yellow angular spots in shape bounded by leaf veins (Sherf and Macnab, 1986). As the disease progresses the yellow angular spots may remain yellow or become tan to brown or almost black and necrotic with age (Plates 2.2 and 2.3). During moist weather, the underside surface of individual lesions is covered with a faint pale grey to purple growth (Horst, 1979). This mildew is made up of large microscopic lemon shaped spores (sporangia) borne on long branched conidiophores. Occasionally, the purple hue is lacking and the colour ranges from white to almost black (Sherf and Macnab, 1986; Zitter *et al*., 1996). Diseased leaves may turn brown and in a few days the entire leaf is dead. Fruits may be dwarfed and have poor flavour but seldom infected directly (Horst, 1979). Usually the older leaves are infected first, then symptoms appear on progressively younger leaves (Zitter *et al*., 1996).

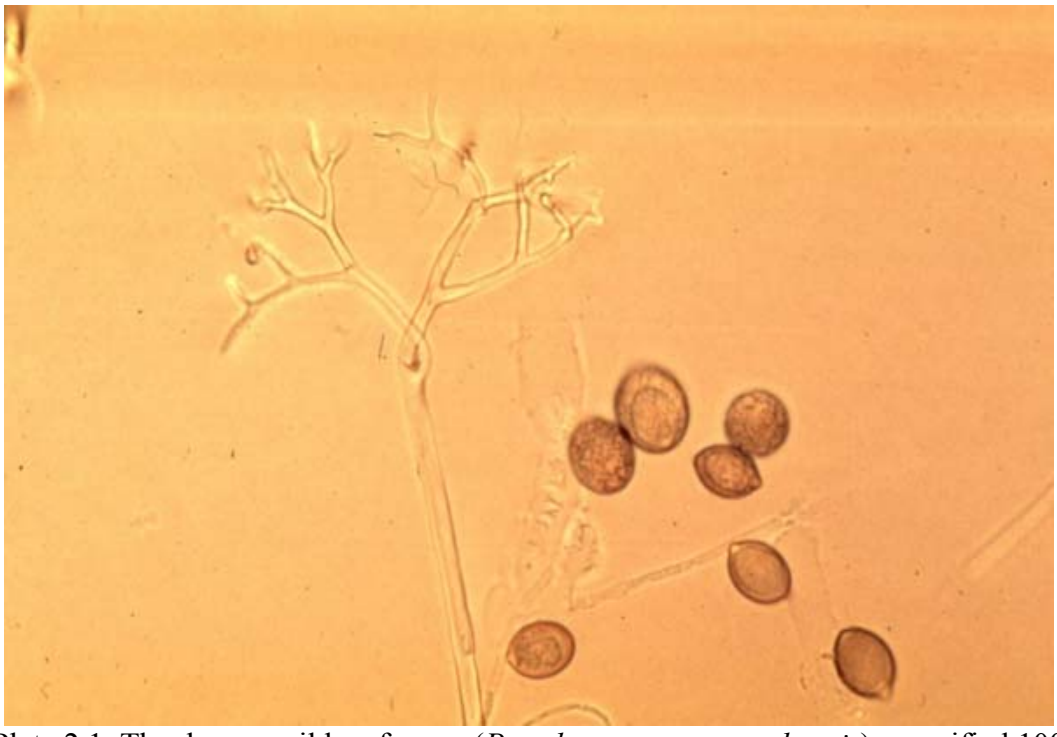

Plate 2.1. The downy mildew fungus (*Pseudoperonospora cubensis*) magnified 100 times. North American Plant Disease Forecast Center. NC State University.

Plate 2.2. Healthy cucumber leaf.

Plate 2.3. Infected cucumber leaf by *P. cubensis*.

2.2.3 Conditions for downy mildew development

Disease development can occur over a wide temperature range under conditions of high humidity resulting from dew, fog, or rainfall for different downy mildews. For example *P. parasitica* on cabbage can be very destructive at 10–15°C, and symptoms can develop at 24°C, but sporulation is limited beyond 24°C. In sorghum and maize symptoms of downy mildew infection develop at 25°C, but incidence is reduced < 20 and > 35°C (Thakur and Mathur, 2002). The disease is favoured by high humidity but the specific fungus (*P. cubensis*) does not require cool weather for reproduction as other downy mildews (Horst, 1979; Sherf and Macnab, 1986). It can infect plants at temperatures between 8-27 ^{o}C, the optimum lying between 18-23 ^{o}C (Blancard, 1994). According to Shi *et al*. (2005) the optimum temperature for the occurrence of cucumber downy mildew disease is 25-35 ^{o}C. *P. cubensis* can withstand high temperatures; it can survive several days when temperatures are over 37 ^{o}C (Blancard, 1994).

All Peronosporaceae require surface wetness for spore germination and infection, and high relative humidity for spore production (Malathrakis and Goumas, 1999). According to Cappelli and Buonaurio (2003), occurrence of wet climatic conditions due to frequent storms during the summer provide favourable environmental conditions for the development of secondary spread. Standing water on the cucumber leaves is necessary for at least short periods before infection can take place. (Blancard, 1994). Most sporulation occurs when temperature is around 20 °C and relative humidity over 95% (Thakur and Mathur, 2002). Infection success on cucumber plants depends on temperature which is not as critical as it is with many other vegetable diseases and dew periods which are the most important environmental factor affecting infection, development and spread of the disease (Shetty *et al*., 2002). Rouzet and Jacquin (2003) reported that extended dry periods can block oospore maturation. So sporangia must remain wet until they germinate, otherwise they die (Sherf and Macnab, 1986; Zitter *et al*., 1996). Except for these two environmental conditions, sporangial concentration is also a major factor for infection success (Panagopoulos, 1995).

2.2.4 Disease Cycle

The time of successful initial infection depends on the presence of the ideal weather conditions and the availability of inoculum because spore production must be efficient (Zitter *et al*., 1996). Reproduction in members of the Oomycetes class is usually both sexual and asexual. During the sexual phase, oospores are formed by the union of an oogonium, which according to Horst (1979) is a large globular multinucleate female cell, and an antheridium, a smaller male cell. After the host decay the oospores are set free (Snowdon, 1990). Oospores are thick-walled and long-lived, can withstand extreme environmental conditions in temperature and dryness and grow again when a susceptible crop is present and when the weather permits the infection. So downy mildew may overwinter as oospores which are the primary inoculum source (Thakur and Mathur, 2002; Sherf and Macnab, 1986). It is not fully understood how the pathogen survives from season to season and if the oospores play an important role (Zitter *et al*., 1996; Blancard, 1994).

The asexual spores of most Oomycetes (the lemon shaped sporangia that are borne on long branched sporagiophores) result in the formation of zoospores which are wall-less, uninucleate cells possessing two flagella of unequal length. Zoospores are dispersed in water drops, water films or through water-rich soils. The mechanism by which these spores are attracted to the host is not fully clear (Latijnhouwers *et al*., 2003). The sporangia are disseminated from plant to plant or to adjoining fields by splashing rains, moist air currents, insects, tools, farm equipment, the clothing of workers and through the handling of infected plants (Zitter *et al*., 1996). So, when a film of moisture is present on a leaf surface, the sporangia germinate and give rise to swarm zoospores, which swim for a while and then penetrate cucurbit leaves though stomata by producing germ tubes (Sherf and Macnab, 1986). Sporangia may also germinate directly producing germ tubes, thereby acting as conidia (Horst, 1979). The form of one or another kind of sporangial germination depends on the kind of the pathogen and the weather conditions (Tzamos, 2004). The greatest number of sporangial production occurs at 15-20 ^{o}C in day time and 15 ^{o}C at night (Panagopoulos, 1995). The primary source of inoculum in the field generally comes from airborne sporangia, which also serve

as a secondary source of inoculum. The pathogen can also overwinter in southern areas with mild winter temperatures, in the form of active mycelium in either cultivated or wild species of cucurbits (Zitter *et al*., 1996). It appears in the North in July or August, or it winters in greenhouses and later sporangia are spread to neighbouring cucumbers (Horst, 1979).

The minimum time of dew period that is necessary for a successful infection increases gradually as the inoculum concentration or the temperature is reduced (Panagopoulos, 1995). Infection can occur following dew periods of two hours at 20 ^{o}C, six hours at 15-20 ^{o}C, twelve hours at 10-15 ^{o}C and twenty four hours at 5-10 ^{o}C (Sherf and Macnab, 1986). The fungus cycle is relatively short. A few days after the infection a new crop of sporangia are produced. According to Zitter *et al*. (1996) this period is four to twelve days but Blancard (1994) reported that three to four days after the infection the conidiophores appear.

2.2.5 Disease Control

Since the disease expands rapidly and causes a great amount of damage to the cucumber cultivations it is essential to take precautionary measures like wide spacing between plants, planting in places with good soil drainage, air movement and exposure to sunlight. Avoidance of overhead watering especially at night and in the morning hours which can extend the period that the leaves will remain wet, thus producing conditions favourable for spores germination, should help check disease development (Zitter *et al*., 1996; Blancard, 1994). To minimise the amount of free standing water on the leaf surfaces, atmospheric humidity especially in the greenhouses should be reduced by ventilation (Blancard, 1994; Panagopoulos 1995). To help reduce the chance of spread from early plantings to late plantings, it is advised to avoid making successive plantings in nearby fields during a single season (Sherf and Macnab, 1986). It is also recommended to avoid growing the same variety in particular fields to reduce the inoculum build up and to restrict virulence selection in the pathogen population. A two-year crop rotation with a non-host crop in clean, well-drained soils is suggested (Thakur and Mathur, 2002).

As Blancard (1994) reported, drainage in soils that retain a great deal of water should be improved and badly contaminated soil must be disinfected as well as all the surfaces in the greenhouse. According to Thakur and Mathur (2002) since the pathogens survive in the form of oospores in the host tissues, removal, destruction and burning of the infected plant debris along with weeds serves to reduce the primary inoculum.

The use of resistant plant varieties to downy mildew, whenever possible, is an effective precautionary measure for controlling the disease development (Panagopoulos, 1995). Host plant resistance is a practical and economic method of control for downy mildews (Thakur and Mathur, 2002).

Major control measures include the use of fungicides. These applications may be necessary on susceptible varieties during weather conditions that are conducive to the disease occurrence. Since infections commonly take place on the undersides of the leaves, it is very important to get good coverage of the lower leave surface (Zitter *et al*., 1996). Both systemic and protective fungicides are available for control of downy mildew of cucurbits. Protective fungicides that provide good control include maneb plus sulphur, mancozeb and chlorothalonil (Sherf and Macnab, 1986). Protectively sprayings begin when the plants have two or three leaves and go on every four or seven days depending on the humidity conditions (Panagopoulos, 1995).

The advent of metalaxyl, a new systemic fungicide, provided a real breakthrough against the pathogen, although resistance to the fungicide exists (Sherf and Macnab, 1986). Since the 1970s, metalaxyl-based fungicides have been widely used for the management of downy mildews (Chaluvaraju *et al*., 2004; Panicker and Gangadharan, 1999). According to Latijnhouwers *et al*., (2003) this fungicide is highly effective against Oomycetes. Metalaxyl is absorbed through the leaves, stem and roots and inhibits protein synthesis in the fungus. It has various formulations, and can be applied as a seed treatment or foliar spray. Propamocarb and prothiocarb are two systemic fungicides that kill sporangia, inhibit sporulation on lesions and limit symptom severity. One of the three frequently used fully systemic fungicides besides metalaxyl and propamocarb is fosetyl-Al with very

good effectiveness against *P. cubensis* (Urban and Lebeda, 2006, 2007). Successful management seems to be by applications with combinations of metalaxyl and mancozeb or propamocarb and prothiocarb (Panagopoulos, 1995). Gupta and Shyam (1996) also reported that metalaxyl combined with mancozeb was the most effective treatment in reducing the number of sporangia. On the other hand Robak (1995) stated that the combination of cymoxanil, mancozeb and chlorothalonil gave the best control of cucumber downy mildew *P. cubensis*.

2.3 INFLUENCE OF NUTRITION ON PLANT DISEASE DEVELOPMENT

2.3.1 Nitrogen and plant disease

The mineral nutrition has been long recognized as an important component of control of plant diseases and has been studied for decades (Dordas, 2008; Reuveni and Reuveni, 1998; Walters and Bingham, 2007; Huber, 1980). Nitrogen is one of the most important nutrients for plant growth and disease development and there are many reports about its influence on disease incidence for various host-pathogen systems (Table 2.2).

Many researchers have reported contradictory conclusions about the significance of the amount of nitrogen and the time of its application. Thus, Leitch and Jenkins (1995) mentioned that the actual quantity of N applied to wheat rather than the timing of its application had greater influence on Septoria disease development. On the contrary Huber (1980) stated that delayed N application to wheat resulted in predisposition to take-all root rot. There are also inconsistencies in relation with the type of the pathogen. Dordas (2008) reported that there is a difference in response to N supply between obligate and facultative parasites. Regarding the obligate parasites there is an increase in disease severity with high N supply, however high N application decreases the severity of the infection caused by facultative parasites. In contrast Reuveni *et al*. (1996) reported that N fertilizers can induce systemic protection against both obligate and facultative parasites in maize.

Table 2.2. Some plant diseases affected by nitrogen (Huber and Thompson, 2007).

Disease or disorder	Pathogen or causal agent	Host	Effect of nitrogen[a] in the form of:		
			N (unspecified)[b]	NH_4	NO_3
Aflatoxin	*Aspergillus flavus* group	Corn, peanut	D	D	—
Anthracnose	*Gnomonia leptostyla*	Black walnut	D	D	D
Black root rot	*Thielaviopsis basicola*	Tobacco	—	D	I
Black root rot	*Rhizoctonia fragariae*	Strawberry	—	D	I
Black rot	*Cylindrocladium crotalariae*	Peanut	D	—	—
Blast	*Pyricularia oryzae*	Upland rice	I	—	—
Blast	*Magnaporthe grisea*	Rice	—	D	I
Blight	*Rhizoctonia* sp.	Tall fescue	I	—	—
Blight	*Xanthomonas* sp.	Syngonium	D	—	—
Blossom-end rot	Physiological disorder	Tomato	—	I	—
Brown patch	*Rhizoctonia solani*	Ryegrass	D	D	—
Bunch rot	*Botrytis cinerea*	Grapes	I	—	—
Canker	*Hypoxylon mammatum*	Aspen	I	—	—
Clubroot	*Plasmodiophora brassicae*	Cabbage	D	D	—
Corky root	*Rhizomonas suberifaciens*	Lettuce	I	—	—
Corky root	*Pyrenochaeta lycopersici*	Tomato	I	I	I
Crown and root rot	*Fusarium oxysporum*	Tomato	—	I	D
Crown rot	*Rhizoctonia solani*	Beets	—	I	D
Damping-off	*Rhizoctonia solani*	Snap bean, lima bean	I	—	—
Early blight	*Alternaria solani*	Potato	D	—	—
Eyespot	*Pseudocercosporella herpotrichoides*	Wheat	—	I	D
Gray leaf spot	*Cercospora zeae-maydis*	Corn	I	—	—
Leaf blast	*Magnaporthe grisea*	Rice	I	—	—
Leaf blight	*Erwinia chrysanthemi*	Philodendron	D	—	—
Leaf blight	*Erwinia chrysanthemi*	Chrysanthemum	I	I	I
Leaf spot	*Alternaria macrospora*	Cotton	—	D	—
Leaf spot	*Pseudomonas cichorii*	Chrysanthemum	I	—	—
Leaf spot	*Xanthomonas hederae*	Schefflera	D	—	—
Lesion nematode	*Pratylenchus penetrans*	Strawberry	—	D	I
Mildew	*Erysiphe graminis*	Wheat	I	—	—
Mosaic	Brazilian *Soybean mosaic virus*	Soybean	I	—	—
Mummy berry	*Monilinia vaccinii-corymbosi*	Blueberry	—	D	—
Nematodes	*Criconemella* spp. and others	Turfgrass	—	—	I
Nodorum blotch	*Stagonospora nodorum*	Wheat	D	—	—
Pod rot	*Pythium, Rhizoctonia, Fusarium* spp.	Peanut	I	—	—
Red thread	*Laetisaria fuciformis*	Turfgrass	D	—	—
Root rot	*Sclerotium rolfsii*	Carrot	D	—	—
Root rot	*Gibberella zeae*	Carnation	I	—	—
Root rot	*Fusarium oxysporum, F. moniliforme*	Asparagus	—	I	D
Root rot	*Pythium* spp.	Geraniums	I	—	—
Root rot	*Phytophthora parasitica*	Tomato	I	—	I
Root rot	*Fusarium* spp.	Pea	D	I	D
Root rot	*Thielaviopsis basicola*	Pansy	—	D	I
Root rot	*Phytophthora* spp.	Oak, beech	I	—	—
Root rot	*Rhizoctonia solani*	Rape, canola	D	—	—
Root rot	*Phymatotrichum omnivorum*	Cotton	D	—	—

[a] D=decreased severity, I=Increased severity, — =No effect or not reported.
[b] The form of N was not reported or was inconsequential.

It has been reported by Huber (1980) that the greatest disease response to mineral elements is usually with tolerant or moderately resistant plants, while disease reactions of highly resistant or highly susceptible plants are not altered by nutrition. On the contrary, Long *et al*. (2000) stated that nitrogen increased lesion area of rice blast on very susceptible cultivars, while N application did not affect the highly resistant cultivar. According to Reuveni and Reuveni (1998) N fertilizer application to resistant varieties of cucumber leads to significantly higher powdery mildew control compared with susceptible ones.

The form of nitrogen is an important factor that affects plant diseases. According to Huber and Watson (1974) the effect of specific forms of N on disease severity is not the same for all host-parasite associations. A given form of N may reduce one disease but increase another (Table 2.2) or a specific N form may decrease one disease while other N form may not affect this disease (Hatcher *et al.* 1997). The influence of the different forms of N on various plant diseases such as seedling diseases, root rots, cortical diseases, vascular wilts, foliar diseases, gall, canker and virus diseases are well documented by the previous authors. The situation, though, is quite complicated because of biological activities in soil such as nitrification. Thus, different forms of N may be available by commercial fertilizers but the predominant N form in soil is probably determined more by the soil environment than by the form of N applied (Huber and Thompson, 2007).

Mechanisms of nitrogen effect on plant disease

According to Huber and Thompson (2007) the way that N influences plant disease is determined by three factors:

(a) *Changes in plant physiology*: Nitrogen affects metabolic pathways, growth, plant constituents or exudates, i.e. nitrogen is the main mineral factor that reduces cellulose content of the cell wall and thereby affects the mechanical strength of them. Foliar pathogens are able to penetrate and develop more rapidly.

(b) *Effect on growth of pathogen*: The amount and form of N may affect the growth and virulence of pathogens by stimulating or inhibiting enzyme synthesis or activity required for pathogenesis.

(c) *Modification of the biotic and abiotic environment*: Root absorption of NH_4-N reduces the rhizosphere pH while pH is increased as NO_3-N is absorbed. Changes of pH have been proposed as the mechanism controlling some diseases, e.g. take-all of wheat (Huber and Watson, 1974).

Most scientists agree that nitrogen generally increases plant susceptibility to diseases (Huber and Thompson, 2007; Reuveni and Reuveni, 1998; Simoglou, 2004). However, it is rather unwise to generalize about the effects of N on plant diseases without taking into account information about N supply, the pathogen and the kind of crop that is under consideration. Thus, the rate and time of application, form of N, soil conditions, interactions with other elements (Huber and Thompson, 2007), pathogen type (Dordas, 2008; Hoffland *et al*. 2000), amount of N applied (Leitch and Jenkins, 1995) should be taken into account. Hence, each disease must be considered on an individual basis.

2.3.2 Potassium and plant disease

In general most authors consider that potassium tends to improve plant health and reduce development of many diseases caused by various pathogens. (Table 2.3; Amtmann *et al*., 2008). This is reported to occur mainly for fungal diseases. However, as in the case of nitrogen, this generalization should be avoided without giving adequate consideration to associated anions, nutrient balance and status (Prabhu *et al*. 2007).

Prabhu *et al*. (2007) also stated that K is associated more with host resistance than its direct affect on the pathogen, and that in some cases the positive effect of K on plant diseases is evident only when the element is deficient in soil. It is also reported by the same authors that the effect of excess potassium depends on the crop, the disease and the environment.

Table 2.3. Number of published papers reporting effects of potassium on disease (Prabhu *et al*. 2007).

	Decrease in disease	Increase in disease	No effect	Total
Fungal diseases	89	33	8	130
Bacterial diseases	19	5	… [a]	24
Virus diseases	9	5	3	17
Nematode diseases	3	6	1	10

[a] Data not available.

Many studies agree that the balance between nutrients, the ratio N: K or ratio between cations greatly influences disease development in various pathosystems (Dordas, 2008; Reuveni and Reuveni, 1998; Bains and Jhooty, 1984). Prabhu *et al*. (1999) also showed that K fertilization in the absence of N significantly decreased panicle blast development of rice caused by *Pyricularia grisea.* On the other hand in the medium level of N applied, disease severity increased up to a limit and beyond this decreased with increasing rates of K_2O. They concluded that the effect of K_2O on blast severity depended upon the rates of N fertilization, given that when N was applied at the high rate, K fertilization did not affect the disease. Consequently, they speculated that the K: N ratio is more important than the effect of each individual nutrient in the panicle blast development.

Prabhu *et al*. (2007) reviewed many cases in which interactions of K with other nutrients have been demonstrated for various plant diseases. Thus, some diseases were increased when K was applied alone but were reduced by adding other nutrients such as Mg and Zn or in some cases a combination of nutrients (K and Si) or a complete fertilizer (N, P, K) had the lowest disease severity.

An interesting statement is that the reduced disease severity obtained by K application might have been because of Cl of the added KCl fertilizer and not because of K (Huber, 1980; Prabhu *et al*., 2007; Sweeney *et al*., 2000). Therefore it is often difficult to distinguish between the effect of K and the effect of the anion in

the fertilizer salt used. As a consequence, the effect on disease may be erroneously attributed to one or the other element. This is reinforced by the fact that it is well known that Cl can affect various plant diseases independently of K. Elmer (2007) discusses many reports about disease suppression with chloride fertilization for various crops. Moreover Sanogo and Yang (2001) reported that KCl reduced development of sudden death syndrome of soybean but conversely other potassium salts as KNO_3, K_2PO_4 and K_2SO_4 increased disease severity.

In some cases the disease development was correlated with the concentrations of nutrients in plant tissues. Filippi and Prabhu (1998) studied the relationship between panicle blast severity and mineral nutrient content of plant tissue in rice and found that K and Ca in plant tissues were negatively correlated with the disease severity whereas N, P and Mg were positively correlated with it.

Mechanisms of K effect on plant disease

K nutrition affects the reaction of a plant to diseases in the following ways:

(a) Direct effect on the pathogen by inhibiting its germination, penetration or enzymatic activity and survival (Huber, 1980), e.g. Prabhu *et al*. (2007) concluded that K stimulates the germination and formation of appressoria of *P. grisea* and conidia of *Phomopsis juniperovora*.

(b) Effects on the plant physiology or plant metabolism, thus affecting food supply for the pathogen, e.g. K deficient plants accumulate N compounds such as amides which are used by invading pathogens (Dordas, 2008)

(c) Effects on the establishment and development of the pathogen within plant. This is achieved through the K influence on plant defence responses and cell-wall ultrastructures and function of stomata (Reuveni and Reuveni, 1998), i.e. K promotes the development of thicker cell walls which prevent disease attack (function as mechanical barriers to infection by pathogens) or limits the pathogens' growth (as a result of higher proportion of more sclerenchyma tissue development) (Prabhu *et al*., 2007; Huber, 1980).

2.3.3 Concluding remarks

The severity of most diseases can be reduced by management of mineral nutrition through modifying the availability of particular nutrients. K application was found to be beneficial for resistance against fungi in the majority of the cases. However the mechanism involved in disease suppression by K is not very well understood. In the case of N, it is well recognized that diseases differ in their response to N forms. In order to enhance plant disease control not only the N form but also the timing and the rate of N application should be considered. Balanced nutrition has a great importance in plant resistance to diseases, as well. Finally, each disease-host nutrition interaction should be studied in the specific environmental conditions.

2.4 ECONOMIC IMPORTANCE OF THE CUCUMBER CROP IN GREECE

A lot of crops are cultivated in Greece due to the Mediterranean climate. Vegetables occupy a high position in agricultural production which brings Greece into twenty-fourth position among 185 countries, according to the FAO. Table 2.4 shows the fruits and vegetables production in Greece and share in world. Cucumber (*Cucumis sativus*) is the third vegetable crop in terms of production after tomato and watermelon in Greece (Table 2.5) and according to the Department of Agricultural Development (Heraklion) almost 70% of the total production of the country is cultivated in the island of Crete. It is widely known that cucumber is susceptible to diseases (Neykov and Dobrev, 1988). Thus, downy mildew is an important cucumber disease not only in Greece but also in most cucumber production areas worldwide as reported by Shetty *et al*. (2002). This disease causes major yield reductions and has been a severe problem on cucumbers in Europe since 1984 (Lebeda and Widrlechner, 2003a). The pathogen is a serious threat and in years of epidemics and under favourable conditions, it can destroy 100% of plants (Neykov and Dobrev, 1988).

The most effective current way of controlling downy mildew consists of using resistant cultivars (Neykov and Dobrev, 1988; Shetty *et al*., 2002) and fungicide applications that are necessary on susceptible varieties. Although resistant varieties of cucumber have been developed in several countries, various races of the pathogen have been found (Shetty *et al*., 2002). In addition the pathogen (*P. cubensis*) is characterized by large variation in pathogenicity (Lebeda and Widrlechner, 2003a, b). Thakur and Mathur (2002) stated that stability of resistant varieties to downy mildews depends on the evolution of new pathotypes or races of the pathogen. Furthermore, according to Robak (1995) even a resistant cucumber variety, under high infection pressure, has to be chemically protected against downy mildew disease.

On the other hand fungicide applications are very common in cucumber cultivations. Their use has increased dramatically in recent years (Table 2.6). There is no doubt that fungicides use plays a significant role in plant protection against diseases and pests and therefore in stabilizing our food supply. However, it is certain that the excessive fungicide application threatens human health and causes environmental pollution. Nowadays there is widespread public concern about the use of pesticides, including fungicides and their effect on the environment. Besides, the continuous use of fungicides has produced resistance in many pathogens. Thus, strains of *P. cubensis* resistant to fungicides have been found in several countries (Blancard *et al*., 1994). Thus, a more sustainable approach to fungicide use combined with other methods of control would be beneficial.

These limitations associated with the methods routinely used (resistant varieties and fungicides) give emphasis to the need for newer methods of downy mildew control as an alternative. A perusal of the literature on diseases reveals that there is enough evidence to show that crop nutrition can influence disease development in a range of pathosystems. The relationship of cucumber nutrition to downy mildew though has not been much studied especially in field conditions. If control of this disease through nutrient manipulation is to be identified then minimization of both chemical expense and environmental hazards could be achieved.

Table 2.4. Production in Greece of fruits and vegetables and share in world (FAOSTAT, 2008).

	1979-1981	1989-1991	1999-2001	2003	2004
Production (1000 tonnes)	7073	8076	8591	7072	7782
Share in world %	1.12	0.99	0.71	0.53	0.56

Table 2.5. Production and area harvested of the main vegetable crops in Greece in the last three years (FAOSTAT, 2008).

Vegetable crop	2005		2006		2007	
	Production (tonnes)	Area Harvested (Ha)	Production (tonnes)	Area Harvested (Ha)	Production (tonnes)	Area Harvested (Ha)
Tomato	1707376	35621	1568183	33577	1450000	26500
Watermelon	697490	17452	703654	16609	635000	14200
Cucumber	157275	2757	152900	2693	171700	2200
Melon	167922	8083	157823	7888	143500	6300
Pumpkin	99860	5082	91900	4000	92300	4100

Table 2.6. Use of fungicides and bactericides in Greece (FAOSTAT, 2008)

Year	Use (tonnes)	Year	Use (tonnes)
1992	2670	1997	3047
1993	3474	1998	4731
1994	1423	1999	3707
1995	2554	2000	4676
1996	2591	2001	4860

CHAPTER THREE
GENERAL MATERIAL AND METHODS

3.1 EXPERIMENTAL PLOTS AND LAYOUT

The experiments that were conducted in this thesis were established during the 2006-2008 period in a greenhouse at the farm of the Technological Educational Institute of Heraklion in Greece (Figure 3.1). Throughout the experiments, plants were cultivated in 7 L volume plastic pots. The distance between the pots in each plot was 50 cm and the plots were 80 cm from each other. The replicates of each experiment were kept 100 cm apart (Figure 3.2). All the pots were filled with 7800 g of surface soil (0 – 20 cm) taken from the farm of TEI, which was well mixed and sieved using a 4mm sieve first. After its placement in the pots, they were watered until full soil water capacity. The soil had the following properties; texture=SL (sandy loam), s.g.=1.3 g cm^{-3}, pH=7.4, EC=2.6 mS/cm, $CaCO_3$ =24% (total calcium carbonate).

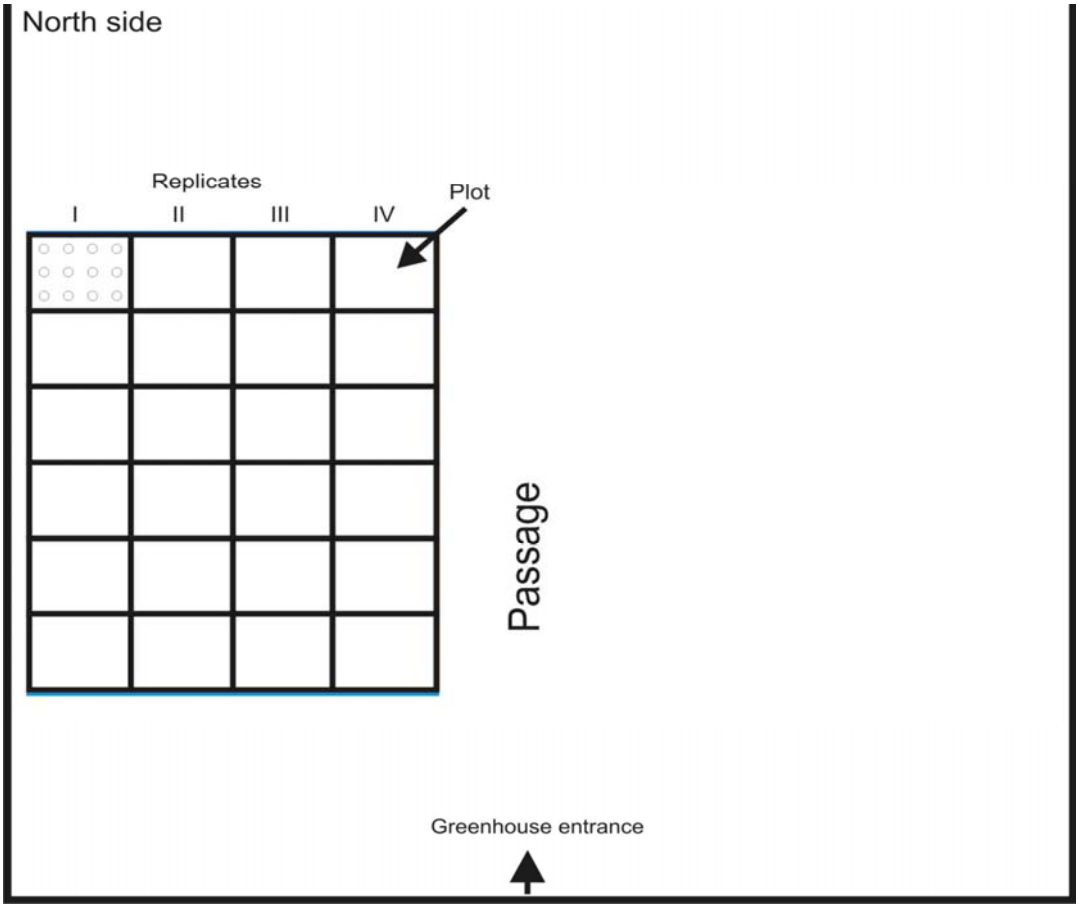

Figure 3.1. Experiments position in the greenhouse.

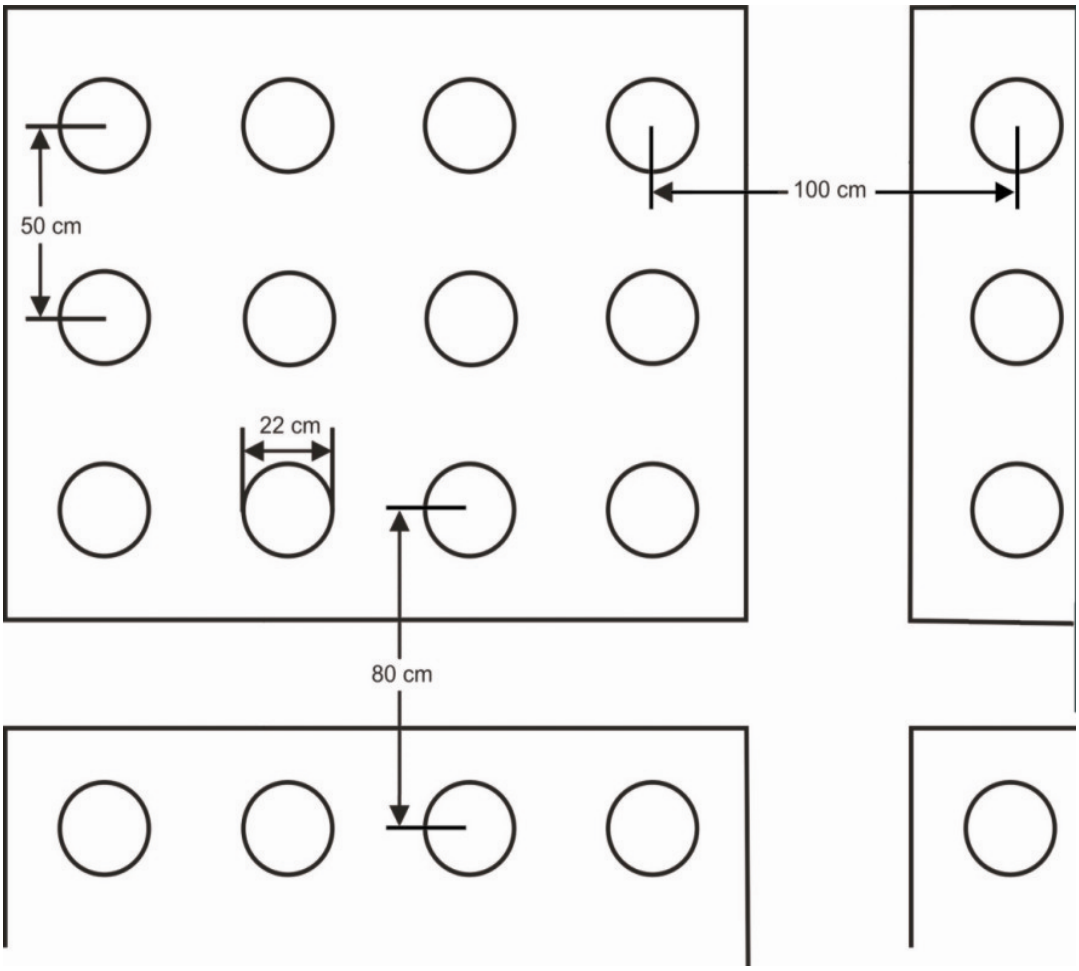

Figure 3.2. Distances in the experimental design.

3.2 CULTIVATION OF CUCUMBER PLANTS

Cucumber plants, *Cucumis sativus* L. of Knossou variety (Plate 3.1; short cucumbers, length 15-22 cm, diameter 3.5-5.5 cm) were used in all the experiments. This variety is susceptible to *P. cubensis* and commonly cultivated in Crete. To obtain a highly uniform seedling population the seeds were pre-germinated in Petri dishes with maximum moisture content at 30 ^{o}C for two days. Uniformly sized (3-5 mm radicle length) germinated seeds were planted into compost in plastic disks. The disks were placed in the greenhouse and watered when needed with tap water until the first leaf of the plants was fully expanded and then transplanted into the pots. During this procedure 100 cm^3 of compost was added to each pot around the plant roots to prevent breaking. During the first week the cucumbers were each irrigated when necessary with 500 mls water. With this amount of water some water drainage was observed. Plate 3.2 shows the plants at the 5th leaf stage. Afterwards the fertilizer application regime was made according to the treatments of each experiment. The quantity of solution added was determined by the point where run off from the pots occurred (500 mls). If no drainage was observed then an additional small amount of the appropriate treatment solution was added (30-50 mls). In this way, the elements concentration in the soil solution in each pot was maintained at the required concentrations. The rate of nutritional additions was increased as the plants grew because of the rate of plant growth and because of the higher transpiration as consequence of the increased temperature (especially in experiments during the spring period).

The plants were trained upwards so that the main stem was allowed to climb to the overhead wire along a polyethylene twine. The horizontal wires were attached three meters above the ground. All the cultural practices such as training, weeding, laterals removing, were applied in the same way in all the plots. The environmental conditions inside the greenhouse were recorded with the aid of Data Loggers. Tiny Talks (Gemini Data Loggers) monitored temperature and relative humidity (RH) all along the experiments (Appendix A, Plate A1). They were programmed to take records at one hour intervals inside the greenhouse during each experiment (Appendix C, Tables C1, C2, C3 and C4).

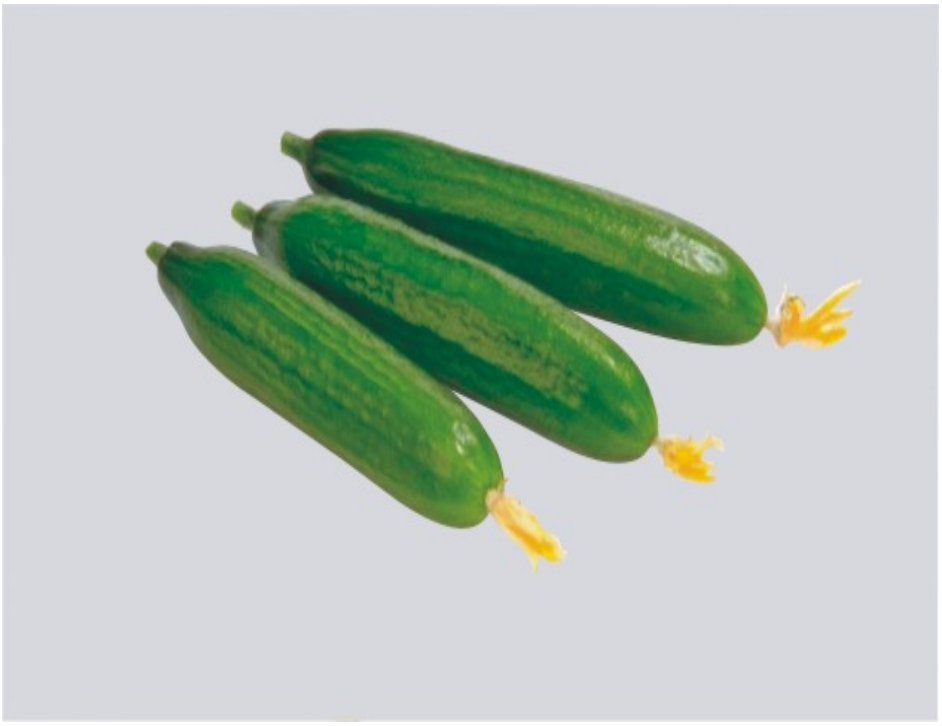

Plate 3.1. Fruit of cucumbers cv. Knossou.

Plate 3.2. Plot of cucumber plants at the fifth leaf stage.

3.3 PATHOGEN MAINTENANCE

In order to ensure that the same inoculum level was maintained for each inoculation, ten cucumber plants of the same variety were planted every twenty days in the plastic pots. An isolate of *Pseudoperonospora cubensis* (Berk. and M.A. Curtis) Rostovzev was obtained from a local greenhouse and subsequently maintained on these cucumber plants. After inoculation, the plants were incubated in a dew chamber at 20 ^{o}C, in the dark, for 48 hrs. Plants were then returned to the greenhouse conditions for disease development. Inoculum was obtained from freshly sporulating leaves two weeks after inoculation. Afterwards the next ten plants were inoculated and the cycle repeated.

3.4 PLANT INOCULATIONS

All the plants were inoculated with the pathogen *P. cubensis*. The inoculum was collected from freshly sporulating leaves of the plants in which the inoculum was maintained. Zoospores were gently brushed into a small quantity of distilled water containing two drops of Tween-20. The suspension was diluted and adjusted to a known concentration of spores per milliliter with the aid of a haemocytometer (details for the final spore concentration are given in each experiment). The fungus was applied by placing five droplets of the suspension on the upper side of the leaves, using a Pasteur pipette. The leaf that was going to be inoculated was previously marked with a tape in order to be distinguishable during the inoculation. After inoculation, the plants were put in a humidity chamber (average relative humidity of 24 hrs RH=95%) for 24 h at 25 ^{o}C in the dark. Plants were then kept in greenhouse conditions for symptom production. These conditions are necessary for disease development because most sporulation occurs when temperature is around 20 ^{o}C and at > 95% RH (Thakur and Mathur, 2002; Zitter *et al*., 1996). A week later the first symptoms/lesions were observed.

3.5 MEASUREMENTS – ANALYTICAL METHODS

3.5.1 Disease assessment

A digital photo of all inoculated leaves was taken at various intervals ten days after inoculation (Appendix A, Plates A2-A7). A black thick sheet of paper was used as a background with one centimeter calibration line on it as a measure in order to calibrate the relative software. These images were later used to assess disease development with the aid of special software (Bersoft Image Measurement, 4x Professional). Thus, the total leaf area as well as the lesion area was measured at each sampling and the results reported as cm^2. The relative leaf and lesion area was also calculated.

3.5.2 Leaf and soil sampling

In order to determine macro- and micro-elements, leaf and soil samples were taken two days before the plant inoculation. The fifth or sixth leaf (most recent fully developed leaf) from the apex of each plant was selected. Six, eight or twelve leaves were taken from each plot depending on the number of pots in each experiment and then combined to form a mixed sample. The leaf samples were then put into paper bags, labeled with the treatment number and replicate (plot characteristics) and transferred to the laboratory for analyses.

The soil sampling was carried out by taking cores from each pot using a sampling tool. Each sample (one sample per plot) was composed of mixed soil cores. The soil samples were put in bags with their identification numbers written on them and removed to the laboratory for analyses.

3.5.3 Nutrient determination

In order to determine the nutrients in cucumber leaf tissues and soil the analyses were carried out twice according to the international standards procedures of the laboratories.

(a) Leaf analysis

Leaf samples (blades) were washed by placing them in basins containing water and 2% detergent solution (Jones *et al*., 1991). The leaves were lightly brushed in order to detach any dust particles without breaking the plant tissues. They were then cleaned with water, rinsed with distilled water and finally left on a sieve for draining. Each leaf sample was placed on filter paper with the record of sample on it and oven dried at 80 ^{o}C for 48 hrs (oven with forced air, Binder). Enzyme activity interception and grinding was done after drying. An agate mill (Retsch, RM 100) was used with the aim of obtaining a homogeneous sample. Ground leaves were put into plastic boxes.

In order to determine the moisture of the plant tissues a small quantity of each sample (approx. 1g) was weighed and transferred to a 100 ml Erlenmeyer flask after its net weight had been recorded with the aid of an analytical balance. The flasks were then oven dried at 104 ^{o}C for 24 hrs (Memmert). They were then kept in desiccators and the final dry mix weighed with an analytical balance. The net weight was obtained by subtraction.

In order to destroy the organic matter, wet oxidation was carried out by digesting the samples. HNO_3, H_2SO_4 and $HClO_4$ in the proportion 5:1:2 respectively was used (Chapman and Pratt, 1961; Tsikalas, 2003). A volume of 20 mls of this mix of acids was dispensed in each Erlenmeyer flask and positioned on a hot plate inside the fume cabinet (Appendix A, Plate A8). The samples were heated at low temperature (approx. 100 ^{o}C) for several minutes taking care to avoid frothing and thus boiling over of the sample. After this the temperature was raised and samples heated until fumes evolved. The pots were covered with watch glasses during the digestion until a clear coloured liquid was observed. The digestion was

continued until the volume of the solution inside the flask was reduced to about 3 mls. At this point the flasks were taken off the hot plate and left to cool. A small quantity of distilled water was added afterwards to the cooled samples through the wall of the flask in order to dilute the solution. The samples were then filtered and acid washed, using 125 mm diameter filter papers into 100 ml volumetric flasks (Whatman No 41, ashless). A small volume of distilled water was rinsed into each Erlenmeyer flask twice and left to flow through the funnel with the filter paper by gravity. The solutions (stock) were decanted into 100 ml volume plastic bottles with the identification number recorded on them and stored in the refrigerator for later analysis.

An aliquot of the stock solutions was placed into small tubes in order to determine the nutrient levels. Calcium, magnesium, potassium, iron, manganese, copper, and zinc were determined with Atomic Absorption Spectrophotometer (Perkin Elmer 2100). For potassium determination the emission technique was used. At first, dilutions were made for calcium, magnesium and potassium determinations in order for the sample measurements to be within the linear part of the corresponding curve of each one of the above three elements. The dilutions were prepared using a Sample Changer with Dilutor (Sample Changer model 221 and Dilutor model 401, Gilson). The stock solution was diluted twice with the same dilution factor (DF=14), thus the total DF was 196. For this purpose 700 μls of each stock solution was diluted with 9100 μls distilled water and the final solution was shaken vigorously and then was diluted again in the same way. The standard solutions for calibration were made by diluting the appropriate volumes of 1000 ppm standard of each element in order to have the suggested range of the calibration curve. The results that were obtained from Atomic Absorption Spectroscopy (AAS) were expressed as ppm relative to the stock solution. The results were calculated as percentage of DM for macroelements and as ppm of dry matter for microelements.

Total phosphorus in leaves was determined by using the phosphomolybdovanadate yellow method (Chapman and Pratt, 1961). From each one of the above stock solutions 2 mls were received in a new test tube in which

1.6 mls molybdovanadate acid was added by using sample changer with dilutor. A volume of 4.4 mls distilled water was added in the tubes afterwards with the same apparatus. The final solutions were shaken well and after 30 min (time necessary for full yellow colour development) the P concentration was measured at 470 nm wave length on a spectrophotometer (VIS-UVI Single – Double beam, Hitachi U-2000). Standards solutions for calibration were made by taking 0, 5, 10, 15 and 20 mls aliquots of the 50 ppm standard phosphorus solution, diluting to 50 mls volume and developing the colour. Phosphorus concentration was calculated as ppm of solution. The final results were reported relative to percentage of DM.

Ammonium nitrogen and nitrate nitrogen of leaves was extracted from plant tissue using KCl and analyzed by Direct Distillation. Approximately 0.5g of ground tissues were weighed using an analytical balance (Sartorius handy) (four decimals measurements were recorded) into a watch glass and transferred to a 50 mls Erlenmeyer flask. A volume of 15 mls of 2 N KCl solution was added to each flask using a constant suction pipette. Samples were shaken for 15 minutes on an oscillating shaker and filtered immediately. Ammonium nitrogen and nitrate nitrogen were analyzed by steam distillation using Kjeltec apparatus (Kjeltec Auto 1030 Analyzer). Thus, each solution was transferred into tubes and attached to the distillation apparatus. Distillation was commenced with the ammonia being absorbed in H_3BO_3. Hydrochloric acid (0.05M) was used to titrate the distillate. After NH_4-N measurement, 0.2g Devardas Alloy reagent was transferred rapidly into the same Kjeldahl tube and distillation commenced again. Nitrate nitrogen of the same sample was reduced to ammonia by Devardas Alloy and ammonia was steam distilled into H_3BO_3 solution as above. The distillate was titrated with 0.05M hydrochloric acid. Nitrate nitrogen was thus determined. A blank sample was measured every five samples. The results were obtained using the following formula for each one of the nitrogen forms; % N = (A-B)xMx1.401/W, A = ml of 0.05M HCl needed for titration of sample, B = ml of 0.05M HCl needed for titration of Blank sample, M= Molarity of 0.05M HCl solution, W = Dry Weight of sample reported to grams. The final results reported as ppm of nitrogen in the form of NH_4-N and NO_3-N in plant tissues.

(b) Soil analysis

The soil samples were spread out on trays of stout paper in the laboratory, stones were removed and large soil aggregates broken up. After the samples were well mixed they were spread out in a thin layer and divided into four parts with a large spatula. Two opposite quarters were discarded and the remainder of each sample was labeled. The soil samples were left to air dry and regularly mixed to ensure an even drying process. Then gentle crushing followed using a porcelain mortar and pestle to avoid breaking up gravel. The air dried soil sample was sieved through a 2mm sieve, returned to its tray and left in a temporary storage space until analysis.

Ammonium acetate was used to extract available potassium from the soil (Dewis and Freitas, 1970). Thus, 5g of air dried soil of each sample was used. For this purpose trays with samples were wheeled in on a trolley from the soil preparation room to the soil weighing room and were placed, one at a time, next to the balance during weighing, thus avoiding contamination of samples by accidental spillage. In order to ensure that each small portion taken for analysis contained as far as possible, the same proportion of different sized particles as in the main sample, the well mixed sample was spread out on its tray and small portions were taken at random from the full depth of the soil layer with a spatula, until the required weight was obtained. The samples were weighed out on a scoop of metal and transferred to 250 mls Erlenmeyer flask where 100 mls 1 N CH_3COONH_4 solution was added by using measuring cylinders. Samples were shaken on a reciprocating rotator (Thermolyne) for 30 minutes and filtered through funnel tubes with 125mm diameter filter papers (Whatman No 41).

The potassium concentration of the extracts was measured by flame photometry, calibrating the photometer (Jenway) with the standards 0, 10, 25, 50, 75 and 100 ppm K containing 0-100 ppm K in 1 N CH_3COONH_4. Thus, a standard potassium solution (100 ppm) and 1N CH_3COONH_4 were sprayed alternately and the sensitivity controls was operated until the standard read a selected point (100) on the photometer point and the ammonium acetate read zero (blank value). The other standard potassium solutions were then sprayed and the corresponding scale

readings recorded. The test solutions were sprayed afterwards and their values recorded, checking the photometer performance every five samples. The concentrations of the test solutions were obtained by reference to the calibration graph (Figure 3.3). The final results were reported as ppm of potassium in soil.

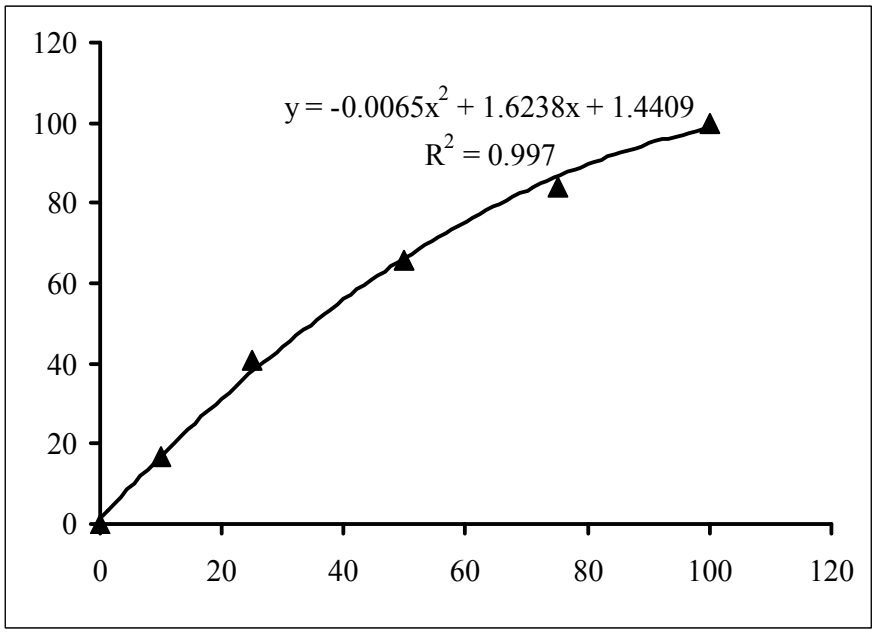

Figure 3.3. Calibration curve of the flame photometer used in soil potassium determination.

NH_4-N and NO_3-N of soil were determined by Steam Direct Distillation (Hesse, 1972). Approximately 2g of soil (exact measurements were recorded by using an analytical balance) were extracted with distilled water (Barker, 1974). The extracts were transferred into the tubes of the Kjeltec apparatus. The liberated NH_4-N was steam distilled into H_3BO_3 solution. The distillate was titrated automatically with standard hydrochloric acid (0.05M) (green color changed to pink). The volume of the titrant needed was recorded for NH_4-N determination. Into the same tube after NH_4-N distillation, Devardas Alloy reagent was added for reduction of nitrate to ammonia. Therefore, 0.2g Devardas Alloy was added rapidly to the tube and ammonia was steam distilled into a fresh portion of H_3BO_3 solution. HCl was then used as titrant (0.05M). Its volume was recorded in order to

determine NO_3-N. The formula % N = (A-B)xMx1.401/W was used as above (NH_4-N and NO_3-N determination of leaves) and the final results reported as ppm of NH_4-N and NO_3-N in soil.

3.6 STATISTICAL ANALYSIS

In order to detect differences in disease incidence as well as in leaves and soil nutrients between treatments, analysis of variance (ANOVA) was performed for each variable separately in the Randomized Blocks experiments. ANOVA was also carried out for the data obtained after calculation of the existing variables. The analyses were carried out after checking normality of each variable using Kolmogorov-Smirnov and Shapiro-Wilk tests. In order to examine the development rate of leaf and lesion area after the infection of the leaves until the end of each experiment Regression Analysis was performed and the model best fitting the data was investigated in each case. Significant differences between the mean values of the various treatments were determined according to Duncan's Multiple Range test at the P = 0.05 level. In order to study the relationships among the variables Pearson's Rank Correlation was carried out revealing various correlation coefficients. To examine the effect of nutrients on disease development mainly, covariance analysis was conducted with nutrients concentrations in leaves and soil as covariates. Specifically for the factorial experiment, analysis of variance was conducted according to Randomized Block design with all the treatments (eight in total) as well as independently with the six factorial treatments in order to detect interactions between nitrogen and potassium levels.

CHAPTER FOUR

EFFECT OF ZOOSPORE CONCENTRATION ON CUCUMBER DOWNY MILDEW

4.1 INTRODUCTION

It is well understood that for artificial inoculations the spore concentration of the suspension is one of the main factors that has to be considered. Biles *et al*. (1995) reported that a low concentration of *Phytopthora capsici* zoospore suspension was insufficient for optimum infection whereas a high zoospore content did not permit the differentiation of environmental effects on pepper infection and thus the objective could not be accomplished. In order to artificially inoculate cucumber plants with *P. cubensis* Williams and Palmer (1982) suggested that the spore inoculum should be adjusted to $12x10^4$ sporangia ml^{-1} with distilled water. Thus, similar concentrations of this fungus such as $1x10^5$ spores ml^{-1} of suspension has been used (Urban and Lebeda, 2004, 2007). Lindenthal *et al*. (2005) also inoculated cucumber leaves by spraying them with $1x10^5$ zoosporangia ml^{-1} of *P. cubensis* but in other experiment in the same study they used quite higher concentrations of this fungus, such as $5x10^5$ zoosporangia ml^{-1} by placing suspension droplets on the leaf surface. Nevertheless, many scientists used suspensions with much lower spore concentrations and still achieved successful plant inoculations. Thus, Reuveni and Ravin (1997) used $2.5x10^4$ sporangia of *P. cubensis* per ml of suspension. Even lower spore contents have been reported such as 5000 (Portz *et al*., 2008; Baider and Cohen, 2003) or 2000 sporangia ml^{-1} of suspension (Cohen *et al*., 2003).

Furthermore, the leaf age has an essential importance for infection. It has been reported for other pathogens that artificial inoculation of some parts of the plants is hardly achieved. A long time ago authors found that inoculation of cotyledons of cucumber seedlings with *Didymella bryoniae* was unreliable (Wyszogrodzka *et al*., 1986; Van Der Meer *et al*., 1978). A more recent study also showed that susceptibility increased with leaf age for all cucumber cultivars tested

for symptoms of gummy stem blight (St. Amand and Wehner, 1995). Although it is known for cucumber plants that in field conditions usually the older leaves are infected first and then the younger ones (Zitter *et al*., 1996), successful infection has been achieved for cotyledons. Wyszogrodzka *et al*. (1987) reported high reproducible symptoms after inoculation of cotyledons with this fungus. Furthermore, Cohen *et al*. (2003) recently performed successful inoculations with *P. cubensis* on cotyledons of cucurbits cultivars.

The objective of this study was (a) to further investigate zoospore concentration and leaf age that may affect the infection rate of *Pseudoperonospora cubensis* on cucumber plants and (b) to study the nutritional status of cucumber with regard to symptom development of downy mildew.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design

A 2x3 factorial experiment of cucumber leaves (young and old leaf) and spore concentrations of *P. cubensis* was conducted. Eight plants were grown per plot, with five replicates. Given the suggestion of Williams and Palmer (1982) that the concentration of *P. cubensis* spores for cucumber inoculation should be $12x10^4$ sporangia ml^{-1}, the following zoospore concentrations were examined; $48x10^3$, $97x10^3$ and $195x10^3$ sporangia ml^{-1} of suspension. The third and the seventh leaf from the apex of the plants were selected for inoculation. The treatments are shown in Table 4.1 and arranged in greenhouse according to the experimental design of Table 4.2.

Table 4.1. Treatments of the experiment (1-6). The young and the old leaves (third and seventh leaf) were from the top of the plant respectively. Concentration 1, 2, 3 means $48x10^3$, $97x10^3$ and $195x10^3$ sporangia ml^{-1} of suspension respectively.

1	Young leaf +concentration 1
2	Young leaf +concentration 2
3	Young leaf +concentration 3
4	Old leaf +concentration 1
5	Old leaf +concentration 2
6	Old leaf +concentration 3

Table 4.2. Experimental design arrangement (I, II, III, IV, V: replicates, 1 to 6: treatments).

I5	II1	III6	IV6	V5
I3	II5	III4	IV2	V2
I4	II6	III2	IV4	V4
I1	II4	III5	IV5	V3
I2	II2	III3	IV1	V6
I6	II3	III1	IV3	V1

4.2.2 Cultivation Description

In order to grow vigorous and healthy plants fertilization was started a week after transplantation. The fertilization solution consisted of 50, 100, 200 ppm of phosphorus, nitrogen, and potassium respectively. H_3PO_4, NH_4NO_3 (33.5-0-0) and KNO_3 (13.5-0-46.2) fertilizers were used in order to make the above solution. The desirable quantities of the above fertilizers per litre were as follows; 0.11 mls, 0.09 g and 0.52 g respectively. Therefore for plant fertilization, 16.5 mls orthophosphoric acid, 13.5 g ammonium nitrate and 78 g potassium nitrate were weighed and dissolved in 150 L of water. The solution (500 mls) was supplied to cucumber plants whenever necessary. All the other cultivation techniques were applied as described above.

4.2.3 Pathogen Inoculation

The inoculation of *P. cubensis* was made on the third and seventh leaf from the top of the plant (young and old leaf respectively) with inoculum obtained from infected plants that were constantly re-infected. Leaf and soil samplings, nutrients determination and statistical analysis of data were carried out afterwards.

4.2.4 Measurements – Analytical Methods

Four series of photos of all the inoculated leaves were made for both of two leaf ages of each plant. The photos were processed in order to measure leaf and lesion area. The relative leaf and lesion area were calculated afterwards by expressing each measurement relative to the highest value of leaf and lesion area, respectively, in each sampling.

4.3 RESULTS

4.3.1 Effect of leaf age on leaf and lesion area of cucumber

Statistical analysis of the main effects of the tested variables indicated that leaf age resulted in a significant difference in leaf area, in all the samplings (Figure 4.1 A). The older leaf (seventh leaf from the plant apex) had the largest area compared to the young leaf (third leaf from the top of the plant) in each one of the four samplings, regardless of the spore concentration. Similar effects of leaf age were observed in relative leaf area in all the samplings (Figure 4.1 C).

Disease emergence was observed in all inoculated leaves. The Figure 4.1 B shows that there were no significant differences between the seventh and the third leaf in relation to lesion area in any of the four samplings. However, the leaf age resulted in statistical significant differences of relative lesion area in the third sampling, with the young leaves having significantly lower relative lesion area than the older leaves (Figure 4.1 D).

4.3.2 Effect of inoculum concentration on leaf and lesion area

In general, no statistically significant differences were observed in relation to the effect of suspension concentration on the leaf area of cucumber plants (Figure 4.2 A). Nevertheless, in the second sampling (day 3), the higher spore content resulted in statistical significant larger leaf and relative leaf area (Figure 4.2 C) compared with the two lower sporangia concentrations.

Figures 4.2 B and 4.2 D show the effect of inoculum concentration on lesion and relative lesion area caused by *P.cubensis* on cucumber leaves, respectively. All artificial inoculation treatments resulted in successful infection. Statistically significant differences were recorded in all the samplings. The higher spore concentration resulted in statistically significant larger lesion areas with regard to the other spore contents. The intermediate spore concentration level was

also statistically significantly different when compared with the lower level (Figure 4.2 B). This occurred in sampling 1 and 3. In samplings of the day 6 and 10 the two higher spore contents were not statistically significantly different from each other but only when compared to the lower inoculum level. The same results were recorded with regard to the relative lesion area with the exception that in the third sampling there were no statistically significant differences between the inoculum concentrations for disease development (Figure 4.2 D).

Statistical analysis of the data obtained revealed interactions of leaf age x inoculum concentration in the case of leaf area and lesion area, in the first and second sampling respectively (Figure 4.3). The intermediate concentration of the spore suspension increased the area of the young leaf compared with the two other concentrations. In contrast, the area of the old leaf was decreased in the intermediate inoculum suspension and increased at the lower and higher level of suspension. Regarding the relative lesion area similar results were obtained (Figure 4.3).

The lesion area of the young cucumber leaf was increased in the intermediate inoculum level and more increased in the higher, in the second sampling (Figure 4.3). The intermediate and higher inoculum load also increased the infection area of the old leaf but the lower spore content resulted in higher lesion area than the corresponding area of the young leaf.

4.3.3 Assessment of nutritional status of cucumber plants

In general, there were statistically significant differences in the nutrient concentration in cucumber tissue and soil. Means of macro and micro nutrients are shown in Table 4.3. Macroelements in leaves tend to increase with increasing spore content in both leaves although the differences were statistically significant only at two of the three concentration levels or not significant at all. An opposite trend (reduction) was observed for microelements, NH_4-N and NO_3-N in leaves. The differences were also non-significant at the three inoculum concentration levels

tested. Nutrient soil content did not result in statistical significant differences (Table 4.3).

Covariance analysis of the obtained data showed that nutrient content in leaves affected the lesion area caused by *P. cubensis* on cucumber leaves. The leaf content of Fe, Zn and Ca influenced the total and the relative lesion area at $P<0.05$ in the first sampling. In the fourth sampling the same variables (total and relative lesion area) were affected by NH_4-N in soil at $P<0.01$. Leaf area and relative leaf area of cucumbers was also affected by Mn ($P<0.05$) in the second sampling. In all the other cases there were no statistically significant effects of nutrients as covariates on the leaf and lesion area of cucumber plants.

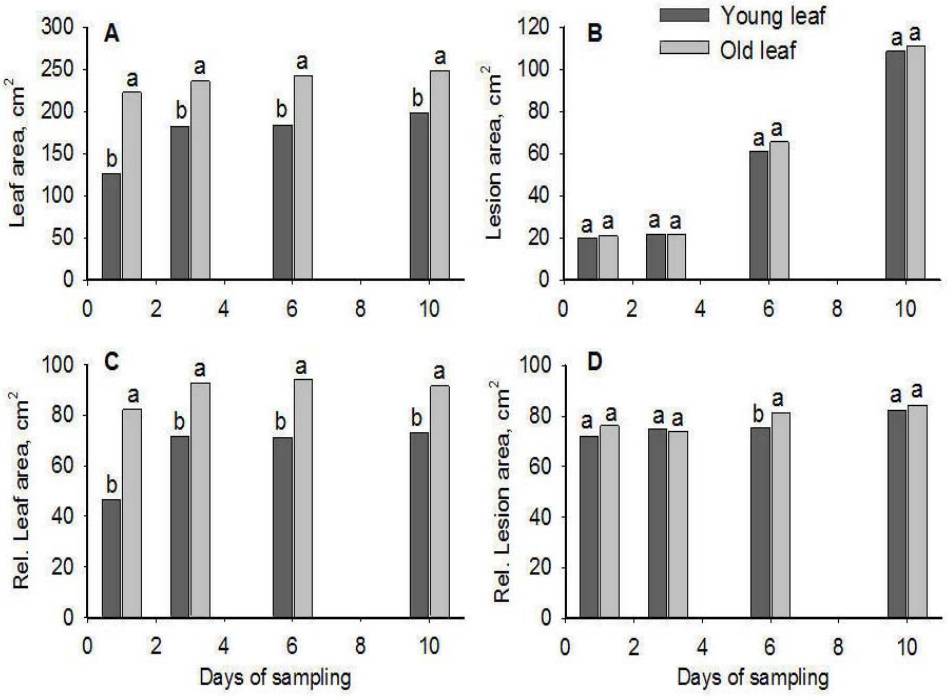

Figure 4.1. Effect of leaf age (young and old leaf) on leaf (A) and lesion (B) area, relative leaf (C) and relative lesion area (D), during the samplings (Day 1, 3, 6, 10 after appearance of downy mildew of cucumber).

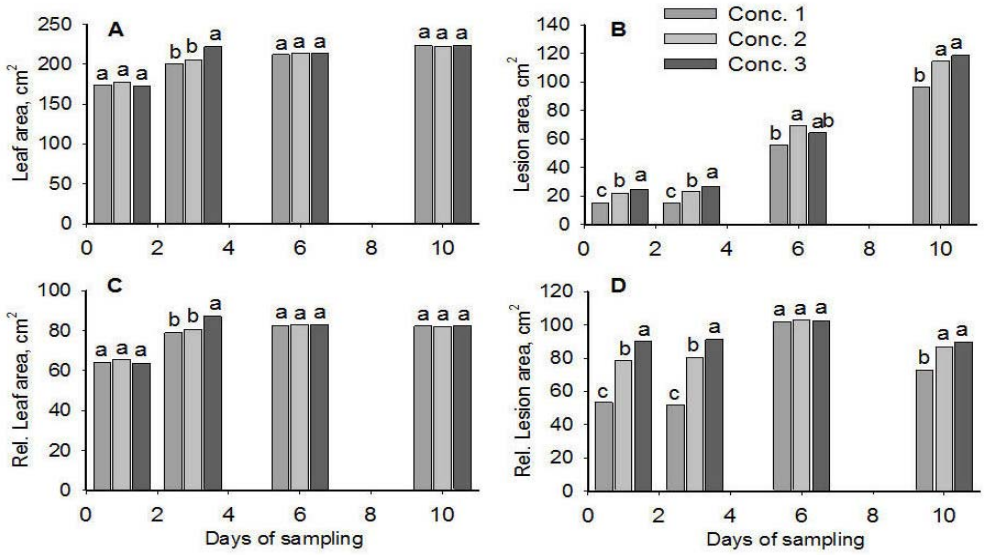

Figure 4.2. Effect of inoculum concentration (Conc.1=48x10^3, Conc.2=97x10^3 and Conc.3=195x10^3 sporangia of *P. cubensis* ml^{-1} of suspension) on leaf (A) and lesion (B) area, relative leaf (C) and relative lesion area (D), during the samplings (Day 1, 3, 6, 10 after appearance of downy mildew of cucumber).

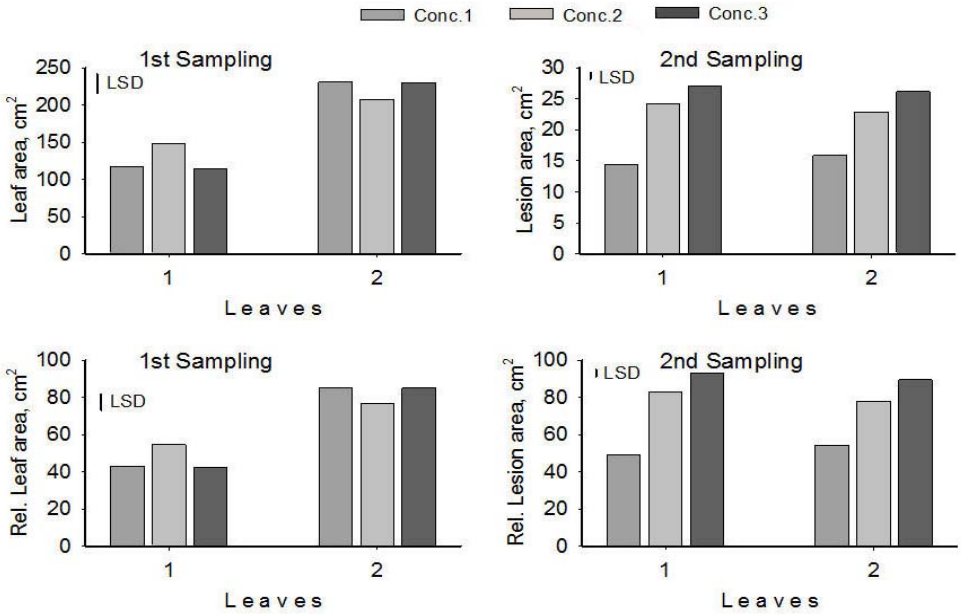

Figure 4.3. Interaction of leaf age x inoculum concentration with regard to the leaf and relative leaf area at the first sampling (day 1 after appearance of cucumber downy mildew) and in the case of lesion and relative lesion area at the second sampling (day 3 after appearance of cucumber downy mildew). (Conc.1=48x10^3, Conc.2=97x10^3 and Conc.3=195x10^3 sporangia of *P. cubensis* ml^{-1} of suspension; Leaf 1, 2 = young, old leaf respectively). The significant difference (P=0.05) among inoculum concentrations is presented with LSD bars.

Table 4.3. Effect of treatments (Leaf 1, 2: young and old leaf respectively; Concentrations reported in sporangia of *P. cubensis* ml^{-1} of suspension) on macro and micro nutrients in cucumber leaves and soil. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Treatments		Macro – elements in leaves			
Leaf	Conc.	Ca %	Mg %	K %	P %
1	48 x 10^3	6.456 b	1.041 ab	6.479 a	0.869 ab
1	97 x 10^3	6.042 b	0.887 bc	4.620 b	0.899 a
1	195 x 10^3	8.316 a	1.095 a	4.699 b	0.745 c
2	48 x 10^3	5.854 b	0.852 c	5.241 b	0.798 c
2	97 x 10^3	5.996 b	0.905 bc	4.854 b	0.898 a
2	195 x 10^3	6.525 b	0.817 c	4.765 b	0.812 bc
		Micro – elements in leaves			
		Fe ppm	Mn ppm	Cu ppm	Zn ppm
1	48 x 10^3	535.2 a	146.5 b	187.5 a	67.7 a
1	97 x 10^3	339.1 c	90.8 d	153.2 b	54.7 b
1	195 x 10^3	450.7 b	204.1 a	105.3 d	51.7 bc
2	48 x 10^3	365.3 c	160.1 b	121.0 cd	43.6 cd
2	97 x 10^3	395.1 bc	140.0 bc	138.4 bc	58.7 b
2	195 x 10^3	334.7 c	108.6 cd	118.8 cd	39.7 d
		NH_4-N	NO_3-N	NH_4-N	NO_3-N	K
		ppm in leaves		ppm in soil		
1	48 x 10^3	82.8 a	129.2 ab	6.4 c	19.8 a	418.3 ab
1	97 x 10^3	33.7 c	71.9 c	8.6 b	14.4 a	399.6 b
1	195 x 10^3	51.6 b	104.5 bc	6.4 c	19.2 a	438.0 a
2	48 x 10^3	81.7 a	146.4 a	10. a	16.9 a	409.2 ab
2	97 x 10^3	84.7 a	135.9 ab	7.0 bc	16.1 a	424.7 ab
2	195 x 10^3	50.4 b	121.7 ab	6.8 bc	17.6 a	411.8 ab

4.4 DISCUSSION

The results of this study indicate that there were statistically significant differences in size between the young and the old leaf. This is apparent because of the different aged leaves that were selected for inoculation. It is clear that the younger leaves (third leaf from the apex of each plant) were not fully developed and thus always smaller when compared with the older leaves (seventh leaf from the plant apex) that had reached their maximum size. It can also be concluded that the spore concentration had no significant effect on leaf area of either of these leaves. Thus, leaf development was independent of the amount of inoculum. However, an interaction of leaf age and inoculum concentration was observed for leaf area in the first sampling, where the higher spore content decreased the area of the young leaf while the old leaf's area was not influenced and developed independently. This suggests that the development of the younger leaf and not the old was prevented by the high inoculum load.

In the present work it was very evident that both young and old leaves were successfully infected with no statistically significant differences between them regardless of the spore concentrations used. The results showed that *P. cubensis* affected lesion development on both types of leaves in the same way. Wyszogrodzka *et al*. (1987) confirmed that even cotyledons of cucumber seedlings of a susceptible cultivar expressed symptoms of downy mildew disease after inoculation with *P. cubensis*. They also reported that in the multiple pathogen inoculations conducted with cucumber cotyledons, symptoms were produced by *P. cubensis*, although the cotyledons were inoculated simultaneously with another three pathogens. The successful infection of both the third and seventh leaf (from the plant apex) is similar to other studies carried out where successful inoculations where made on cotyledons, first, second or third leaf of cucumber plants. Normally the methods used involved spraying the suspension on both surfaces (Portz *et al*., 2008; Cohen *et al*., 2003) but also leaving droplets on the leaf containing the fungus (Lindenthal, 2005) as in the present study. The symptoms produced on the third leaf resulted in no significant differences with regard to those of the seventh although in field conditions where plants are naturally infected, usually the

symptoms appear on the older leaves first and then progressively on the younger leaves (Zitter *et al*., 1996). This shows how destructive downy mildew could be for the whole cucumber plants.

Downy mildew symptoms on cucumber leaves appeared even at the lowest sporangia concentration ($48x10^3$ sporangia ml^{-1} of suspension). This is not surprising since artificial inoculation of cucumber leaves with lower spore contents of *P. cubensis* have been reported. Reuveni and Ravin (1997) inoculated cucumbers with $2.5x10^4$ sporangia of *P. cubensis* ml^{-1} suspension. However, they actually, sprayed 2 mls of this suspension on each leaf resulting in potentially $5x10^4$ sporangia being attached to the target leaf area. Baider and Cohen (2003) and Portz *et al*. (2008) reported cucumber inoculations with even lower contents ($5x10^3$ sporangia of *P. cubensis* ml^{-1}) but they also used the spraying method with spores sprayed on the upper or even both leaf surfaces. With this technique probably a lower spore concentration was needed for successful infection. In contrast, in the present study, the droplet method was used and perhaps more spores might be required in order to obtain germination before the droplet dries. In similar inoculation studies with droplets $5x10^5$ sporangia of *P. cubensis* ml^{-1} were successfully applied on the lower leaf surface (Lindenthal *et al*., 2005). The droplet method was chosen in the present studies because the experimental objective was an accurate monitoring of downy mildew development.

When inoculum concentration was increased disease severity statistically significantly increased when compared with the lower spore concentrations. However, as the infection progressed and disease was spread there was no significant difference between the symptoms obtained at the two higher concentrations. The increase in disease severity when inoculum concentration was increased was predictable and has been reported for other pathogens e.g. *Alternaria brassicicola* (Doullah, 2006). However, in the case of the research on dark leaf spot disease of *Brassica rapa,* assessment was by rating once for lesions, and not at several intervals during the symptom development as in the present study.

There was evidence that nutrients in leaves (Fe, Zn and Ca) had statistically significant effect on the lesion area of cucumbers as covariates. The important role

that nutrients play in infection of plant has also been reported for other cucumber diseases. A study by St. Amand and Wehner (1995) about the effect of various factors such as leaf age, stomata opening on cucumber gummy stem blight stated that nutrients provided by guttation were more important in the infection process than other factors like stomatal opening.

An interaction of leaf age x inoculum concentration was ascertained for infection area and therefore the effect of the inoculum concentration on the lesion area of the young leaf was not the same with that for the old leaf. Thus, the two higher spore contents affected in the same way the disease severity of both the young and old leaves while the lower concentration caused larger lesion areas on the older leaves than the younger ones (Figure 4.3). Low numbers of sporangia were possibly better developed on an old cucumber leaf wherein due to its large size and lower position on the plant probably the microclimate conditions for infection were more favourable (long dew periods, not good ventilation) while the high amount of spores reproduced independently of the leaf size consequently leaf age.

4.5 CONCLUSION

The conclusions which came from this study point out to the fact that all the tested inoculum concentrations resulted in successful infection of both young and older cucumber leaves, after artificial inoculation in greenhouse conditions. It was also clear that the pathogen attacked leaves of cucumber regardless of age, causing the same infection area. The lesions of all the leaves tend to have the same total area, as the disease spread regardless of the amount of inoculum. This was apparent for the two higher inoculation concentrations while the lower concentration continued to result in significantly less infection. The importance of leaf nutrients in infection has also been noted. The current study suggests that around $15x10^4$ spores of *P.cubensis* ml^{-1} is the optimal inoculum concentration with which successful inoculation of cucumbers to be achieved under greenhouse conditions in Crete. This result is consistent with the studies of Williams and Palmer (1982) who suggested adjustment of *P.cubensis* suspension to $12x10^4$ sporangia ml^{-1}.

CHAPTER FIVE

EFFECT OF NITROGEN FERTILIZATION ON DOWNY MILDEW DEVELOPMENT OF CUCUMBER

5.1 INTRODUCTION

Downy mildew is caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostovzev, an obligate biotrophic oomycete which is one of the most destructive diseases of cucumber plants and causes major yield reductions (Lindenthal *et al*. 2005; Neykov and Dobrev, 1988). The pathogen infects the leaves after penetration via stomata and extracts nutrients from the plant by forming haustoria (Latijnhouwers *et al*., 2003; Panstruga, 2003). The first symptoms appear as small angular yellow spots on the upper side of the leaves, which are often bounded by the leaf veins (Sherf and Macnab, 1986). Under favorable conditions (RH > 90%) fluffy purplish mildew appears on the lower sides and subsequently the yellow lesions become brown and necrotic (Horst, 1979). Progressively the entire leaf may become prematurely senesced.

A number of factors can influence the occurrence and severity of downy mildew in cucumbers such as the environmental conditions (temperature and humidity), crop establishment and management practices (Zitter *et al*., 1996; Panagopoulos, 1995). Among these factors could be the inorganic fertilizer supply. It is widely recognized that there may be complex relationships between nutrients and the incidence of plant diseases. An examination of the literature on such diseases reveals that the influence of nutrition on disease development has received increasing interest in recent years (Reuveni and Reuveni, 1998; Walters and Bingham, 2007; Dordas, 2008).

According to Vitug (1997) nitrogen fertilization increased the incidence of sugarcane downy mildew. Ash and Brown (1991) stated that increased rates of nitrogen led to increase incidence of *Puccinia striiformis* on wheat. Hoffland (2000) also reported that nitrogen increased tomato susceptibility to *P. syringae*

and *O. lycopersicum*. Leser and Treutter (2005) came to the same conclusion about scab of susceptible varieties of apple trees (Golden delicious). In contrast, Sivaprakasam *et al.* (1974) stated that downy mildew of pearl millet was not markedly affected by nitrogen nutrition. According to Rodgers-Gray and Shaw (2000) there was no clear relationship between N and foliar diseases of wheat. However, other studies (Bains and Jhooty, 1978) have reported that downy mildew development caused by *Pseudoperonospora cubensis* in muskmelon was lower on the plants grown in high nitrogen solutions. Rotenberg *et al*. (2005) more recently reported that total soil N was inversely related to cucumber angular leaf spot incidence. Besides, addition of N and NP decreased the severity of dark kernel of wheat, while P had no effect (Tian *et al*., 1997). Kolota and Osinska (2001) observed that downy mildew (*P. cubensis*) was significantly decreased on cucumber leaves by applying a foliar fertilizer containing 11 essential nutrients.

The host-pathogen associations are very multifarious and as Hoffland (2000) reported the balance between nutrition and resistance of tomato depended on the invading pathogen. In addition, soil and leaf nitrogen status is associated with plant disease through several ways (Walters and Bingham, 2007; Dordas, 2008). As Huber and Watson reviewed (1974), the form of nitrogen and the ratio of NH_4-N to NO_3-N play an important role in plant disease but since no one form of it controls all diseases on any group of plants each disease must be studied individually. Although the above data come to the conclusion that a great deal of information is available for various pathosystems, however cucumber-*P. cubensis* has not been much investigated. There are thus complex relationships between type and time of fertilizer application and various cucumber diseases (Reuveni and Reuveni 1998). The objectives of this study were (a) to examine the effect of nitrogen fertilization on downy mildew appearance in cucumbers, (b) determine the disease progress curves and (c) to investigate the impact of nutritional factors of leaf tissues themselves and substrate on the rate of disease spread.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Design

Six nitrogen treatments were tested in a randomized block design with four replicates. Each plot consisted of twelve pots which were located in the Greenhouse according to the experimental design (Table 5.1). The cultivation techniques have been mentioned in chapter 3. The experimental treatments were composed of six nitrogen concentrations which were applied to plants as a nutritional solution (100, 200, 300, 400, 500 and 600 ppm N). P and K were added in the above solution in a standard concentration (50 and 200 ppm respectively), so the plants received a balanced nutrition. Table 5.2 shows the treatments analytically.

Ammonium nitrate (33.5-0-0), orthophosphoric acid (H_3PO_4) and potassium nitrate (13.5-0-46.2) fertilizers were used in appropriate proportions to achieve the desirable concentrations. Thus, 0.52g potassium nitrate per litre and 0.11 mls orthophosphoric acid per litre was mixed with each one of the following quantities 0.09, 0.39, 0.69, 0.99, 1.28 and 1.58g l^{-1} ammonium nitrate. The 48 pots (12 pots per plot x 4 replicates) of each treatment needed 24 L fertilization solution given that 500 ml solution was supplied in each pot whenever necessary, according to the plants' requirements. Depending on the above the quantities that needed in order to be dissolved in 30 L of water (little more was made for waste reasons) were calculated (Appendix B, Table B1). The solutions were made just before the irrigation accomplishment and kept in six 40 L plastic vessels (one per treatment). A 500 mls vessel was used to transfer the solution from each big vessel to the plants, according to treatments. Applications were made in the order of 100 to 600 ppm N.

5.2.2 Pathogen Inoculation

In order to inoculate the plants, the fifth leaf from the top of each plant was marked as mentioned above. The inoculum preparation as well as the inoculation of *Pseudoperonospora cubensis* has been described in chapter 3. Zoospores were counted with the aid of a haemocytometer to give a suspension of $15x10^4$ sporangia ml^{-1}.

Table 5.1. Experimental design arrangement (I, II, III, IV: replicates, 1 to 6: treatments).

I5	II1	III6	IV3
I3	II5	III4	IV2
I4	II6	III2	IV5
I1	II4	III5	IV4
I2	II2	III3	IV1
I6	II3	III1	IV6

Table 5.2. Treatments of the experiment (1-6). Six nitrogen concentrations in the fertilization solution with simultaneous addition of standard concentrations of P and K in each treatment.

1	100 ppm N + 50 ppm P + 200 ppm K
2	200 ppm N + 50 ppm P + 200 ppm K
3	300 ppm N + 50 ppm P + 200 ppm K
4	400 ppm N + 50 ppm P + 200 ppm K
5	500 ppm N + 50 ppm P + 200 ppm K
6	600 ppm N + 50 ppm P + 200 ppm K

5.2.3 Measurements – Analytical Methods

Digital photos of the infected leaves were taken 10 days after the inoculation. Five series of photos (samplings) were followed at second, third, fifth, seventh and eleventh day after the first sampling. The measurements about the leaf and lesion area as well as the leaf and soil sampling were accomplished. Statistical analysis of data followed.

This experiment was conducted twice in two subsequent years. The experimental design, the nitrogen treatments, the cultural practices, the pathogen inoculation, the disease assessment, the leaf and soil sampling and the nutrients determination in plant tissues and soil were accomplished in the same way at both experiments. The difference was that at the first experiment one photo sampling was taken instead of six (second experiment). Thus, in order to get more detailed information in relation with the disease progress and to verify results this experiment was established twice.

5.3 RESULTS

5.3.1 First Experiment: Effect of nitrogen fertilization on downy mildew development of cucumber

The leaf and lesion area of cucumber caused by *P. cubensis* in each one of the N treatments are shown in Figure 5.1. The lesion area was decreased up to the third N level (300 ppm N) which was statistically significantly different when compared with the other treatments and then increased up to the higher N concentration. The reverse was observed for the leaf area measurements (Figure 5.1). The N dose of 300 ppm in the fertilization solution resulted in statistical significant largest leaf size in relation with the other N treatments.

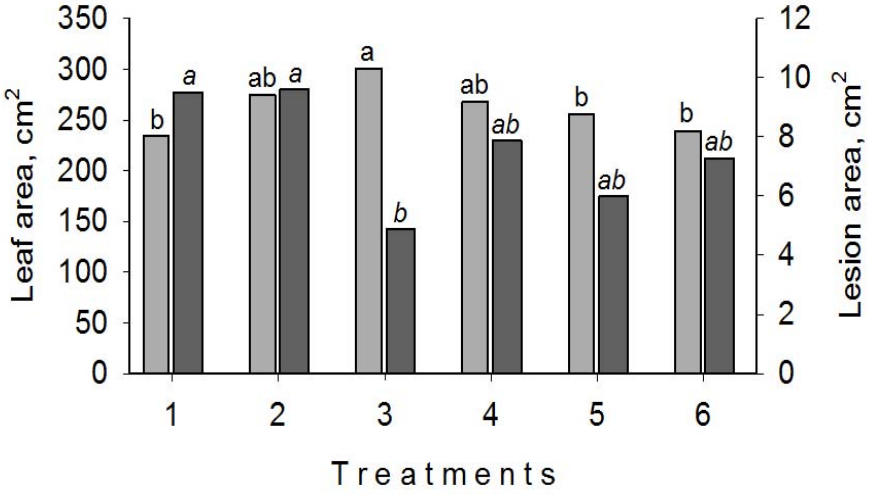

Figure 5.1. Leaf (light grey columns-left axis) and lesion area (dark grey columns-right axis) of cucumber plants treated with six N fertilization treatments (1-6; 100-600 ppm, first experiment). Columns which differ significantly from one another are marked with a different letter (italics for lesion area and regular letters for leaf area, Duncan, P=0.05).

Statistical analysis of the obtained data indicated that nitrogen treatments resulted in significant (P=0.05) differences in nutrient accumulation in cucumber leaves and soil (Table 5.3). Mn and Cu leaf content tended to increase with increasing N doses in soil although in the higher N treatments there were no statistical significant differences. The opposite result occurred for Zn, while Fe had no statistically significant effects. With regard to macronutrients in leaves there was a tendency for Ca, Mg and K content to reduce as the N was being higher in soil, although only lower N levels were statistically significantly different when compared with the higher treatments. The third treatment (300 ppm N) resulted in higher P leaf concentration although the difference was not statistically significant when compared with the other treatments. Statistically significant increases were observed for NH_4-N and NO_3-N of leaves due to N treatments. Soil NH_4-N was also increased in the higher N levels while NO_3-N in soil had statistically significant differences in almost all the treatments. K soil content in the last two N doses was statistically significantly different when compared with the other treatments.

Table 5.3. Effect of treatments (1-6: 100-600 ppm N in the fertilization solution) on nutrient concentration in cucumber leaves and soil (first experiment). Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Tr	Fe		Mn		Cu		Zn	
	ppm in leaves							
1	71.6	a	15.7	c	7.1	c	36.2	a
2	94.2	a	30.2	b	9.2	bc	30.4	b
3	86.4	a	35.8	a	10.7	abc	28.5	b
4	105.9	a	37.8	a	12.2	ab	30.6	b
5	74.3	a	37.6	a	13.2	a	31.2	b
6	87.9	a	38.9	a	10.0	abc	30.0	b

	Ca		Mg		P		K	
	% in leaves							
1	4.76	a	1.10	a	0.46	a	3.78	ab
2	3.76	b	0.93	b	0.46	a	3.50	abc
3	4.40	ab	0.97	b	0.59	a	3.94	a
4	4.04	ab	0.91	b	0.46	a	3.19	bc
5	4.06	ab	0.90	b	0.46	a	3.26	bc
6	4.26	ab	0.95	b	0.46	a	3.01	c

	NH_4-N		NO_3-N		NH_4-N		NO_3-N		K	
	ppm in leaves				ppm in soil					
1	74.97	bc	33.21	d	66.88	b	83.79	e	211.59	c
2	70.60	c	41.58	d	56.40	b	124.38	e	187.81	c
3	111.03	ab	93.76	b	63.49	b	264.73	d	196.49	c
4	110.36	ab	66.49	c	71.32	ab	435.42	c	193.78	c
5	131.12	a	82.66	bc	90.99	a	644.69	b	242.06	b
6	111.07	ab	128.71	a	90.45	a	751.22	a	285.32	a

5.3.2 Second Experiment: Effect of nitrogen fertilization on downy mildew development of cucumber

- **The effect of nitrogen fertilization on downy mildew progress**

The results obtained show that the leaf area increased with increasing nitrogen levels in the soil with statistical significant differences between treatments as shown in all the samplings (Fig. 5.2). The highest nitrogen level resulted in the biggest leaves which were significantly different relative to the other treatments. In the middle nitrogen concentrations the leaf area remained stable (Fig. 5.2). The respective lesion area seems to be lowest in the 300 ppm N with statistical significant differences as compared with the other treatments, in the samplings 2, 3, 4 and 5 (Fig. 5.3 B, C, D, E). There is a reduction trend in lesion area at the first two levels of nitrogen, although no significant differences were found (sampling 3, Fig. 5.3 C). There was no statistically significant effect of higher N levels in all the samplings. Similar results were observed in the cases of relative leaf and relative lesion area (Figures 5.4, 5.5).

It is also concluded that leaf area index increased with time in all treatments (Fig. 5.6 A-F) with the polynomial curve best fitting the data. Symptoms of downy mildew were observed in all the plots. The total lesion area increased during the sampling dates in all treatments, reaching a maximum at the final sampling (entire leaf infected) (Fig. 5.6 A-F). These Figures also show the disease progress curves and their equations in each case. The cubic model was observed in all treatments.

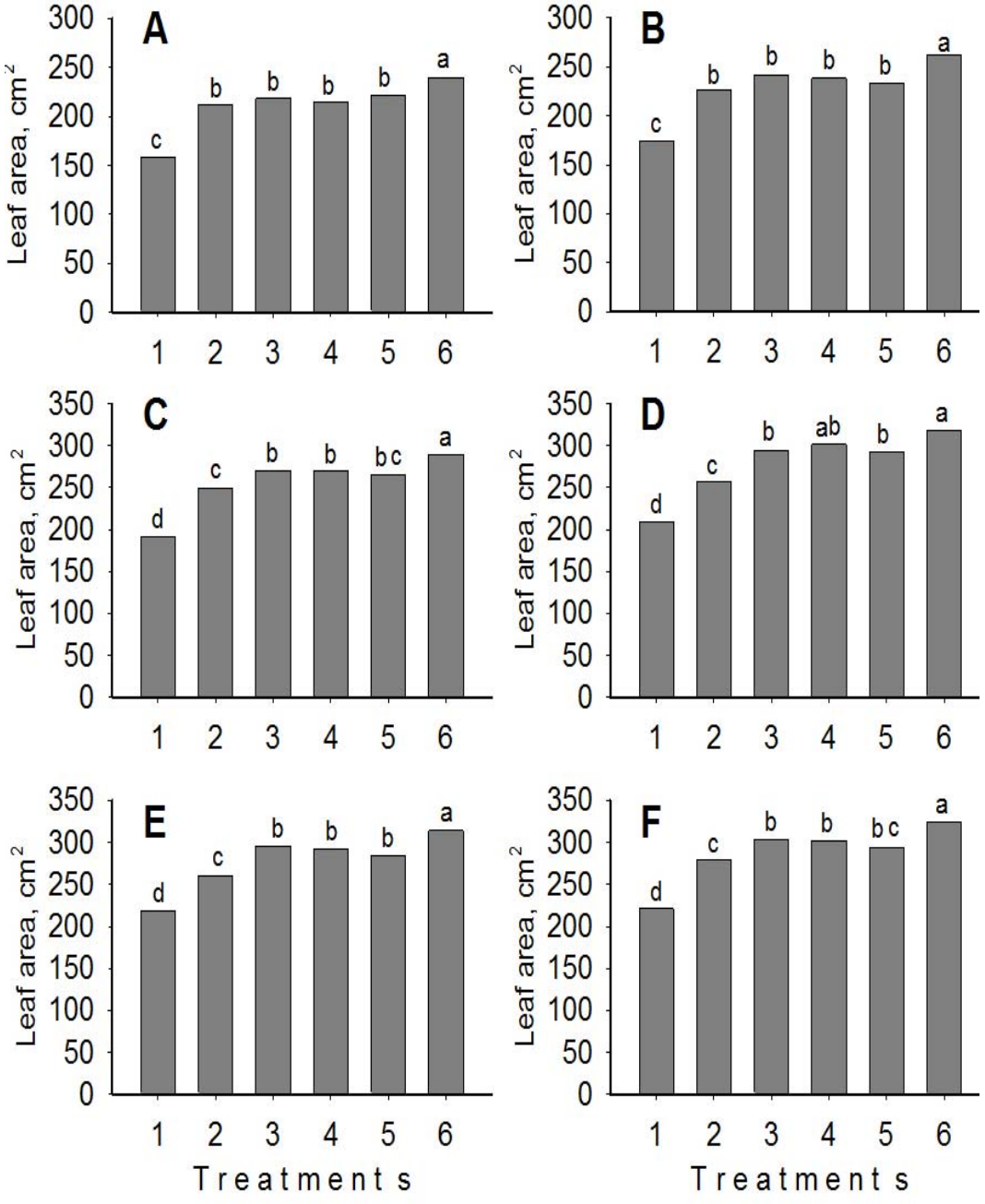

Figure 5.2. Effect of N fertilization (1-6; 100-600 ppm) on leaf area of cucumber plants at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

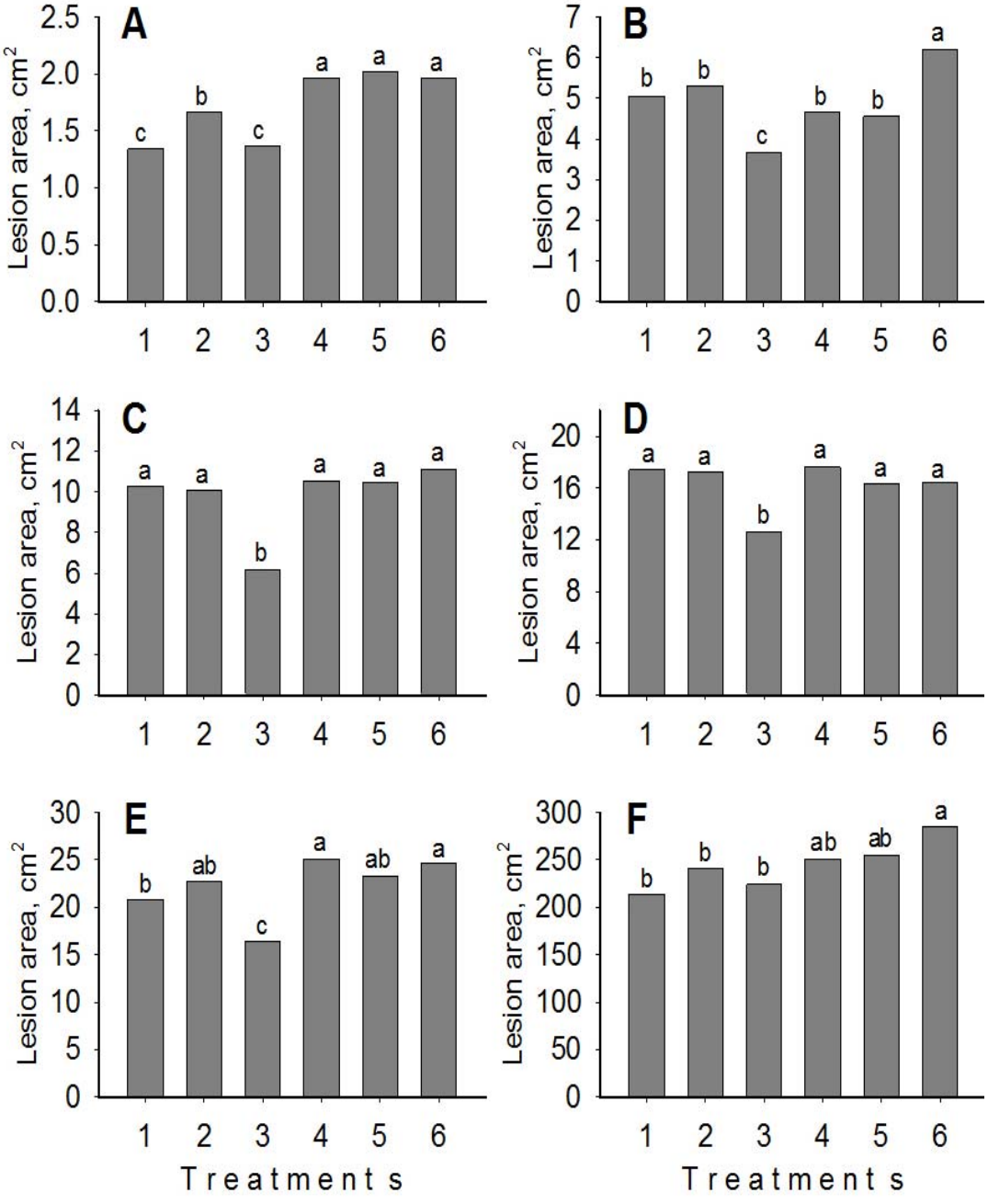

Figure 5.3. Effect of N fertilization (1-6; 100-600 ppm) on lesion area of cucumber leaves at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

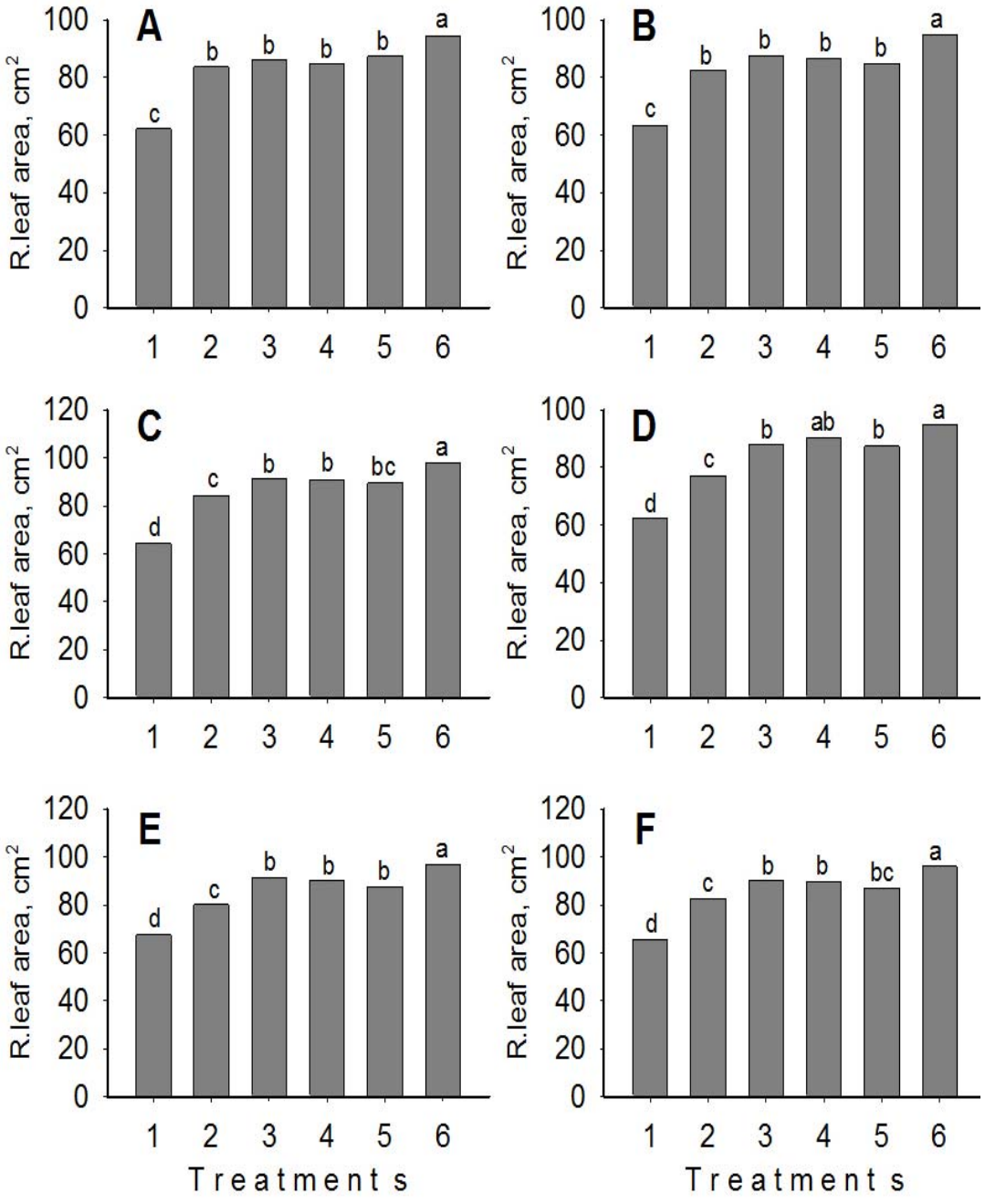

Figure 5.4.Effect of N fertilization (1-6; 100-600 ppm) on relative leaf area of cucumber plants at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

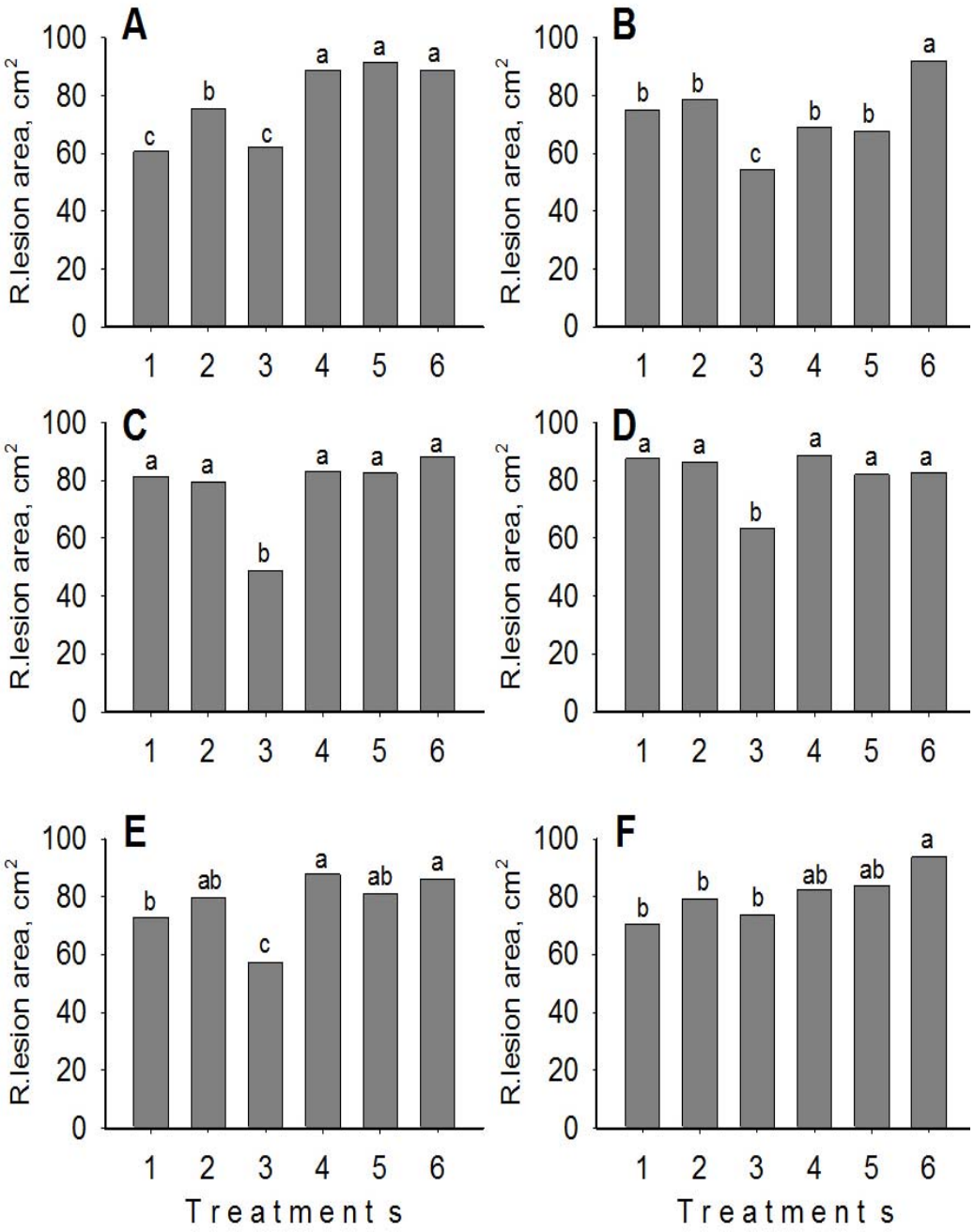

Figure 5.5 Effect of N fertilization (1-6; 100-600 ppm) on relative lesion area of cucumber leaves at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

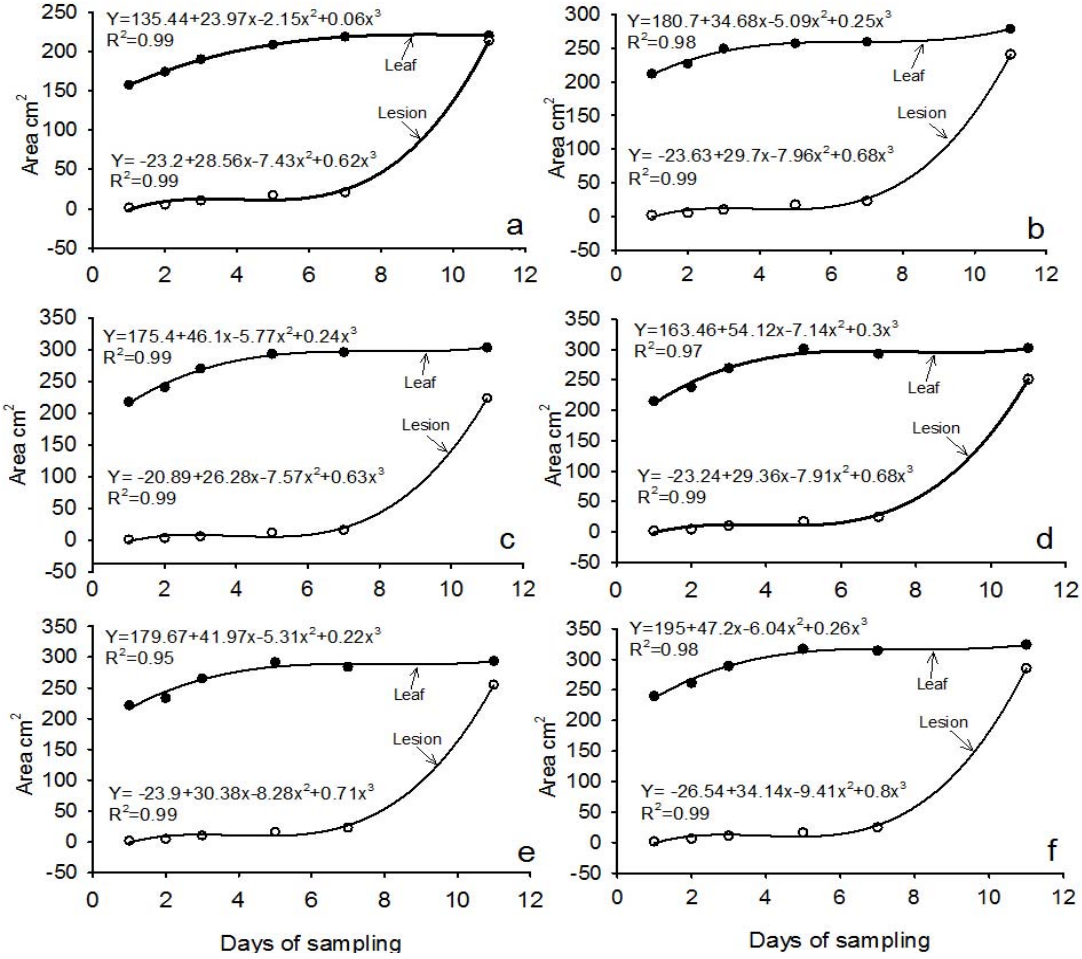

Figure 5.6. Progress of the treatments (A-F; 100-600 ppm N in the fertilization solution) effect on leaf and lesion area of cucumbers during the samplings.

Covariance analysis of the data revealed that nutrient content in leaves influenced the leaf area and lesion development caused by *P.cubensis* (Tables 5.4, 5.5). The means of the tested variables (leaf and relative leaf area, lesion and relative lesion area) without this influence (by nutrients of leaves) are shown in the columns marked with 'cov', meaning that the corresponding nutrient in each case was a covariate. Thus, the lesion area and the relative lesion area were affected principally by leaf Mn concentration in almost all the samplings (Tables 5.4, 5.5). The following nutrients: NH_4-N, P, Fe, Mg and P affected the leaf area of cucumbers in the samplings 1, 3, 4, 5, 6 respectively (Table 5.4). The same nutrient influence occurred for relative leaf area (Table 5.5).

Table 5.4. Effect of nutrients on leaf and lesion area of cucumber plants, at the N fertilization treatments (100-600 ppm) at the six samplings (Tr: 1-6; Sam: 1-6), after covariance analysis (Leaf and lesion area Cov.).

Sam	Tr	Leaf Area		Leaf Area Cov.	Lesion Area		Lesion Area Cov.
				NH_4N leav			Mn
1	1	157.63	c	134.59	1.34	c	0.96
	2	212.15	b	197.76	1.66	b	1.73
	3	218.33	b	216.20	1.37	c	1.51
	4	215.02	b	222.54	1.96	a	2.01
	5	221.90	b	236.24	2.02	a	2.12
	6	239.63	a	257.35	1.96	a	2.00
							Mn
2	1	174.31	c		5.06	b	3.44
	2	226.97	b		5.30	b	5.58
	3	241.22	b		3.66	c	4.23
	4	238.27	b		4.66	b	4.86
	5	233.86	b		4.56	b	4.96
	6	261.47	a		6.20	a	6.36
				P			
3	1	190.54	d	188.73	10.30	a	
	2	249.54	c	236.48	10.05	a	
	3	270.35	b	269.72	6.17	b	
	4	269.21	b	269.30	10.53	a	
	5	265.38	bc	268.40	10.44	a	
	6	289.31	a	301.70	11.13	a	
				Fe			Mn
4	1	209.01	d	210.56	17.42	a	13.93
	2	257.29	c	258.50	17.20	a	17.81
	3	293.55	b	294.55	12.61	b	13.85
	4	301.35	ab	294.27	17.64	a	18.08
	5	291.87	b	292.64	16.32	a	17.18
	6	317.17	a	319.71	16.48	a	16.83
				Mg			Mn
5	1	219.04	d	219.54	20.80	b	14.01
	2	259.67	c	255.30	22.74	ab	23.92
	3	296.01	b	294.63	16.39	c	18.80
	4	292.70	b	296.15	25.11	a	25.97
	5	284.44	b	285.64	23.20	ab	24.88
	6	314.00	a	314.61	24.65	a	25.32
				P			
6	1	220.78	d	217.88	214.11	b	
	2	279.04	c	258.05	240.96	b	
	3	303.64	b	302.62	223.83	b	
	4	302.10	b	302.25	251.24	ab	
	5	293.95	bc	298.80	255.41	ab	
	6	324.07	a	344.00	285.51	a	

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Table 5.5. Effect of nutrients on percentage leaf and lesion area (Leaf and lesion area PC) of cucumbers, after covariance analysis (Leaf and lesion area PC Cov.). (Samplings: 1-6; Treatments 1-6: 100-600 ppm N).

Sam	Tr	Leaf Area PC	Leaf Area PC Cov. NH_4N leav	Lesion Area PC	Lesion Area PC Cov. Mn
1	1	62.15 c	53.06	60.78 c	43.25
	2	83.64 b	77.96	75.34 b	78.39
	3	86.08 b	85.24	62.02 c	68.22
	4	84.77 b	87.74	88.84 a	91.06
	5	87.48 b	93.13	91.50 a	95.82
	6	94.47 a	101.46	88.63 a	90.37
					Mn
2	1	63.35 c		75.08 b	51.11
	2	82.48 b		78.69 b	82.86
	3	87.66 b		54.35 c	62.84
	4	86.59 b		69.11 b	72.14
	5	84.99 b		67.69 b	73.60
	6	95.02 a		92.01 a	94.39
			P		
3	1	64.31 d	63.70	81.40 a	
	2	84.23 c	79.82	79.43 a	
	3	91.25 b	91.04	48.78 b	
	4	90.86 b	90.89	83.22 a	
	5	89.57 bc	90.59	82.51 a	
	6	97.65 a	101.83	87.98 a	
			Fe		Mn
4	1	62.52 d	62.98	87.53 a	69.98
	2	76.96 c	77.33	86.45 a	89.51
	3	87.81 b	88.11	63.36 b	69.57
	4	90.14 ab	88.03	88.63 a	90.85
	5	87.31 b	87.54	82.01 a	86.34
	6	94.88 a	95.64	82.83 a	84.56
			Mg		Mn
5	1	67.54 d	67.69	72.80 b	49.05
	2	80.06 c	78.71	79.59 ab	83.72
	3	91.27 b	90.84	57.38 c	65.79
	4	90.25 b	91.31	87.88 a	90.88
	5	87.70 b	88.07	81.22 ab	87.07
	6	96.81 a	97.00	86.28 a	88.63
			P		
6	1	65.43 d	64.56	70.36 b	
	2	82.69 c	76.47	79.18 b	
	3	89.98 b	89.68	73.55 b	
	4	89.52 b	89.57	82.56 ab	
	5	87.11 bc	88.55	83.93 ab	
	6	96.04 a	101.94	93.82 a	

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

- **Total accumulation of nutrients in the leaves and soil**

The experimental analysis of the macro nutrients in the cucumber leaves and soil indicated that there were differences in their accumulation dependent on the nitrogen dose. There were however no significant differences in the concentrations of Fe, Mn, Zn, Cu, Ca, Mg and K in the leaves (Tables 5.6, 5.7). Figure 5.7 shows the influence of treatments on P, NH_4-N and NO_3-N concentrations in cucumber leaves and that of NO_3-N in soil. This demonstrated that in the 300 ppm N treatment the concentration of P was the highest and significantly different when compared to the other treatments (Fig. 5.7 A). The results also indicated that NH_4-N as well as NO_3-N was gradually increasing in leaves as the N supply was increased in the soil (Fig. 5.7 B, C). For the macro elements in soil, the concentrations of K and NH_4-N were not differentiated by treatments (Table 5.7) but NO_3-N appeared to have the most statistically significant effect (P=0.05). Thus, the application of higher nitrogen levels increased the concentration of NO_3-N in the soil for almost all the treatments (Fig. 5.7 D).mm

Correlation analysis of the data showed that there was a high positive correlation between NH_4-N and NO_3-N in leaves (r=0.92), NH_4-N in leaves and NO_3-N in soil (r=0.87) as well as NO_3-N in leaves and NO_3-N in soil (r=0.93).

Table 5.6. Effect of treatments (1-6: 100-600 ppm N) on micronutrients in leaves. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Tr	Fe ppm		Mn ppm		Zn ppm		Cu ppm	
1	87.10	a	18.86	b	17.93	a	12.73	a
2	88.36	a	36.50	a	18.06	a	9.74	a
3	89.19	a	39.21	a	16.41	ab	10.98	a
4	102.15	a	35.79	a	16.63	ab	9.63	a
5	90.06	a	37.59	a	16.31	ab	10.63	a
6	83.29	a	35.38	a	15.72	b	12.72	a

Table 5.7. Effect of treatments (1-6: 100-600 ppm N) on macronutrients in leaves and soil. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

	Macro – elements in leaves						Macro – elements in soil	
Tr	Ca %		Mg %		K %		K ppm	NH_4N ppm
1	2.47	a	0.86	a	4.00	a	242.12 a	72.12 a
2	2.21	a	0.91	a	4.37	a	184.90 b	50.93 c
3	2.08	a	0.88	a	4.06	a	190.80 b	67.08 ab
4	1.93	a	0.82	a	4.13	a	196.76 b	58.81 bc
5	2.05	a	0.85	a	3.94	a	205.31 b	64.67 ab
6	2.05	a	0.86	a	3.61	a	187.75 b	60.12 bc

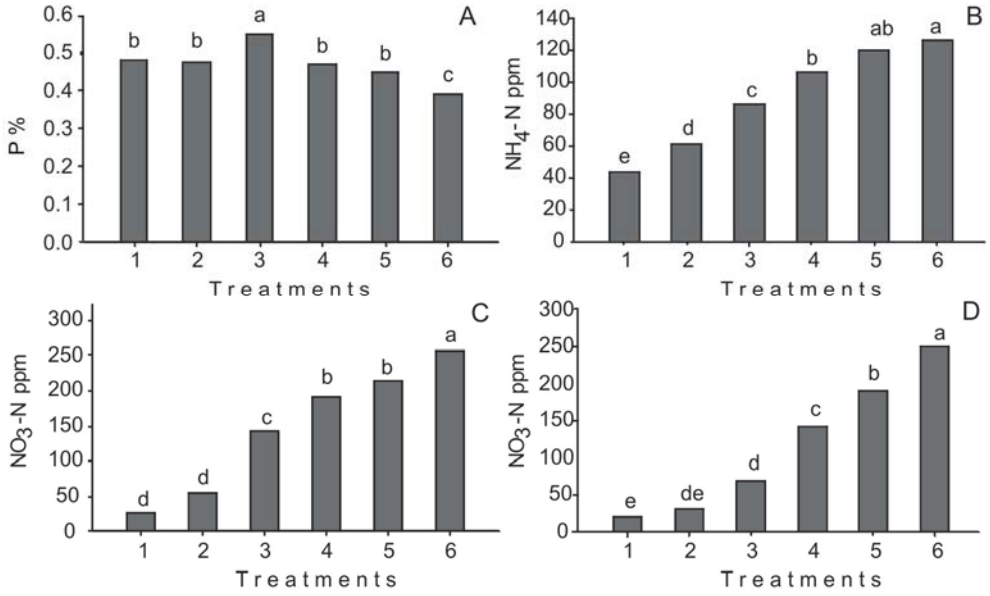

Figure 5.7. Effect of treatments (100-600 ppm N) on P, NH_4-N and NO_3-N (A, B and C respectively) concentrations in leaves and NO_3-N in soil (D).

5.4 DISCUSSION

It was concluded that the two experiments led to the same results in relation to nutrient status in cucumber leaves and soil. There were statistical significant differences in the accumulation of nutrients in leaves and soil but NH_4-N and NO_3-N content in leaves and soil NO_3-N seemed to be more important. In the first experiment, P concentration in the 300 ppm of N had the higher value although the difference was not found to be statistically significant when compared with the other N levels. In the second experiment, the same result was observed with the exception that here leaf P content was statistically significantly different when compared with the other treatments. However, the trend for P was clear in the first experiment. The intermediate N concentration (300 ppm) resulted in the lowest lesion area in both experiments. The corresponding leaf area (in this N treatment) was the largest in the first experiment but in the second experiment leaf area was increased with increasing N levels and the higher N treatment (600 ppm) resulted in the largest leaf area.

This study has shown that increased nitrogen supply in soil resulted in a gradually increasing of NO_3-N and NH_4-N in the cucumber leaves. Furthermore, ammonium nitrate lowered the severity of downy mildew at the medium dose (300 ppm nitrogen) used. These results are similar to other reports on cereal plants where pathogen severity reduction can occur after ammonium nitrogen fertilizer application (Brennan, 1992). Brennan also found that where nitrogen was applied, the nitrogen concentration in wheat plants resulted in high levels. In the case of cereals, which are very different from cucumbers, this did not seem to play a role in disease severity. The suggestion was however made that the form of nitrogen was the most important factor influencing take all disease. The significance of nitrogen type for various plant-pathogen interactions has also been reported by other authors (Huber and Watson, 1974; Simoglou, 2004). We do not have information to support or not this because of ammonium nitrate addition (both nitrogen forms; NH_4-N and NO_3-N).

The hypothesis that disease increased at the high nitrogen rates (400, 500 and 600 ppm) due to increased leaf N content (Fig. 5.3) is not supported because

this was also happening in low nitrogen (100 and 200 ppm) where the N status was low. In the review articles of Walters and Bingham (2007) and Dordas (2008) it is reported that nutrients can affect infection by altering crop characteristics and hence changing microclimate conditions. Also, this does not seem to interpret our results since both smaller and bigger leaves (Figures 5.2, 5.3) resulted in disease increase.

It is suggested that the influence of nitrogen on downy mildew depended on the type of pathogen rather than due to effect on microclimate or leaf nutrient status that others reported about various plant – pathogen pathosystems (Walters and Bingham, 2007). Thus, nitrogen supply possibly affected disease as a result of an effect on the pathogen's requirements and secondary host metabolites (defence compounds) that prevented fungus growth. Therefore, the supposition that low nitrogen application may have increased susceptibility in cucumber plants and slightly elevated nitrogen enhanced the host's ability to escape disease but further nitrogen increase promoted disease development appears consistent.

Furthermore, Tanaka *et al*. (2000) reported that the increase in the quantity of N in the nutrient solution had a corresponding positive effect on the content in the leaves in susceptible and resistant cucumbers although the leaf N content was not closely correlated with the total lesion area of downy mildew. However they used NO_3-N at three levels which was given to plants grown in hydroponics systems.

Nam *et al*. (2006) recently reported that elevated nitrogen concentrations in the fertilizer solution increased anthracnose severity on strawberry, in contrast to phosphorus and calcium, in cultivation under a noncirculation hydroponics system. Nevertheless, previous studies have shown the reduced effect of nitrogen on plant diseases. Thus, Strengbom and Reich (2006) showed that although increased N input did not cause accumulation of N on the leaves of *Solidago rigida*, decrease of foliar disease occurred. Consequently, crop nutrition may influence disease development in a different way. Simoglou (2004) also reviewed that high nitrogen decreases defended compounds production from plants and hence increases susceptibility and on the other hand nitrogen deficiency results in weak plants

which grow and come to maturity quickly and thus are more susceptible to diseases. In our study this seems to find acceptance because both low and high nitrogen levels led to increase of plant susceptibility to diseases.

David *et al*. (2003) stated that nitrogen fertilization had a positive effect on powdery mildew disease severity of *Hiemalis begonia*, primarily at the later production stages. In their study three levels of nitrogen and potassium were examined in a factorial experiment. In the same study it was also demonstrated that N fertilization at 120 ppm, which was also a medium treatment concentration value among to those that were examined, could help limit powdery mildew incidence on begonia. However, Berry *et al*. (1988) showed that 450 ppm N did not result in a significant increase in bacterial canker disease of tomato in comparison with the medium levels (240 ppm N).

Although it is mostly believed that nitrogen generally increases crop susceptibility to diseases, the importance of the balance between various nutrients is also well established (Reuveni and Reuveni, 1998). Even though 300 ppm of nitrogen is considered high concentration the ordinary rates that are used in Greece are between 150-300 ppm. Thus in our experiment, the higher nitrogen concentrations increased lesion area of downy mildew as compared with the middle dose (300 ppm of nitrogen) which was also stated by Walters and Bingham (2007). In their review paper it was reported that nitrogen supply above recommended rates can increase lesion area which is caused from both biotrophic and necrotrophic pathogens.

Our findings also revealed that the leaf area was increasing as the nitrogen fertilization that was started at the early stage of the cultivation, was increased (Fig. 5.2). Walters and Bingham (2007) arrived at the same conclusion that the early nitrogen application not only led to an increase of disease severity but also to a larger leaf area of winter wheat.

Nutrient status of cucumber plants affected both the area of leaves and lesion area caused by *P. cubensis*. This was evident after analysis of covariance (ANCOVA) in which the nutrients were the covariates. The infection area was

affected mainly by Mn content in leaves which is a much studied microelement (Dordas, 2008). The leaf area development was influenced by several nutrient concentrations in leaves (Table 5.4). The affect of mineral nutrient application in soil on leaf area of plants has been recognized as immediate and evident (Reuveni and Reuveni, 1998). Thus, the changing of the nutrient balance in soil due to fertilizers supply could had a corresponding influence on nutrients in leaves which may affected the leaf area of cucumbers.

5.5 CONCLUSION

The conclusions which come from this study point out to the fact that 300 ppm nitrogen in the fertilization solution is a critical concentration value not only for the leaf size but also for the spread of downy mildew disease. Thus, there are indications that the slightly elevated nitrogen concentrations above the ordinary rates resulted in downy mildew reduction, but the excessive doses had negative effect in disease development. The influence of nutrients in leaves not only on infection area but also on leaf surface is rather existent. The nitrogen supply via ammonium nitrate fertilizer is being considered for the control of downy mildew on cucumbers. However additional research is needed to get positive results in the use of fertilizer as an inducing agent for disease protection.

CHAPTER SIX

EFFECT OF POTASSIUM FERTILIZATION ON DOWNY MILDEW DEVELOPMENT OF CUCUMBER

6.1 INTRODUCTION

Cucumber downy mildew, caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostovzev, is a major disease attacking both field and greenhouse grown cucumber plants. Effective control is generally achieved by the use of fungicidal chemicals. However, in many cases disease management problems have been compounded due to the development of fungicide-resistant strains of the pathogen. Moreover, the concern for public health and a sound environment lead to the need for reduced pesticide levels on food crops and emphasise the need to find alternative control methods of downy mildew of cucumbers.

The effect of nutrients on plant disease control has been long recognized (Huber and Watson, 1974; Bains and Shooty, 1978; Walters and Bingham, 2007; Dordas, 2008). Thus, plant nutrition management techniques have been considered as a good alternative approach. Numerous examples have been reported about the interaction of plant nutrition and disease development especially for cereals (Tian *et al.,* 1997; Reuveni *et al*., 1996; Brennan, 1992). Nevertheless, the relationship between cucumber nutrition – downy mildew interaction has not been studied extensively. In the past the results of these experiments have in many cases been contradictory. For example, potassium was generally found to decrease disease, however there are studies that suggest the opposite. Thus, Sweeney *et al*. (2000) suggested that K fertilizer decreased leaf rust severity of wheat. Reuveni *et al*. (1996) also reported a positive effect of NPK fertilizers sprayed on leaves against both a facultative and an obligate parasite of maize plants (*Exserohilum turcicum* and *Puccinia sorghi*, respectively). Wang *et al*. (1998) also concluded that the disease resistance against *Valsa ceratosperma* in apple was increased by the addition of K fertilizer. In contrast, less recently Burge and Simmons (1982) found

that tomato was particularly resistant to *Verticillium dahliae* when grown without K.

The objectives of the present study were (a) to examine the effect of various K doses in the fertilization solution on downy mildew development of cucumber leaves, (b) to determine the temporal disease progress curves and (c) to examine under which nutritional status of cucumber would downy mildew spread more rapidly.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Design

The experiment was arranged in a Randomized block design with 6 treatments and 4 replicates. Each plot was composed of 12 pots, resulting in 288 pots in total for the experiment. The experimental design is given in Table 6.1. The experimental treatments consisted of 6 K doses which were applied to plants as a nutritional solution which also contained phosphorus and nitrogen. The potassium concentrations were 200, 300, 400, 500, 600 and 700 ppm, the phosphorus concentration was 50 ppm, and nitrogen concentration 250 ppm (Table 6.2).

The three elements above were added by mixing ammonium nitrate (33.5-0-0), orthophosphoric acid (H_3PO_4), and potassium nitrate (13.5-0-46.2) fertilizers. In Appendix B, Table B.2 details the quantities of the above fertilizers, the volume of the solution used and the corresponding nutrient concentrations given. A volume of approximately 30L solution was needed per fertilization in order to irrigate each treatment and kept in 40L plastic vessels (one per treatment). From this solution 500 mls were transferred to plants using a half liter volume vessel each time that irrigation was necessary.

Table 6.1. Experimental design arrangement (I, II, III, IV: replicates, 1 to 6: treatments).

I6	II3	III5	IV5
I2	II2	III6	IV6
I4	II5	III3	IV1
I5	II4	III4	IV3
I1	II1	III2	IV2
I3	II6	III1	IV4

Table 6.2. Treatments of the experiment (1-6): six increased potassium rates in the fertilization solution with standard concentrations of P and N.

1	200 ppm K + 50 ppm P + 250 ppm N
2	300 ppm K + 50 ppm P + 250 ppm N
3	400 ppm K + 50 ppm P + 250 ppm N
4	500 ppm K + 50 ppm P + 250 ppm N
5	600 ppm K + 50 ppm P + 250 ppm N
6	700 ppm K + 50 ppm P + 250 ppm N

6.2.2 Pathogen Inoculation

The fifth leaf from the top of each plant was marked and inoculated with *P. cubensis* by leaving droplets of spore suspension on it (see Section 3.4). The suspension concentration was adjusted to $15x10^4$ sporangia ml^{-1} (concentration obtained from the experiment described in chapter 4).

6.2.3 Measurements – Analytical Methods

A series of six photos (samplings) were taken when the leaves where infected. The days of sampling beginning from the day that the lesions appeared and subsequently on day 1, 2, 3, 5, 7 and 11 where the entire leaf was infected. Leaf and lesion area was measured. Plant tissue and soil analysis for all the plots was conducted. The data obtained were statistically analyzed.

6.3 RESULTS

6.3.1 Effect of potassium fertilization on the downy mildew progress

From the statistical analysis of the data there were indications that the lesion area on leaves was significantly different due to K doses in the fertilization solution. Figure 6.1 shows the effect of K on the downy mildew infection caused by *P. cubensis* on cucumber leaves in all the samplings. The use of 300 and 400 ppm K in the fertilization solution gave the most statistically significant decrease in lesion area in comparison with the other treatments. The disease was increased in the lower and higher K doses (200, 500-700 ppm respectively). The largest infected area on cucumber leaves was observed at the highest dose (700 ppm K) in all the samplings although statistically significant differences when compared with the other treatments observed at sampling 3. The K treatments did not result in significant differences in lesion area at sampling 6, when the entire leaf was infected. Similar results were observed in the case of relative lesion area (Figure 6.2).

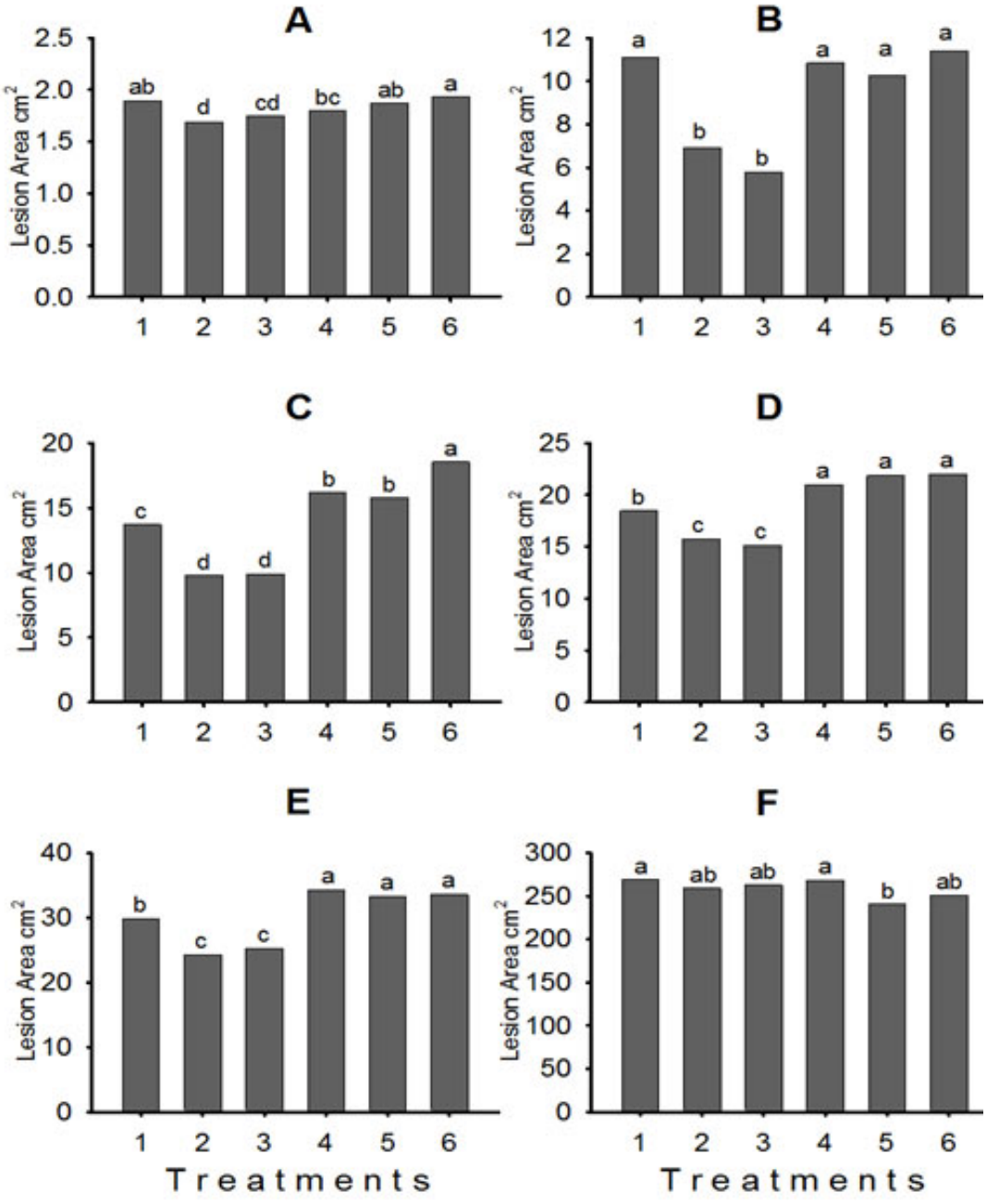

Figure 6.1. Effect of treatments (1-6; 200-700 ppm of K fertilization) on lesion area of cucumber leaves at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

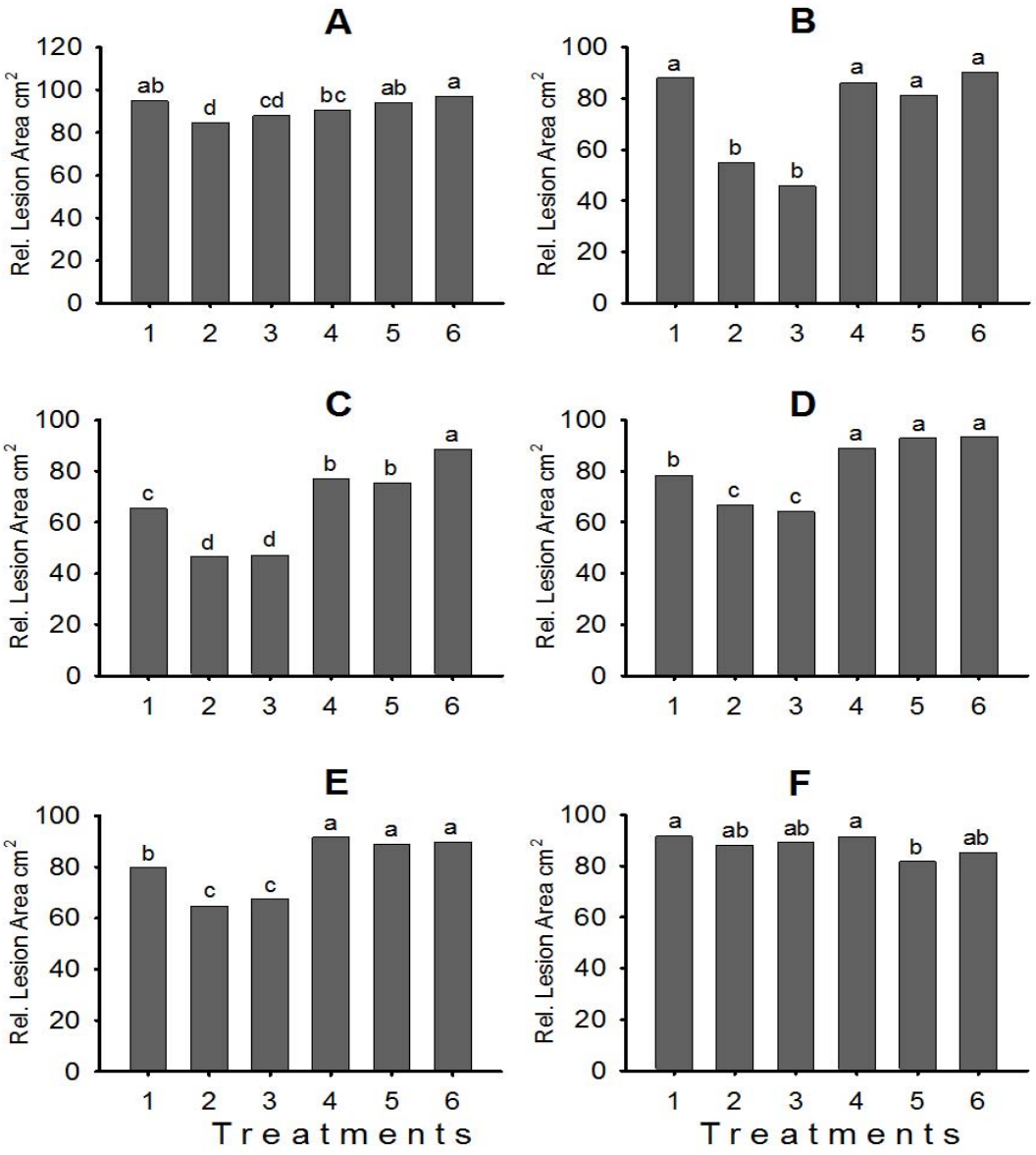

Figure 6.2. Effect of treatments (1-6; 200-700 ppm of K fertilization) on lerative lesion area of cucumber leaves during at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

In general K fertilization did not result in statistically significant differences on leaf size of cucumber plants (Figure 6.3). However, the third treatment (400 ppm K) in all the samplings resulted in the biggest leaves although the differences were not statistically significant. Statistically significant differences were found with the higher K concentrations at the 3rd and 5th sampling. Thus, there was a tendency for increasing leaf area as the K rate was increased from 200 to 400 ppm. Higher concentrations above this threshold decreased leaf area when applied to soil. Comparable results were obtained in relation to K treatments on relative leaf area (Figure 6.4).

Overall, the leaf area of cucumber plants increased with days of sampling at all K levels (Figure 6.5). A polynomial curve was found to best fit the data in all the cases. In relation to disease development, the polynomial model was also most appropriate for all the treatments. Therefore, both leaf and lesion area of cucumbers, increased with time, regardless of K concentration in soil. Figure 6.5 shows the leaf and lesion area progress curves with the cubic function in each case.

Tables 6.3 and 6.4 show the effect of nutrients on leaf area, lesion area and the relative leaf and lesion area respectively after covariance analysis. It is clear that nutrients in some cases influenced the leaf size and disease development. Covariates that had significant effects on these variables were mainly elements of leaves (K, Ca, Mg, Cu and Zn) and K of soil. Thus, soil K had a significant effect on leaf area and Cu in leaves affected the infection of cucumbers in the first sampling. The mean values without the K and Cu influence are presented in the next column (Leaf Area Cov. and Lesion Area Cov., respectively). In the second sampling Zn and K of leaves seem to affect the leaf size. In the third and fourth sampling Ca and Mg of leaves and only Mg respectively influenced the disease development with the corresponding mean values to be shown next to the initials. Finally in the sixth sampling are shown the differentiated mean values of leaf area by leaf K. Comparing the two tables, the same nutrients affected both the leaf and lesion area of cucumbers (Table 6.3) and relative leaf and lesion area (Table 6.4) in samplings 1, 2, 3, 4 and 6.

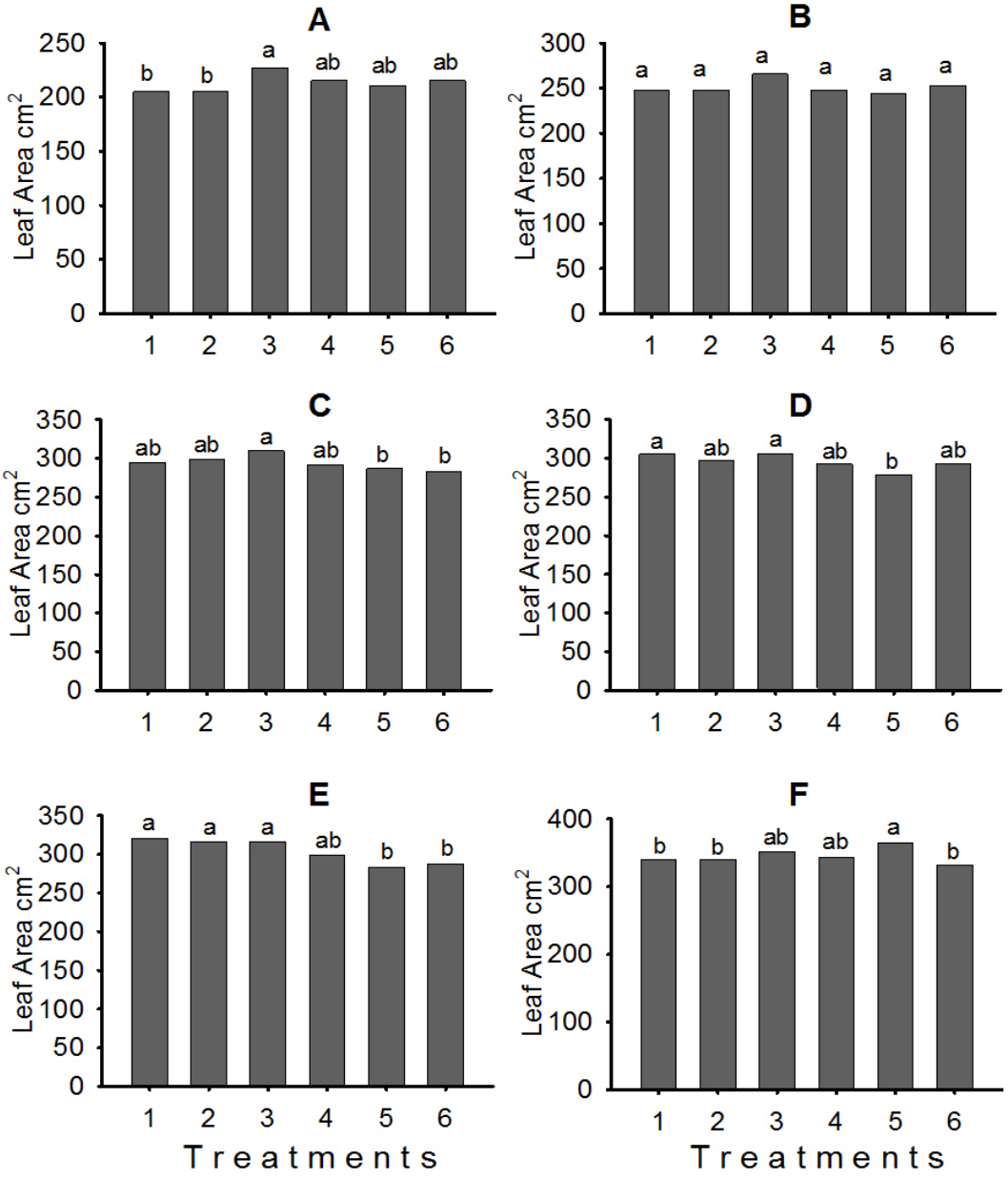

Figure 6.3. Effect of treatments (1-6; 200-700pmm of K fertilization) on leaf area of cucumber plants at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

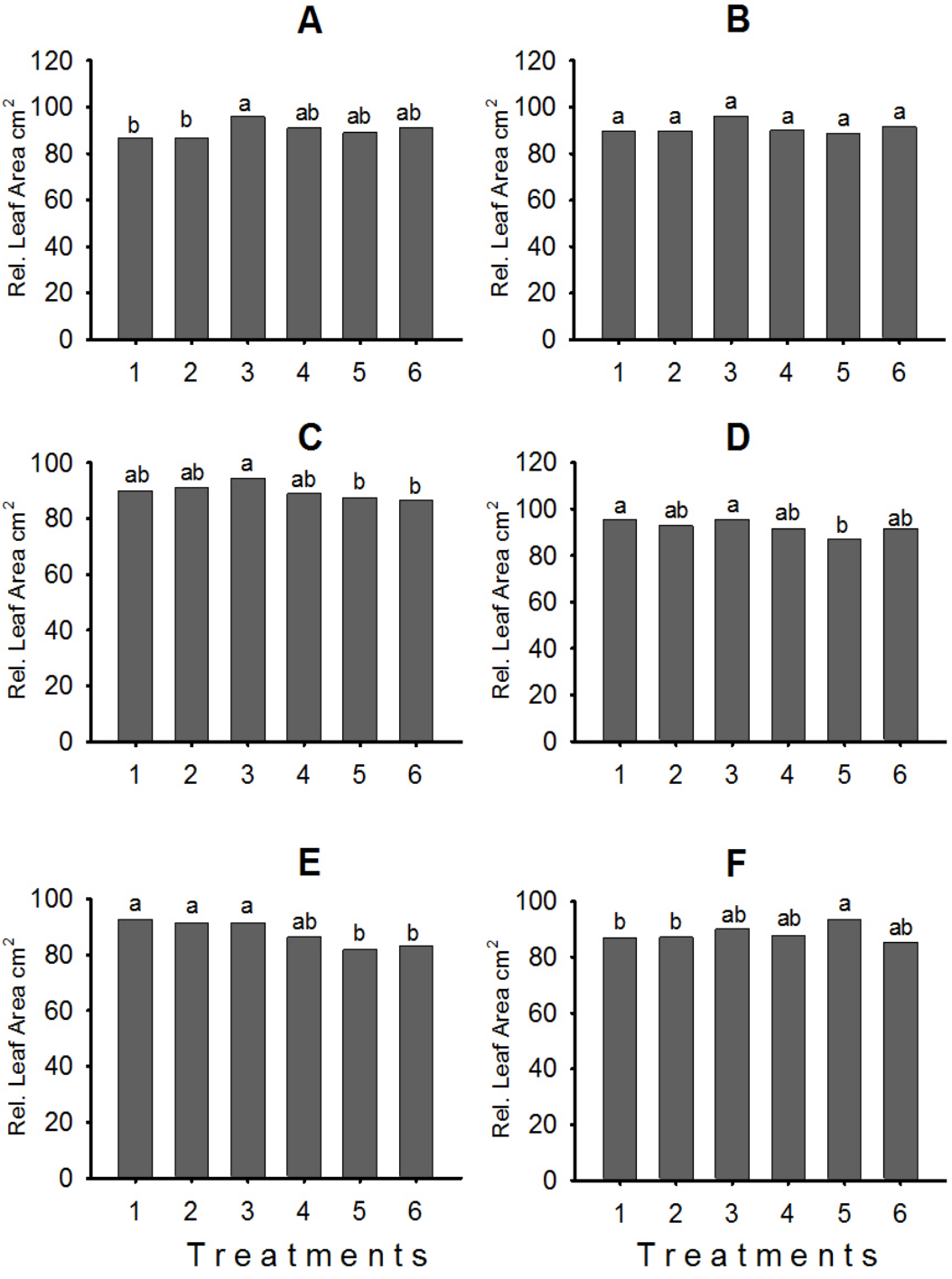

Figure 6.4. Effect of treatments (1-6; 200-700 ppm of K fertilization) on relative leaf area of cucumber plants at the six samplings (A-F; days 1, 2, 3, 5, 7 and 11). Columns which differ significantly from one another are marked with a different letter (Duncan, P=0.05).

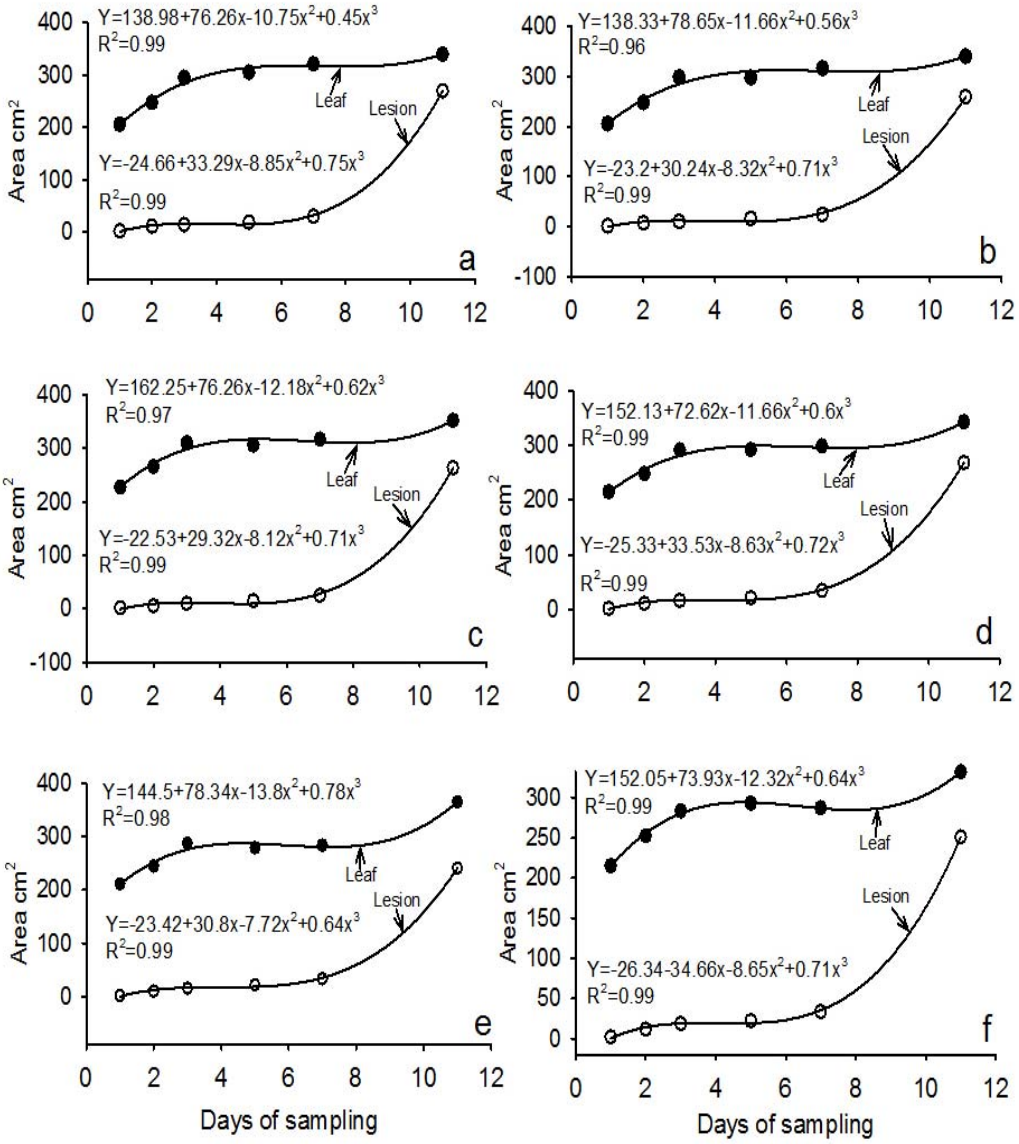

Figure 6.5. Progress of the treatments (a-f; 200-700 ppm of K fertilization) effect on leaf and lesion area of cucumbers during the samplings.

Table 6.3. Effect of nutrients on leaf and lesion area of cucumber plants, at the K fertilization treatments (200-700 ppm) at the six samplings (Tr: 1-6; Sam: 1-6), after covariance analysis (Leaf and lesion area Cov.).

Sam	Tr	Leaf Area	Leaf Area Cov.		Lesion Area	Lesion Area Cov.	
			K soil			Cu	
1	1	205.29 b	230.83		1.89 ab	1.87	
	2	205.48 b	223.77		1.69 d	1.69	
	3	226.88 a	233.18		1.75 cd	1.76	
	4	215.10 ab	208.97		1.80 bc	1.84	
	5	210.88 ab	192.99		1.88 ab	1.89	
	6	215.34 ab	189.23		1.93 a	1.88	
			Zn	K			
2	1	247.07 a	246.59	240.63	11.10 a		
	2	247.71 a	246.73	244.27	6.92 b		
	3	265.57 a	268.47	263.00	5.77 b		
	4	248.19 a	246.37	252.54	10.84 a		
	5	244.48 a	247.81	248.96	10.25 a		
	6	252.45 a	249.50	256.08	11.41 a		
						Ca	Mg
3	1	294.82 ab			13.73 c	13.79	14.43
	2	298.18 ab			9.78 d	9.08	9.29
	3	309.99 a			9.91 d	9.90	9.33
	4	291.62 ab			16.18 b	16.66	16.93
	5	286.84 b			15.84 b	16.58	15.94
	6	283.54 b			18.56 a	17.96	18.06
						Ca	
4	1	305.10 a			18.47 b	18.53	
	2	297.44 ab			15.77 c	15.06	
	3	305.92 a			15.12 c	15.11	
	4	292.34 ab			21.01 a	21.50	
	5	278.50 b			21.89 a	22.66	
	6	293.15 ab			22.03 a	21.42	
5	1	320.68 a			29.87 b		
	2	316.09 a			24.25 c		
	3	316.61 a			25.25 c		
	4	298.76 ab			34.29 a		
	5	283.49 b			33.29 a		
	6	287.14 b			33.56 a		
			K				
6	1	339.24 b	332.69		269.45 a		
	2	339.98 b	336.49		259.29 ab		
	3	351.35 ab	348.73		262.85 ab		
	4	342.84 ab	347.26		268.59 a		
	5	364.77 a	369.32		240.41 ab		
	6	332.21 b	335.90		251.07 b		

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Table 6.4. Effect of nutrients on percentage leaf and lesion area (Leaf and lesion area PC) of cucumbers, after covariance analysis (Leaf and lesion area PC Cov.). (Samplings: 1-6; Treatments 1-6: 200-700 ppm K).

Sam	Tr	Leaf Area PC	Leaf Area PC Cov.		Lesion Area PC	Lesion Area PC Cov.	
			K soil			Cu	
1	1	86.75 b	97.54		94.90 ab	93.70	
	2	86.83 b	94.56		84.60 d	84.99	
	3	95.87 a	98.53		87.91 cd	88.44	
	4	90.89 ab	88.30		90.37 bc	92.26	
	5	89.11 ab	81.55		94.04 ab	94.97	
	6	91.00 ab	79.96		96.87 a	94.33	
			Zn	K			
2	1	89.46 a	89.29	87.13	88.03 a		
	2	89.69 a	89.34	88.45	54.82 b		
	3	96.16 a	97.21	95.23	45.77 b		
	4	89.86 a	89.20	91.44	85.96 a		
	5	88.52 a	89.73	90.14	81.26 a		
	6	91.41 a	90.34	92.72	90.44 a		
						Ca	Mg
3	1	89.93 ab			65.39 c	65.68	68.73
	2	90.95 ab			46.55 d	43.26	44.22
	3	94.56 a			47.19 d	47.16	44.45
	4	88.95 ab			77.05 b	79.35	80.64
	5	87.49 b			75.41 b	78.97	75.89
	6	86.49 b			88.37 a	85.54	86.02
						Ca	
4	1	95.25 a			78.34 b	78.60	
	2	92.85 ab			66.86 c	63.85	
	3	95.50 a			64.12 c	64.10	
	4	91.26 ab			89.09 a	91.19	
	5	86.94 b			92.83 a	96.09	
	6	91.51 ab			93.42 a	90.84	
5	1	92.68 a			79.79 b		
	2	91.35 a			64.79 c		
	3	91.50 a			67.46 c		
	4	86.35 ab			91.60 a		
	5	81.93 b			88.95 a		
	6	82.99 b			89.65 a		
			K				
6	1	86.97 b	85.29		91.62 a		
	2	87.16 b	86.26		88.17 ab		
	3	90.07 ab	89.40		89.38 ab		
	4	87.89 ab	89.03		91.33 a		
	5	93.51 a	94.68		81.75 b		
	6	85.17 b	86.11		85.37 ab		

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

6.3.2 Total accumulation of nutrients in the leaves and soil

Findings obtained from this experiment showed that for concentrations of Ca, Mg, P, K, Fe, Cu and Zn in the leaves there was no statistically significant differences between treatments (Tables 6.5 and 6.6). K levels of 300 and 400 ppm (concentrations that reduced downy mildew severity) resulted in the lowest NH_4-N and NO_3-N content in leaves, respectively, although the differences were not significant when compared with almost all the other levels (Table 6.5). However, Mn content in leaves was statistically decreased in the 400 ppm K. A decreasing trend was for this element up to intermediate K dose (400 ppm) and then an increase was observed until the highest concentration, although the two intermediate treatments (400–500 ppm K) were not statistically different.

Furthermore, there were indications that addition of K influenced the soil content of NH_4-N and K but not that of soil NO_3-N. Increasing K doses in the fertilization solution resulted in higher concentration of K in soil (Table 6.7) with statistically significant differences for almost all the treatments. Thus, the lower soil K content observed in treatment 1 (200 ppm K) and the highest in treatment (700 ppm K). The concentration of NH_4-N in soil in the 300 and 400 ppm K fertilization treatments were statistical significant decreased as compared with the rest of the treatments. With the exception of these two treatments, none of the other gave statistically significant differences in content of NH_4-N (Table 6.7).

Correlation analysis of the data showed that leaf area of cucumber plants was positively correlated with lesion area caused by *P. cubensis* on leaves (r=0.67). The following positive correlations were also obtained: Fe and Zn in leaves (r=0.80), P and K in leaves (r=0.74).

Table 6.5. Means of macroelements concentration in cucumber leaves.

tr	Ca %	Mg %	K %	P %	NH_4N ppm	NO_3N ppm
1	2.48 a	0.95 a	4.67 a	0.47 a	115.44 a	218.50 c
2	2.08 a	0.85 a	4.80 a	0.46 a	93.17 b	259.38 abc
3	2.45 a	0.84 a	4.84 a	0.47 a	107.53 ab	210.96 c
4	2.71 a	0.95 a	5.15 a	0.50 a	107.48 ab	292.95 ab
5	2.85 a	0.90 a	5.15 a	0.51 a	117.26 a	298.42 a
6	2.14 a	0.85 a	5.12 a	0.50 a	110.72 ab	239.37 bc

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Table 6.6. Means of microelements concentration in cucumber leaves.

tr	Fe ppm	Mn ppm	Zn ppm	Cu ppm
1	75.80 a	40.99 a	14.85 a	8.72 ab
2	84.06 a	38.50 ab	14.97 a	9.30 ab
3	74.77 a	34.76 c	14.07 a	9.35 ab
4	73.52 a	37.13 bc	15.16 a	9.86 a
5	76.06 a	39.79 ab	13.97 a	9.50 ab
6	73.92 a	38.03 ab	15.42 a	8.22 b

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Table 6.7. Means of macroelements concentration in soil.

tr	K ppm	NH_4N ppm	NO_3N ppm
1	190.86 d	36.32 a	36.83 a
2	236.55 d	24.91 b	32.87 a
3	312.30 c	22.86 b	34.26 a
4	390.72 b	37.06 a	36.64 a
5	464.98 a	40.30 a	38.43 a
6	516.94 a	39.16 a	35.34 a

Means within columns followed by different letters are significantly different (Duncan, P=0.05).

6.4 DISCUSSION

Overall, the concentrations of K in the fertilization solution at around 350 ppm was found to play an important role in relation to leaf infection by downy mildew, leaf size and nutrients content in cucumber leaves and soil. The results indicated that increasing rates of K in the fertilization solution led to downy mildew reduction in cucumber leaves. Treatments of 300/400 ppm of K resulted in a statistically significant decrease of lesion area on leaves and also significantly lowered the concentration of NH_4-N in soil when compared to the other K concentrations (see Figure 6.1 and Table 6.7 respectively). The third treatment alone (400 ppm K) decreased Mn content in leaves with statistically significant differences when compared with all the other treatments except the slightly higher K concentration (500 ppm). The use of 400 ppm K also resulted in the largest leaf area of the cucumber plants.

This negative effect of K on plant diseases development has been also reported by several other studies in other crops. For example, recently, work on alfalafa showed that leaf spot disease was also reduced by K applications (KCl; Grewal and Williams, 2002). However, the plants used in the present study belong to a different family, the fungus to a different class than theirs (Fabaceae and Leotiomycetes respectively) and the plants were inoculated with uniformity here and not naturally infected. Amtmann *et al*. (2008) also reviewed that in most cases K fertilization generally suppressed plant disease incidence, although many authors (Sweeney *et al*., 2000; Huber, 1980) have suggested that the suppression effect of K on disease severity might have been because of Cl^- in the KCl fertilizer. In the current study K significantly decreased the susceptibility of cucumber plants (up to 400 ppm) but beyond this concentration no further decrease of disease suppression was observed. This threshold effects were recently also suggested by Dordas (2008).

The disease suppressing effect of K salt application was probably due to its influence on plant metabolism. The metabolic functions of K in plant physiology or promotion of thicker outer walls of epidermal cells has been implicated (Dordas, 2008). Excess K fertilization (treatments 4 to 6) possibly changed the nutrient

balance and especially the N:K ratio. This may explain the statistically significant increase in downy mildew infection at the higher K rates when compared with the intermediate concentrations. Huber (1980) and more recently Prabhu *et al*. (1999) also found this ratio to be important with regard to K fertilization on severity of maize stalk rot and rice blast, respectively. Another factor that could explain the increase of lesion area of cucumber leaves in the higher K concentrations treatments in the fertilization solution added to cucumbers may be the type of fertilizer used. The importance of the influence of the fertilizer type on plant disease development is known in other host-parasite relationships (Huber, 1980). Thus, KCl was found to reduce *Diplodia* stalk rot while similar rates of KNO_3 increased severity of this disease and application of K_2SO_4 had little effect on stalk rot of maize. In the present experiment K was added as KNO_3. Therefore the downy mildew increases found in treatments >400 ppm could be due to this although cucumbers belong to a different taxonomical group than maize (Cucurbitaceae – Poaceae, respectively).

Covariance analysis of the data indicated that nutrients affected the leaf area of cucumbers and the lesion area caused by *P. cubensis*. This shows that the mean values of these variables would be different without the effect of the elements. The nutrients that were involved in changing the effect of different K doses on the leaf area were soil K, leaf content of Zn and K. The infection development was influenced by Cu, Ca and Mg leaf content. The influence of these nutrients on leaf and lesion area progress was an indirect action of each separate element which consisted a covariate.

Mineral nutrient supply has been generally considered to have an immediate, evident effect in increasing the leaf area of the plants grown in soil that can utilise this type of fertilization (Reuveni and Reuveni, 1998). Thus, in the present study the best treatment (400 ppm K) increased the leaf area of cucumbers when compared with the lower K rates although the differences were not statistical significant. Higher K doses (treatments 5, 6) lowered the leaf area of cucumbers with statistical significant differences compared with that at 400 ppm K. This was probably related to the significant increase of lesion area observed in these

treatments and could be explained by the leaf shrinkage as a result of high diseased leaves.

Potassium fertilization resulted in no significant statistical differences on macroelement content (Ca, Mg, P, K) in cucumber leaves. However, higher K levels in soil (500, 600 and 700 ppm) increased K concentration in plant tissues. This phenomenon was also observed in the case of a quite different plant, alfalfa (Grewal and Williams, 2002). Decreased concentrations of nutrients in cucumber leaves and soil were observed in fertilization treatments with 300 and 400 ppm K. Thus, NH_4-N in soil was decreased in the 300/400 ppm K and leaves of cucumber grown in 400 ppm K also had the lowest Mn content. Mn is one of the most studied micronutrients in relation to influences on plant diseases. It was reported that high Mn content in wheat seeds produced plants with less take all (*Gaumannomyces graminis*) compared with plants grown from seeds of the same cultivar which had lower Mn concentration (Huber and McCay-Buis, 1993).

6.5 CONCLUSION

Potassium fertilization has been generally recommended to diminish disease incidence in many host plants. This trend of K was more pronounced with respect to fungal diseases in contrast to viral infections that were found to be more frequent in plants with high K application. The results of the present study were consistent with the above suggestion but this disease suppression effect of K had an optimum limit and beyond this downy mildew was significantly increased on cucumber leaves. Nutrient status in soil and leaves appeared to affect infection and leaf size. The elements NH_4-N (of soil) and Mn (of leaves) seemed to play an important role in downy mildew development. Hence, K fertilization close to 350 ppm via KNO_3 is suggested for cucumber plants because of its positive effect on leaf area and significantly decreasing the lesion area caused by *P. cubensis*.

CHAPTER SEVEN

NITROGEN, POTASSIUM AND FUNGICIDE EFFECTS ON CUCUMBER DOWNY MILDEW

7.1 INTRODUCTION

Downy mildew of cucurbits caused by *P. cubensis* is a destructive disease not only in southern areas of Europe or United States (Keinath *et al*., 2007) but also in some years it was observed even in northern regions such as Finland (Tahvonen, 1985). The disease is difficult controlled because *P. cubensis* belongs to a group of 'the high risk pathogens' with high evolutionary potential. This means that the fungus quickly overcomes the host resistance and efficacy of some fungicides when compared with pathogens with low evolutionary potential (McDonald and Linde, 2002). The pathogen is highly variable in its pathogenicity (Lebeda, 1999) and thus genetic control of it is very difficult. Although effective sources of resistance based on race specificity are known in *Cucumis melo* and *cucurbita spp*., downy mildew control by growing resistant cultivars of cucumbers has not been effective yet (Lebeda and Prasil, 1994; Lebeda and Widrlechner, 2003b).

Therefore the most effective control method of cucumber downy mildew has been for many decades been considered the use of fungicides. For a long time in the past contact fungicides such as copper formulations were the only ones that protected from downy mildew. However the discovery of systemic fungicides (e.g. fosetyl-Al, propamocarb, metalaxyl) with good protection against Oomycetes was a revolution of chemical control (Cohen and Coffey, 1986). Systemic fungicides have many advantages as compared with contact fungicides. Among of them are their application at relatively low rates and that they leave no visible residue. Most of them leave little if any detectable residue in plant parts at harvest or in the environment, because they are applied at such low rates (Dutky, 2008). However the main disadvantage of them is that have a narrower spectrum of activity against multi-site toxicant fungicides that provide broad-spectrum control. Thus systemic fungicides have a specific, single-site mode of action and they act on a single metabolite in growing fungal cells. For this reason the risk of resistance

development to singe-site fungicides is high (Dutky, 2008; Urban and Lebeda, 2006). Several authors in many countries have reported appearance of resistance of *P. cubensis* to single-site toxicant fungicides after their continuous use under high disease pressure (Table 7.1)

Table 7.1. Occurrence of *P. cubensis* strains resistant /tolerant to fungicides (Urban and Lebeda, 2006).

Chemical Group/ Chemical Class	Common name	Countries where resistant/tolerant strains occurr
Phenylamides	Metalaxyl	Israel Greece Italy USA Russia Australia Czech Republic
Strobilurins	Azoxystrobin, kresoxim-methyl	Japan Taiwan
Phosphonates	Fosetyl-Al	Israel Czech Republic
Carbamates	Propamocarb	Israel
Phthalimides	Folpet	Israel
Dithiocarbamates	Mancozeb	Israel

On the other hand the influence of host nutrition to downy mildew disease incidence is established for many crops but the information for cucumbers is limited (Bains and Jhooty, 1978; Chaluvaraju *et al*., 2004; Kolota and Osinska, 2001; Panicker and Gangadharan, 1999; Sivaprakasam *et al*., 1974; Tanaka *et al*., 2000). In some cases applications of nutrients gave such successful control of diseases that can be compared with the efficacy of chemical control (e.g. powdery mildew of pepper, Reuveni *et al.*, 1998). Therefore in an integrated disease management approach host nutrition via inorganic fertilization should play an important role including of course several other factors such as various fungicides, mixtures of them, more disease resistant cultivars, epidemiological studies, weather forecasts etc.

Given that the two previous experiments which involved the effect of nitrogen and potassium fertilization to downy mildew of cucumber indicated positive results in relation to disease control, a factorial experiment was conducted. The objectives of this study were (a) to investigate the interaction of nitrogen and potassium concentrations that gave the best control of cucumber downy mildew according to the randomized block experiments that preceded (b) to study the nutrient status of the soil and leaves of cucumbers and the potential to relate with disease incidence (c) to examine the disease development with time (d) to study the effect of a common used systemic fungicide to disease control (e) to compare the effect of nutrient supply in soil on disease development in relation with that of fungicide application.

7.2 MATERIALS AND METHODS

7.2.1 Experimental Design

A factorial experiment was established with two levels of nitrogen and three levels of potassium in the fertilization solution and two treatments concerning the application of a systemic fungicide. The two nitrogen levels were 200 and 300 ppm while 200, 300 and 400 ppm were the levels of potassium. According to the treatments a volume of 500 mls of the fertilization solution was applied to each pot. A widely used fungicide fosetyl-Al (source Aliette 80 WG, producer: Bayer CropScience SA, France) which has been registered in Greece for field application (6651/04-07-1996) was used as a soil drench at the recommended concentration by the producer (2g L^{-1}). Fungicides drenches were applied to the pot surface (300 mls $plant^{-1}$) using a watering can. Therefore approximately 10 L of fungicide solution (32 plants x 300 ml $plant^{-1}$=9600 mls) was needed for each fungicide treatment. The two treatment applications were made, 24 hrs before inoculation and 24 hrs after downy mildew symptoms emergence. The treatments analytically are shown in Table 7.2 and the experimental design that was followed in Table 7.3. Each plot was consisted of eight pots with four replicates.

The cultivation techniques were similar to those applied to the other experiments and have been mentioned above (Chapter 3). In order to achieve the combinations of N and K concentrations the following fertilizers were used in appropriate proportions; ammonium nitrate (33.5-0-0) and potassium nitrate (13.5-0-46.2) (Appendix B, Table B3). P was added in the fertilization solution via orthophosphoric acid (H_3PO_4). The cucumbers that received the fungicide application were fertilized with standard solution contained 50 ppm P, 100 ppm N and 200 ppm K. The quantities of the fertilizers used and the volume of the solution were also described in Appendix B, Table B3. A volume of 500 mls of each solution kept in plastic vessels was applied in each pot with the aid of a 500 mls volume vessel.

7.2.2 Pathogen Inoculation

The cucumbers were artificially inoculated on the fifth leaf from the apex of each plant by placing five droplets of *P. cubensis* spore suspension using a Pasteur pipette. The suspension concentration was adjusted to $15x10^4$ sporangia ml^{-1} (details are given in paragraph 3.4) which was found that it resulted in quite a satisfactory infection (experiment described in chapter 4).

7.2.3 Measurements

Since symptoms appeared in leaves, digital photos were taken of each leaf and the photography repeated at two or three days intervals (samplings). The leaf and lesion area were calculated as described above. In order to make leaf and soil analysis samples of leaves and cores of soil were also received. Data obtained were statistically analyzed.

Table 7.2. Treatments applied to the cucumber plants in the pots.

1	200 ppm N + 200 ppm K + 50 ppm P
2	200 ppm N + 300 ppm K + 50 ppm P
3	200 ppm N + 400 ppm K + 50 ppm P
4	300 ppm N + 200 ppm K + 50 ppm P
5	300 ppm N + 300 ppm K + 50 ppm P
6	300 ppm N + 400 ppm K + 50 ppm P
7	Fungicide as a soil drench 24 hrs before inoculation
8	Fungicide as a soil drench 24 hrs after disease appearance

Table 7.3. Experimental design arrangement (I, II, III, IV: replicates, 1 to 8: treatments).

I7	II3	III4	IV4
I1	II2	III2	IV3
I8	II6	III6	IV6
I3	II8	III5	IV7
I2	II4	III3	IV2
I5	II1	III8	IV1
I4	II5	III1	IV8
I6	II7	III7	IV5

7.3 RESULTS

7.3.1 Main effects of N and K on leaf and lesion area of cucumbers

Figures 7.1 and 7.2 show the main effects of N and K on leaf, lesion, relative leaf and relative lesion area caused on cucumbers by *P. cubensi*s. Both N levels increased leaf and relative leaf area in the second sampling and decreased it in the next two samplings. The second N level (300 ppm) resulted in statistically significantly largest leaf size and the highest relative leaf area in almost all the assessments (samplings 1, 6, 10) (Fig. 7.1 A, C). N level of 300 ppm resulted in significantly the larger infection area than the lower N level (200 ppm) in the samplings of day 6 and 10 (Fig. 7.1 B, D). Infection area increased in both N concentrations during the samplings. However, the relative lesion area decreased with time in the first N level (200 ppm) while in the 300 ppm of N the relative infection surface increased on cucumber leaves.

The largest leaf surface was observed in the lowest K concentration (200 ppm) with statistically significant differences when compared with the other K levels in all the samplings (Fig. 7.2 A). The three K levels increased the leaf area of cucumbers in the second sampling while the third and fourth sampling resulted in smaller leaf size. However, the relative leaf surface was the highest for all K levels in the fourth sampling (Fig. 7.2 C). The lesion area increased with time in all the samplings (Fig. 7.2 B). K concentration of 200 ppm resulted in the highest infection in the first two samplings while the opposite was observed in the last two samplings. Similar results were recorded for relative lesion area (Fig. 7.2 D).

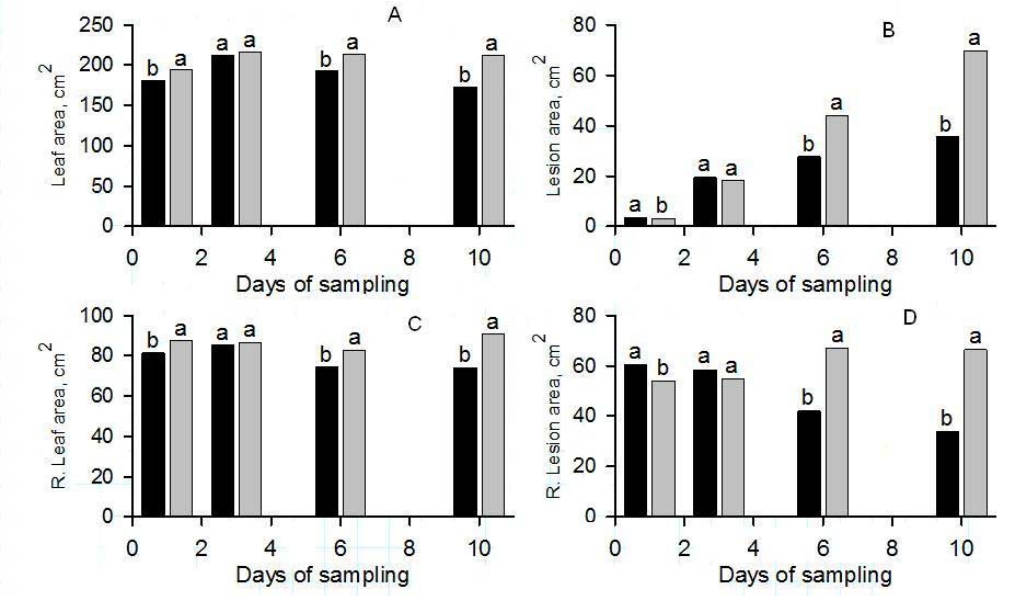

Figure 7.1. Main effects of N levels (200 ppm N: black columns; 300 ppm N: grey columns) on leaf (A), lesion (B), relative leaf (C) and relative lesion (D) area caused by *P. cubensis* on cucumbers grown in fertilization combinations of N and K.

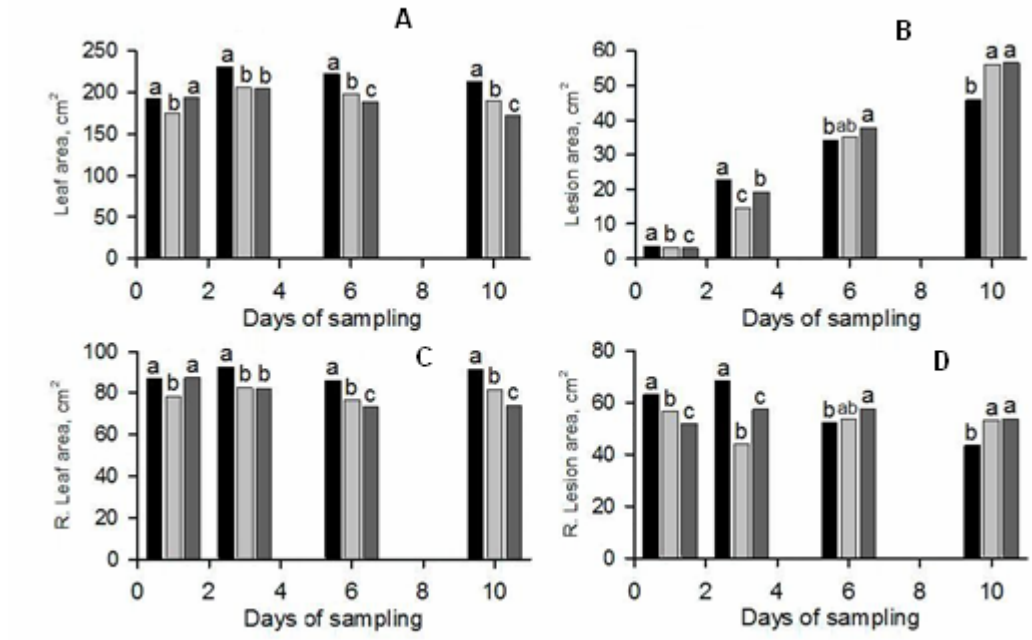

Figure 7.2. Main effects of K levels (200 ppm K: black columns; 300 ppm K: light grey columns; 400 ppm K: dark grey columns) on leaf (A), lesion (B), relative leaf (C) and relative lesion (D) area caused by *P. cubensis* on cucumbers grown in fertilization combinations of N and K.

7.3.2 Interactions of N and K

Figure 7.3 shows the interactions of N x K for the variables leaf and lesion area of cucumbers in the four samplings separately. Thus, both N levels decreased the leaf area of cucumbers in the second potassium dose (300 ppm K) and increased it in the third one (400 ppm K) in the first two samplings. However, only in the second sampling the NxK interaction was statistically significant at P=0.01. In the third and fourth sampling the leaf surface statistically significantly decreased (P=0.05 and 0.001 respectively) in the 200 ppm N with increasing K levels when compared with the higher N level (300 ppm) (Fig. 7.3).

N x K interaction was observed for lesion area of cucumbers in all the samplings at P=0.001 (Fig. 7.3). The infection surface of leaves in the 200 ppm of N decreased as K concentration was increasing in the fertilization solution while the opposite was observed for lesion area in the highest N level (300 ppm).

7.3.3 Fungicide effects on cucumber leaf and lesion area

The effect of the two fungicide treatments (pre and post inoculation) on leaf surface is shown in Figure 7.4. The area of cucumber leaves was non-significantly different in both fungicide treatments during the samplings. A slightly reduction trend of leaf area appeared in both cases as the disease was spreading. Cubic curves were best fitting the data obtained from leaves area of cucumbers treated with fungicide applications.

Lesion area was developed in a different way in each of the two fungicide treatments during time. The pre inoculation application of fungicide resulted in a rather stable progress, slightly increased in the last sampling days (Fig. 7.5). In contrast, the lesion area of cucumbers treated with the fungicide after the symptoms appearance was increased with time reaching a maximum value in the last sampling (Fig. 7.5).

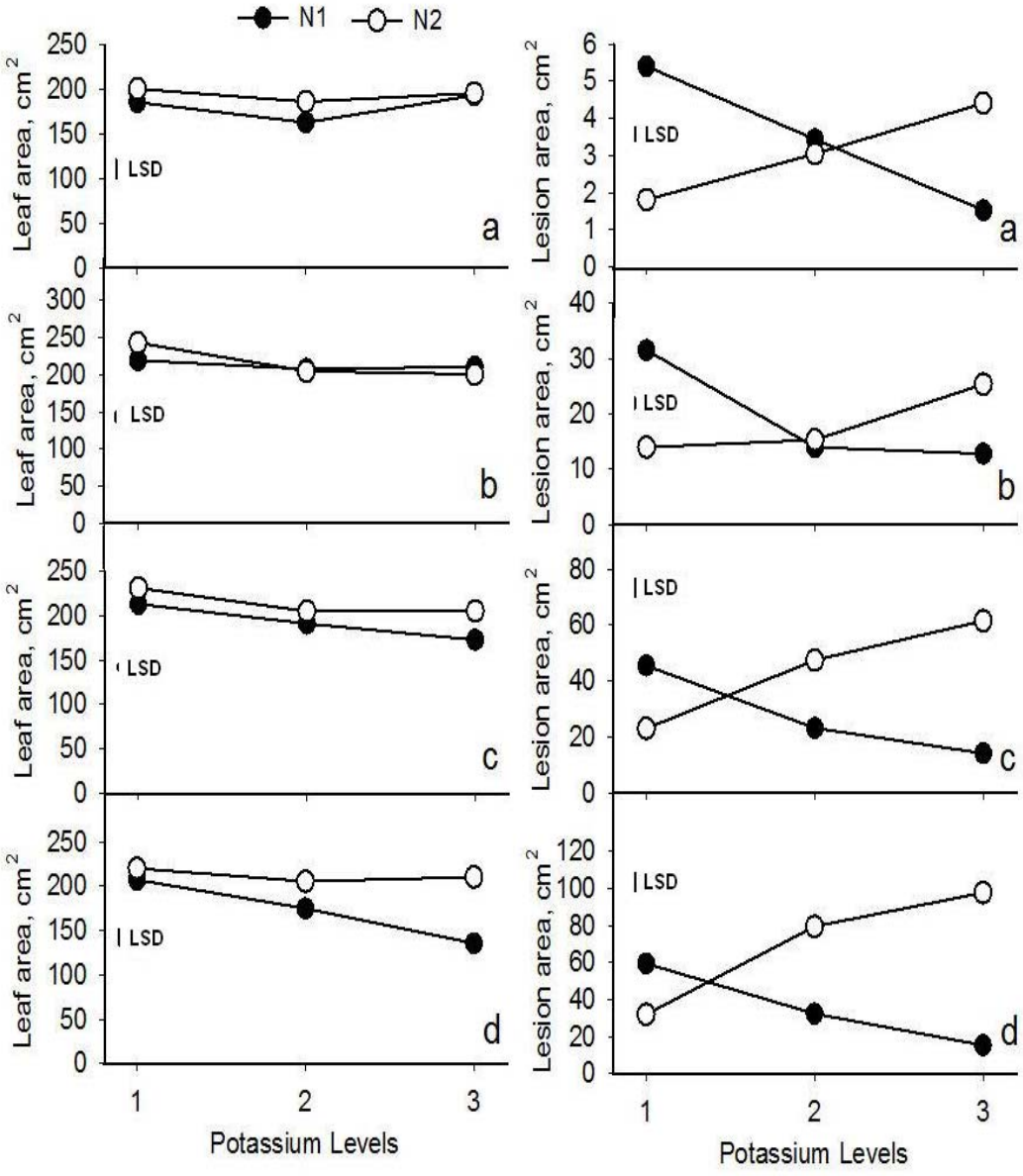

Figure 7.3. Interaction of N (N1: 200 ppm; N2: 300 ppm) and K (1; 2; 3: 200; 300; 400 ppm K) levels with regard to the leaf and lesion area caused of *P. cubensis* on cucumber plants at different stages of disease development (a-d: disease assessment at day 1, 3, 6, 10).

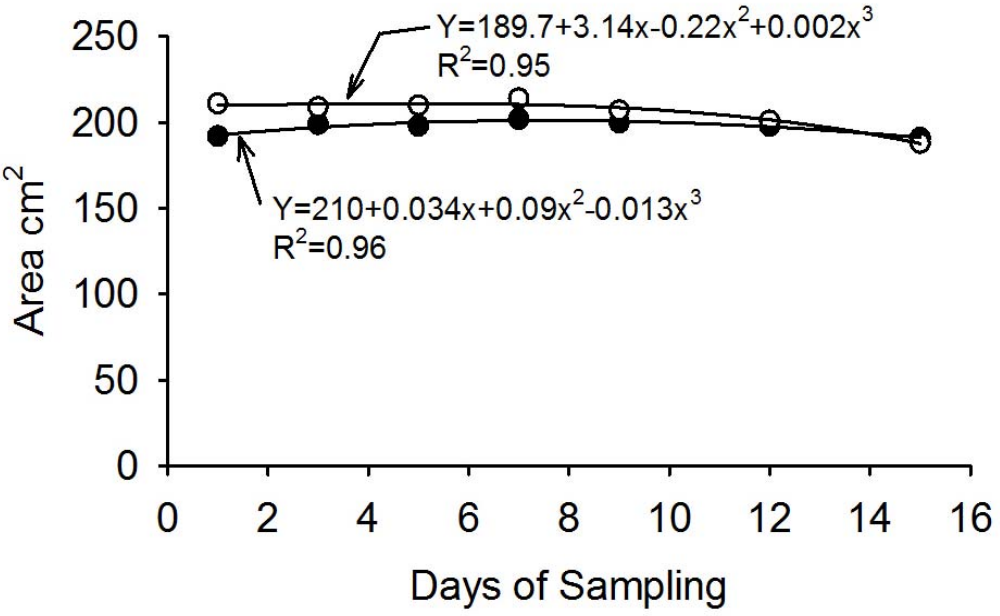

Figure 7.4. Leaf area development with time of cucumbers treated with fungicide applied as soil drench 24 hrs before inoculation (closed symbols) and 24 hrs after symptoms appearance (open symbols).

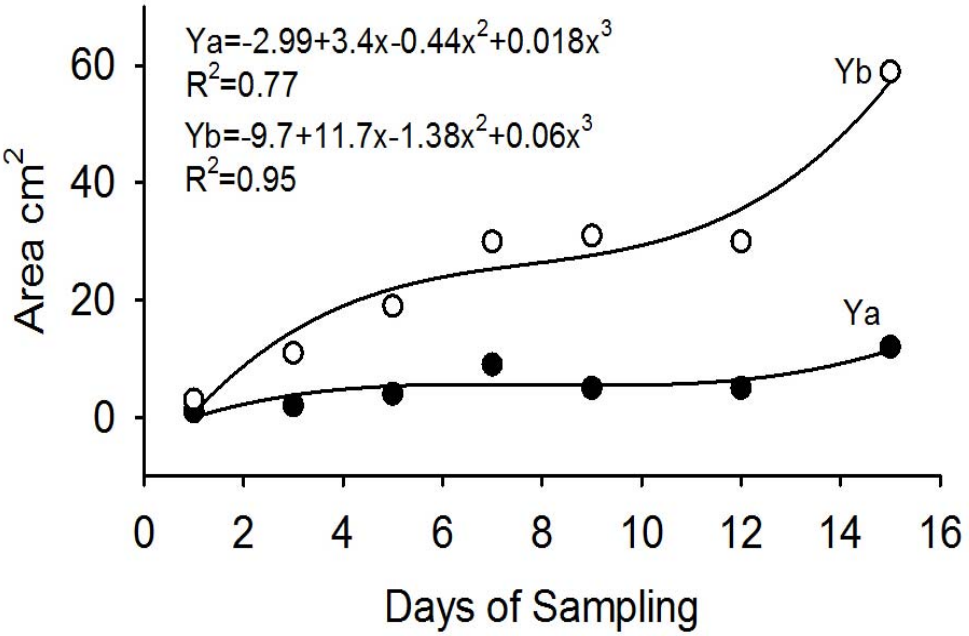

Figure 7.5. Downy mildew progress on cucumber leaves treated with fungicide applied as soil drench 24 hrs before inoculation (Ya) and 24 hrs after symptoms appearance (Yb).

Data presented in Table 7.4 show the comparison between the two fungicide applications and the fertilization treatments on cucumber plants for leaf and lesion area and the corresponding relative areas. Cucumbers fertilized at the same time with 300 ppm N and 200 ppm K had the largest leaf size and relative leaf area although statistically significant differences when compared with the other fertilization combinations and the fungicide treatments were observed only in the second sampling.

Data presented in Table 7.4 also indicate that the application with fungicide before inoculation of leaves with *P. cubensis*, statistically significantly suppressed the lesion area when compared with the other treatments in the samplings 1 and 2. However, in the last two disease assessments, the protective fungicide application continued to give the best control but the differences with the fertilization of 200 ppm N and 400 ppm K were not found statistically significant at P=0.05. The curative fungicide use gave moderate control of downy mildew relative to intermediate fertilization treatments (low N with high K level and high N with low K level in the first and second sampling). In the third sampling, the infection area of leaves in the post inoculation fungicide application was not statistically significantly different when compared with that of 200 ppm N-300 ppm K and 300 ppm N -200 ppm K. Besides, in the next symptoms assessment the above fertilization treatments resulted in better disease control than the curative fungicide application. It was also evident that the lowest N and K concentrations in the fertilization solution significantly increased the lesions surface at low disease levels (sampling 1 and 2) while when the infection was highly developed (samplings 3 and 4) the highest fertilization combination (300 ppm N-400 ppm K) resulted in significant increase of downy mildew.

Table 7.4. Effect of N and K fertilization and fungicide treatments (Fungicide 1 used 24 hrs before inoculation with *P. cubensis*; Fungicide 2 used 24 hrs after symptoms appearance) on leaf and lesion surfaces and relative areas of cucumbers. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Treatments		Leaf area cm^2	Lesion area cm^2	Relative leaf area cm^2	Relative lesion area cm^2
N ppm	**K ppm**		**First sampling**		
200	200	185.6 b	5.404 a	83.4 b	94.643 a
200	300	162.9 c	3.437 c	73.3 c	60.192 c
200	400	193.3 ab	1.525 d	86.9 ab	26.708 d
300	200	200.7 ab	1.805 d	90.3 ab	31.619 d
300	300	186.3 b	3.046 c	83.8 b	53.344 c
300	400	195.8 ab	4.409 b	88.0 ab	77.219 b
Fungicide 1		192.4 ab	0.663 e	86.5 ab	11.613 e
Fungicide 2		210.7 a	2.815 c	94.8 a	49.298 c
			Second sampling		
200	200	219.3 b	31.525 a	87.9 b	94.670 a
200	300	207.7 bc	13.975 d	83.2 bc	41.967 d
200	400	210.4 bc	12.825 d	84.3 bc	38.513 d
300	200	242.8 a	13.978 d	97.3 a	41.975 d
300	300	204.8 bc	15.325 d	82.0 bc	46.021 d
300	400	200.7 c	25.375 b	80.4 c	76.201 b
Fungicide 1		198.2 c	3.707 e	79.4 c	11.132 e
Fungicide 2		210.0 bc	19.411 c	84.1 bc	58.291 c
			Third sampling		
200	200	213.3 ab	45.514 b	82.5 ab	69.320 b
200	300	191.3 bc	23.161 c	74.0 bc	35.274 c
200	400	172.9 c	14.093 d	66.9 c	21.464 d
300	200	231.4 a	22.999 c	89.5 a	35.028 c
300	300	205.1 ab	47.425 b	79.3 ab	72.230 b
300	400	205.4 ab	61.590 a	79.5 ab	93.803 a
Fungicide 1		222.2 ab	8.882 d	85.9 ab	13.527 d
Fungicide 2		214.1 ab	29.822 c	82.8 ab	45.420 c
			Fourth sampling		
200	200	207.3 ab	59.504 c	88.8 ab	56.554 c
200	300	175.0 d	32.346 d	75.0 d	30.742 d
200	400	135.5 e	15.361 e	58.1 e	14.599 e
300	200	220.0 a	32.020 d	94.3 a	30.432 d
300	300	205.6 abc	79.525 b	88.1 abc	75.582 b
300	400	210.3 a	97.804 a	90.1 a	92.955 a
Fungicide 1		190.9 bcd	12.479 e	81.8 bcd	11.860 e
Fungicide 2		188.3 cd	59.182 c	80.7 cd	56.248 c

7.3.4 Assessment of nutritional status of cucumber plants

Table 7.5 show the main effects of N and K and that of fungicide application on nutrients of cucumber leaves and soil. The first level of N (200 ppm) resulted in statistical significant higher P, Cu and NH_4-N leaf content when compared with the second level. The opposite observed for the nutrients Ca in leaves and NO_3-N in both leaves and soil. In all the other cases there were no statistically significant differences. The concentrations of K in leaves and soil, Zn in leaves and NH_4-N in soil in the treatment of 400 ppm K were statistically significantly higher when compared with the other K levels while the soil NO_3-N content was the lowest in this K treatment. Non statistically significant differences between K fertilization doses were observed for the other nutrients.

Fungicide application on cucumber plants 24 hrs before inoculation with *P. cubensis* resulted in statistically significantly lowest concentration of Fe and NO_3-N in leaves when compared with the fungicide treatment 24 hrs after the disease appearance. All the other nutrients in leaves and soil had no statistically significant differences due to fungicide treatments (Table 7.5).

Statistical analysis of the obtained data revealed differences in nutrients accumulation in cucumber leaves and soil among the eight treatments (Table 7.2). Table 7.6 show the effect of these treatments analysed as Randomized block design on macro and micronutrients in cucumber leaves and soil. Leaf Ca and Fe content was the lowest in fertilization treatments 2, 3 and 4. The two fungicide applications and the higher levels of N and K (treatment 6) increased the K and NO_3-N leaf content. The combinations of the higher N concentration with all the K levels resulted in the lowest P and Cu concentration in cucumber leaves. Leaf Mg and soil NO_3-N content increased and decreased respectively when compared with the other treatments in both fungicide applications.

Table 7.5. Main effects of N and K fertilization and fungicide applications on nutrients concentration in cucumber leaves and soil. Fungicide applied as soil drench 24 hrs before inoculation with *P. cubensis* and 24 hrs after downy mildew appearance. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Levels	**Macronutriens in cucumber leaves**			
Nitrogen	**Ca%**	**Mg%**	**K%**	**P%**
200 ppm	3.31 b	0.60 a	4.48 a	0.75 a
300 ppm	3.69 a	0.58 a	4.48 a	0.66 b
Potassium				
200 ppm	3.49 a	0.58 a	4.33 b	0.70 a
300 ppm	3.44 a	0.58 a	4.39 b	0.70 a
400 ppm	3.56 a	0.61 a	4.72 a	0.72 a
Fungicide				
pre inoculation	3.98 a	0.85 a	4.63 a	0.81 a
post inoculation	3.80 a	0.71 a	4.79 a	0.83 a
	Micronutrients in cucumber leaves			
Nitrogen	**Fe ppm**	**Mn ppm**	**Zn ppm**	**Cu ppm**
200 ppm	170.1 a	70.7 a	25.8 a	52.0 a
300 ppm	180.6 a	74.7 a	26.6 a	31.3 b
Potassium				
200 ppm	189.4 a	69.0 a	25.0 b	48.7 a
300 ppm	167.8 a	75.1 a	25.5 b	30.9 b
400 ppm	168.7 a	74.0 a	28.2 a	45.4 a
Fungicide				
pre inoculation	207.8 b	81.7 a	23.7 a	61.6 a
post inoculation	257.2 a	85.1 a	26.7 a	62.4 a
	Nitrogen in leaves		**Nutrients in soil**	
Nitrogen	**NH_4-N ppm**	**NO_3-N ppm**	**NH_4-N ppm**	**NO_3-N ppm**	**K ppm**
200 ppm	42.8 a	51.6 b	14.2 a	28.5 b	430.9 a
300 ppm	33.1 b	73.0 a	12.5 a	42.6 a	423.3 a
Potassium					
200 ppm	43.1 a	61.0 ab	11.0 b	36.9 a	372.1 c
300 ppm	33.6 a	57.2 b	14.1 a	37.1 a	432.9 b
400 ppm	37.1 a	68.7 a	15.0 a	32.7 b	476.3 a
Fungicide					
pre inoculation	16.4 a	58.4 b	12.8 a	14.3 a	393.6 a
post inoculation	19.9 a	75.1 a	13.3 a	16.1 a	409.0 a

Table 7.6. Concentrations of nutrients in soil and leaves of cucumber plants treated with combinations of N and K fertilization solutions and two fungicide applications. Fungicide applied as soil drench 24 hrs before inoculation with *P. cubensis* and 24 hrs after downy mildew appearance. Means within columns followed by different letters are significantly different (Duncan, P=0.05).

Treatments		**Macronutriens in cucumber leaves**			
N	K	**Ca%**	**Mg%**	**K%**	**P%**
ppm	ppm				
200	200	3.90 ab	0.61 c	4.46 cde	0.71 cd
200	300	3.16 c	0.60 c	4.50 cd	0.75 bc
200	400	2.86 c	0.60 c	4.46 cde	0.78 ab
300	200	3.09 c	0.55 c	4.19 e	0.68 d
300	300	3.72 b	0.56 c	4.27 d	0.65 d
300	400	4.25 ab	0.63 c	4.99 a	0.66 d
Fungicide 1		3.98 a	0.85 a	4.63 bc	0.81 ab
Fungicide 2		3.80 ab	0.71 b	4.79 ab	0.83 a
N	K	**Micronutrients in cucumber leaves**			
ppm	ppm	**Fe ppm**	**Mn ppm**	**Zn ppm**	**Cu ppm**
200	200	234.3 ab	71.9 bcd	26.0 b	68.6 a
200	300	134.8 d	72.8 bcd	25.7 b	30.1 c
200	400	141.2 d	67.6 cd	25.7 b	57.2 b
300	200	144.5 d	66.1 d	23.9 b	28.8 c
300	300	200.9 c	77.4 abc	25.2 b	31.7 c
300	400	196.3 c	80.5 abc	30.6 a	33.5 c
Fungicide 1		207.8 bc	81.7 abc	23.7 b	61.6 ab
Fungicide 2		257.2 a	85.1 a	26.7 ab	62.4 ab
N	K	**Nitrogen in leaves**		**Nutrients in soil**	
ppm	ppm	**NH_4-N ppm**	**NO_3-N ppm**	**NH_4-N ppm**	**NO_3-N ppm**	**K ppm**
200	200	51.0 a	56.6 c	11.3 b	30.6 c	381.6 cd
200	300	28.1 cde	49.8 c	14.4 ab	28.7 c	437.2 ab
200	400	49.3 ab	48.3 c	16.9 a	26.1 c	474.0 a
300	200	35.1 bcd	65.4 bc	10.7 b	43.1 ab	362.7 d
300	300	39.2 abc	64.5 bc	13.8 ab	45.4 a	428.6 b
300	400	24.9 cde	89.2 a	13.1 ab	39.3 b	478.5 a
Fungicide 1		16.4 e	58.4 bc	12.8 b	14.3 d	393.6 bcd
Fungicide 2		19.9 de	75.1 ab	13.3 ab	16.1 d	409.0 bc

7.4 DISCUSSION

There was evidence that N interacted with K fertilization in the case of cucumber leaf area and infection surface caused by *P. cubensis*. Thus, the increased K led to the smallest leaf size when N was applied at the lowest concentration (200 ppm), while the leaf area was not changed as the K rate increased from 200 to 400 ppm in the highest N level (300 ppm). The increased K concentration significantly decreased downy mildew symptoms only in the lowest N level, reaching a minimum infection area in the higher K dose. In contrast, the largest infection surface was observed in the higher N and K concentrations. This kind of interaction between N and K nutrients with regard to disease development has also been recorded for other fungal diseases such as rice blast. Prabhu *et al*. (1999) reported that increased potassium fertilization had a significantly linear negative response on rice blast development, only in the absence of nitrogen.

The relationship between nutrients influences the way that plants react to diseases. Especially, the balance between the essential nutrients N and K is known to affect disease susceptibility of plants (Dordas, 2008). For example, the development of another Peronosporales fungus, *Phytophora infestans* in potato plants was determined by the N:K ratio (Prabhu *et al.*, 2007). In this study, the increased infection expressed in the high K level was eliminated by low rates of N application. Huber (1980) reported a similar finding about mildew diseases; balanced levels of P and K may offset the negative effects of high N levels.

It was also concluded that both fungicide treatments did not affect the leaf area of cucumber plants and thus remained stable during the sampling days. However, a light decrease in leaf surface was observed especially for cucumbers soil drenched with fungicide after downy mildew symptoms appearance. This was probably because of the high infection recorded in these leaves which possibly resulted in leaf shrinkage. The lesion area developed in a different way in each fungicide application. The protective treatment prevented the fungus development while the curative fungicide application only slightly delayed the disease progress

which was greatly increased with time. Fosetyl-Al was not also found to inhibit lesion expansion when applications were made on a curative basis for another obligate parasite (*Phytophthora citrophthora*, Alvarez *et al*., 2008). However, the systemic fungicide Fosetyl-Al employed as soil drench is known to have good activity against downy mildews (Cohen and Coffey, 1986).

These results however suggested that although between two fungicide treatments the protective application gave better control against downy mildew of cucumbers than the curative use, the efficacy of this chemical can be compared with that of nutrition in some way. Thus, the single protective fungicide application significantly reduced the infection area relative to the fertilization treatments only in low disease levels (samplings 1 and 2). The fungicide application after the symptoms appearance gave relatively poor control of downy mildew compared to that of the combination of the lowest tested N and highest K level (200 ppm N and 400 ppm K). In addition the above fertilization treatment resulted in the largest leaf area of cucumbers. The comparison between the effect of chemicals and fertilizer application on the control of other foliar diseases under greenhouse conditions showed that the fungicide was the most efficient in controlling the infection (tebuconazole-sulphur foliar fertilizer-soybean powdery mildew, Yorinori *et al*., 2004).

The results also indicated that the downy mildew was less controlled by the combination of the lowest N and K concentrations at the beginning of the fungus development. In contrast, when the infection level was high (last disease assessments) the fertilization with highest N and K content was ineffective in reducing downy mildew of cucumbers.

Nutrients of cucumber leaves and soil were not differentiated in any of the two fungicide applications, except Fe and NO_3-N leaf content. In contrast, N levels resulted in differences of Ca, P, Cu, N (both NH_4-N and NO_3-N) accumulation in leaves and NO_3-N in soil. K, Zn, Cu, NO_3-N contents in leaves and both N forms and K in soil produced statistically significant differences due to K levels. Mg in leaves and NO_3-N in soil were the only nutrients that had the highest and the lowest respectively concentration in fungicide application when compared with the

fertilization treatments. The above differences of leaves and soil nutrients content between treatments imply an indirect effect of these nutrients on leaf and lesion area development of cucumber plants.

7.5 CONCLUSION

These results indicated that the use of NH_4NO_3 and KNO_3 fertilizers has a significant role in downy mildew control of greenhouse grown cucumbers. An interaction was observed between N and K nutrients relative to their effect on disease development. Therefore, the increasing K concentration from 200 to 400 ppm suppressed the lesions area at the low N rate (200 ppm). An important indication was that at low disease levels the infection was less controlled with low N and K concentrations while as the disease was spreading high N and K application was not efficient in reducing the lesion expansion. The preventive use of fosetyl-Al gave better control than the curative. However, at high disease levels the fungicide effect on infection was comparable to one fertilization combination of those tested (200 ppm N-400 ppm K). The inhibitory effectiveness of N and K makes fertilization with these nutrients a potential major component of an integrated pest management program.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

8.1 DISCUSSION

Increasing pesticide levels on food crops have stimulated the consumer demand for food safety. In addition the need for environmental protection has been increased in recent years. On the other hand vegetable crops are attacked by destructive diseases that cause loss of production and thus growers are economically suffering. This has highlighted the need for environmentally friendly methods as alternatives in order to enhance protection of plants against diseases. Attempting to meet this goal inorganic fertilizers were examined as a control strategy against the fungus *P. cubensis* that causes downy mildew disease of cucumber plants (*Cucumis sativus*).

In the past, extensive research has been made for various crop-pathogen interactions and many of these studies had conflicting results but for cucumber-downy mildew not much information is available, especially for cultivation in greenhouse conditions. Studies about the effect of mineral application on other cucumber diseases such as powdery mildew (*Sphaerotheca fuliginea*) (Reuveni *et al*., 1995, 1996; Liang *et al*. 2005), anthracnose (*Colletotrichum lagenarium*) (Gottstein and Kuc, 1989) or downy mildews of other than cucumber crops such as cauliflower (*Peronospora parasitica*) (Becot *et al*., 2000), onion (*Peronospora destructor*) (Ahmad and Khan, 2001), pearl millet (*Sclerospora graminicola)* (Chaluvaraju *et al.*, 2004; Sivaprakasam *et al*., 1974), maize (*Peronosclerospora sorghi*) (Panicker and Gangadharan, 1999) have been established. The published data about the influence of mineral supply on the pathosystem cucumber-*P. cubensis* concern mainly studies in hydroponic systems (Tanaka *et al*., 2000) or spraying applications of inorganic salts on cucumber leaves (Kolota and Osinska, 2001) and not supply via fertilization solution.

It was shown in Chapter 4 that *P. cubensis* could attack cucumber leaves regardless of their age causing symptoms with the same severity on them. It was also concluded that even at the lowest inoculum volume the fungus was very aggressive. However, the content of $15x10^4$ sporangia ml^{-1} was suggested as the optimal concentration for successful infection using the droplet method. There were indications that the lesion area significantly increased with increasing inoculum concentration while as the disease was spreading with time, the infection area did not appear to be affected by spore content.

Thus, using the above inoculum concentration one level of N (300 ppm) and two of K (300 and 400 ppm) were found to reduce downy mildew severity. In the case of N treatments, the suppressing effect of N fertilizer (NH_4NO_3) on downy mildew was probably a result of an effect on the pathogen's requirements that prevented fungus growth. Low and high N levels led to an increase in downy mildew symptoms. It was thus suggested that slightly elevated N concentrations above the ordinary rates enhanced the host's ability to escape disease but excessive N rates promoted disease development.

The suppressing effect of K application (as KNO_3) has been generally recognized especially with respect to fungal diseases. As in the case of N, the disease reduction by K had an optimal limit and beyond this downy mildew was significantly increased. It was concluded therefore that excessive doses of any of these two essential nutrients in the fertilization solution were not capable to limit the disease severity. The increase of lesion area on cucumber leaves in the higher K concentrations may be due to the type of fertilizer used as this type has been found to increase severity of another disease (*Diplodia* stalk rot of maize, Huber, 1980). It is difficult to explain such experimental results because many factors contribute in plant growth and disease development that cannot be controlled and are not stable such as the microorganisms' activities in soil, soil pH, nutrients status in plant and soil, microclimate etc.

Taking into account nutrients as such factors analysis of covariance revealed that some of them consisted covariates and influenced both downy mildew development and the area of cucumber leaves. In the study of the effect of N levels

on disease severity (Chapter 5) nutrients that played this important role were: leaf Mn content affected infection area and NH_4-N, P, Fe, Mg of leaves affected the leaf area of cucumbers. However, in the case of K study (Chapter 6) not only nutrients in leaves but also in soil had this indirect effect on leaf and lesion area of cucumbers (soil K, leaf Zn and K affected cucumber leaf area and Cu, Ca and Mg leaf content affected lesion area).

The area of cucumber leaves in the first experiment (Chapter 4) was not influenced by inoculum load while apparently the old leaf (seventh from the plant apex) had statistically significant larger area than the young leaf (third from the plant apex). The N treatments (Chapter 5) had the most marked effect on leaf area. Thus, the leaf size statistically increased as the N concentrations was increasing in soil, although the intermediate N levels resulted in non statistically significant differences for leaf area. It was clear that the higher N fertilization treatment resulted in the higher N (NO_3-N) content in soil which was probably accumulated in leaves since the NO_3-N content in tissues was also the highest and as a consequence the corresponding leaf had the largest size. This was an expected influence of fertilizers since the direct effect of mainly N on leaf surface of plants is well known (Reuveni and Reuveni 1998). However, K (Chapter 6) did not have the same effect on leaf size. In general, the leaf area was not statistically significantly affected by K increasing levels in the fertilization solution. Nevertheless, the intermediate K concentration (400 ppm) seemed to result in the higher value of leaf area in all the samplings.

The results of the NxK factorial experiment (Chapter 7) indicated that N concentration of 300 ppm increased leaf area of cucumber plants. This was expected since as in the case of N fertilization treatments (Chapter 5) the highest N level (in that study 600 ppm N) resulted in the largest leaf size. The fact that the 300 ppm N also increased infection (Chapter 7) could have been contradictory with the corresponding result of the negative effect of 300 ppm N on the downy mildew of cucumber of the previous study (Chapter 5), if the NxK interaction has not been considered.

It was shown in Chapter 6 that leaf area of cucumbers had no statistically significant differences due to K fertilization treatments. However, as concluded in Chapter 7 the main effect of 200 ppm K statistically significantly increased the leaf area of cucumbers. This result apparently obtained because the effect of K on leaf surface was determined by the concentration of N. The fertilization solutions contained separately 300 and 400 ppm K resulted in the lowest infection area caused by *P. cubensis* on cucumber leaves (Chapter 6). The fact that in the factorial experiment (Chapter 7) the main effects of these K levels on lesion area led to the opposite result seemed to find explanation because of the N and K interaction.

Therefore, the downy mildew development was depended on the statistically significantly interaction of these essential nutrients. Excess K resulted in more severe downy mildew under high N conditions. The infection was inhibited by increased K fertilization levels only when N was applied at low rate, reaching a minimum with the combination of 200 ppm N and 400 ppm K. The above fertilization regime gave satisfactory disease suppression in conditions with high disease spread even when compared with the chemical treatment. The protective fungicide application resulted in better control than the curative. More infected cucumber leaves observed in limited fertilized plants when the disease was low developed while the opposite occurred at high disease levels.

In the case of N applications (Chapter 5) NH_4-N and NO_3-N in leaves and soil produced statistical significant differences due to treatments while leaf P had the statistically significant highest internal concentration in the 300 ppm N where the infection area was the lowest. Application of increased K concentrations in the fertilization solution appeared to affect positively the soil K (Chapter 6). The use of 400 ppm K not only statistically significant lowered the downy mildew incidence and resulted in the largest leaf surface (although statistically significant differences were not observed here) but also significantly decreased the NH_4-N in soil and Mn in leaves. The N and K interaction influenced the nutrients accumulation in leaves and soil. In regard to the fungicide effect on nutrients, leaf Mg index and NO_3-N content in soil were the highest and lowest respectively compared with that of the fertilized cucumber plants.

The downy mildew is a devasting disease for cucumber plants and this makes the use of chemical control necessary. However, it should be noted that the over use of fungicides leads to resistant strains of the pathogen and also constitutes a threat for the environment and the human health. Inorganic fertilization via NH_4NO_3 and KNO_3 found to play a significant role in downy mildew control enabling reduced number of fungicide treatments against this disease. Nevertheless, the pathogen development cannot be controlled by the use of fertilizers alone. Even so, it is important to be used as part of an integrated program in order to reduce disease pressure on cucumber plants grown under greenhouse conditions.

8.2 RECOMMENDATIONS FOR FUTURE EXPERIMENTAL WORK

In the future the application of N and K nutrients through different fertilizer types than NH_4NO_3 and KNO_3 could be studied. A possible alternatives could be $(NH_4)_2SO_4$ and KNO_3. These could give important information about the effect of the ammonium or the nitrate form separately since the N form was found to influence the reaction of a range of other fungal diseases (Huber and Thompson, 2007). The use of both KNO_3 and KCl fertilizer could give knowledge about the effect of K on cucumber downy mildew in relation with the potential of the suppressing influence of Cl. Other essential nutrients like P, Ca, Mg or micronutrients should be investigated in terms of their effect to downy mildew of cucumber.

Examination of fungicides belonging to different chemical groups than alkyl phosphonates (e.g. Carbamates) should be thoroughly examined regarding their influence on this vegetable disease. The evaluation of the protective activity of fungicides (various levels, time of application) can play an important role in their performance under greenhouse conditions.

Interaction between inorganic fertilization and fungicide applications is a possible approach for research. For example experimental treatments could consist of combinations of different fungicide doses or application methods (soil drench or

spaying on leaves) with various nutrients levels. The application of fertilizers sprayed alone and or mixed with fungicides at low rates on cucumber leaves in a protective basis, would give interesting results regarding the inhibition of *P. cubensis* development, which is the main target of the producers. This might help to limit the excessive use of fungicides.

8.3 IMPLICATIONS FOR GROWERS

The non significant differences in infection of both young and old leaves of cucumbers confirms the notion that downy mildew is a devasting disease for these plants. The essential nutrients N and K studied in this project were found to influence the fungus development (*P. cubensis*). Slightly elevated N concentration (300 ppm) given to plants seemed to restrict the downy mildew. A range of 300 to 400 ppm of K suppressed the disease when applied with standard N level. However, the best downy mildew control was observed in plants fertilized with 200 ppm N and 400 ppm K. Therefore, the fertilizers NH_4NO_3 and KNO_3 can be used from growers in Crete in order to achieve the above N and K concentrations in the fertilization solution for greenhouse cucumbers. This could have an economic importance for growers since overfertilization and overuse of fungicides is avoided.

It is also suggested the preventive use of the systemic fungicide fosetyl-Al which gave better control of downy mildew development than the curative application. The finding that at low disease pressure the limited N and K fertilization increased the lesions surface while at high disease levels excess fertilization led to more infection has implications for growers. Thus, at the initial stages of the downy mildew development the plants should be efficiently fertilized with N and K and continue with balanced nutrition while the excess fertilization with these nutrients should be avoided especially when the disease has spread. Some downy mildew control benefit by fertilization should, therefore, be possible by growers if used as part of a disease prevention program.

8.4 CONCLUSIONS

A brief summary of the project conclusions is described below.

● Leaf age of cucumber plants did not play any particular role in downy mildew infection regardless of the inoculum load. The infection increased with increasing zoospores concentration although in conditions of high disease pressure the spore content did not seem to affect the lesions area.

● Slightly elevated N concentration in the fertilization solution (300 ppm N as NH_4NO_3) applied to cucumbers suppressed downy mildew and produced efficient leaf development.

● Symptoms expansion caused by *P. cubensis* decreased when cucumbers were grown around 350 ppm K (via KNO_3). Cucumber plants fertilized with 400 ppm K appeared to have increased leaf area.

● Soil and leaf nutrients had an indirect effect on leaf and lesion area development. Not only they differentiated due to fertilization treatments but also consisted factors which influenced the leaf and the infection surface.

● K fertilization affected the disease depending on the N rate. Downy mildew has inhibited with increasing K concentrations only at low N rate, reaching the least infection in cucumbers fertilized with 200 ppm N and 400 ppm K. The above fertilization treatment was comparable with the efficacy of the chemical application.

CHAPTER NINE

LITERATURE CITED

Ahmad, S. and Khan, H. (2001). Influence of host management on downy mildew control in onion. *Pakistan Journal of Biological Sciences*, 4(9), p. 1126-1128.

Alvarez, L. A., Vicent, A., Soler, J. M., De la Roca, E., Bascon, J. and Garcia-Jimenez, J. (2008). Comparison of application methods of systemic fungicides to suppress branch cankers in clementine trees caused by *Phytophthora citrophthora*. *Plant Disease*, 92(9), p. 1357-1363.

Amtmann, A., Troufflard, S. and Armengaud, P. (2008). The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum*, 133, p. 682-691.

Ash, G., J. and Brown, J. F. (1991). Effect of nitrogen nutrition of the host on the epidemiology of *Puccinia striiformis* f.sp. tritici and crop yield in wheat. *Australasian Plant Pathology,* 20(3), p. 108-114.

Baider, A. and Cohen, Y. (2003). Synergistic interaction between BABA and Mancozed in controlling *Phytophthora infestans* in potato and tomato and *Pseudoperonospora cubensis* in cucumber. *Phytoparasitica*, 31(4), p. 399-409.

Bains, S. S. and Jhooty, J. S. (1978). Relationship between mineral nutrition of muskmelon and development of downy mildew caused by *Pseudoperonospora cubensis*. *Plant and Soil*, 49, p. 85-90.

Bains, S. S. and Jhooty, J. S. (1984). The relation between cation-ratio and host-resistance to certain downy mildew and root-knot diseases. *Plant and Soil*, 81, p. 69-74.

Becot, S., Pajot, E., Le Corre, D., Monot, C. and Silue, D. (2000). Phytogard (K_2HPO_3) induces localized resistance in cauliflower to downy mildew of crucifers. *Crop Protection*, 19, p. 417-425.

Barker, A. V. (1974). Nitrate determinations in soil, water and plants. Massachusetts. Massachusetts Agricultural Experiment Station.

Berry, S. Z., Madumadu, G. G. and Uddin, M. R. (1988). Effect of calcium and nitrogen nutrition on bacterial canker disease of tomato. *Plant and Soil*, 120(1), p. 113-120.

Biles, C. L., Bruton, B. D., Wall, M. M. and Rivas, M. (1995). Phytophthora capsici zoospore infection of pepper fruit in various physical environments. *Proceedings of the Oklahoma Academy of Science*, 75, p. 1-5.

Blancard, D., Lecoq, H., Pitrat, M. (1994). A colour Atlas of Cucurbit Diseases. Observation, Identification and Control. Manson Publishing, London.

Brennan, R. F. (1992). The role of manganese and nitrogen nutrition in the susceptibility of wheat plants to take-all in Western Australia. *Fertilizer research*, 31, p. 35-41.

Burge, M. N. and Simmons, J. R. (1982). The influence of potassium and phosphorus in predisposition of tomato to verticillium wilt. *Chemistry in Ecology*, 1, p. 83-92.

Cappelli, C., Buonaurio, R., Stravato, V. M. (2003). Occurrence of *Pseudoperonospora cubensis* Pathotype 5 on Squash in Italy. *Plant Disease*. Abtract 87, p. 449.

Chaluvaraju, G., Basavaraju, P., Shetty, N. P., Deepak, S. A., Amruthesh, K. N., Shetty, H. S. (2004). Effect of some phosphorous-based compounds on control of pearl millet downy mildew disease. *Crop Protection*, 23(7), p. 595-600.

Chapman, D. H. and Pratt, F. P. (1961). *Methods of analysis for soils, plants and waters*. University of California.

Cohen, Y. and Coffey, M. D. (1986). Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology*, 24, p. 311-338.

Cohen, Y., Meron, I., Mor, N. and Zuriel, S. (2003). A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica*, 31(5), p. 458-466.

David, M., Swiader, J., Williams, K. and Eastburn, D. (2003). Nitrogen nutrition, but not potassium, affects powdery mildew development in *Hiemalis begonia*. *Plant Nutrition*, 26(1), p. 159-176.

Dewis, J. and Freitas, F. (1970). *Physical and chemical methods of soil and water analysis*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development*, 28(1), p. 33-46.

Doullah, M.A.U, Meah, M. B. and Okazaki, K. (2006). Development of an effective screening method for partial resistance to *Alternaria brassicicola* (dark leaf spot) in *Brassica rapa*. *European Journal of Plant Pathology*. 116, p. 33-43.

Dutky, E. M. (2008). Fungicides and other plant disease management approaches. Department of Entomology, University of Maryland. [online]. Available at:http://www.colonialdistrictroses.org/sitebuildercontent/sitebuilderfiles/fungicidesandotherdisease.pdf (Accessed: 3 January 2009).

Elmer, W. H. (2007). Chlorine and plant disease. In: *Mineral nutrition and plant disease*, edited by L. E. Datnoff, W. H. Elmer, D. M. Huber, American Phytopathological Society, St. Paul, Minnesota, USA, p. 189-202.

FAOSTAT. (2008). Food and Agriculture Organization of the United Nations. [online] Available at: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor (Accessed: 28 January 2009).

Fasulo, R. T. and Denmark, H. A. (2000). Twospotted spider mite, *Tetranychus urticae* Koch (Arachnida: Acari: Tetranychidae). Institute of Food and Agricultural Sciences. University of Florida. [online] Available at: http://edis.ifas.ufl.edu/pdffiles/IN/IN30700.pdf (Accessed: 25 April 2008).

Filippi, M. C. and Prabhu, A. S. (1998). Relationship between panicle blast severity and mineral nutrient content of plant tissue in upland rice. *Journal of Plant Nutrition*, 21(8), p. 1577-1587.

Gottstein, H. D. and Kuc, J. A. (1989). Induction of systemic resistance to Anthracnose in cucumber by phosphates. *Phytopathology,* 79 (2), p. 176-179.

Greer, L. (2000). Sustainable Whitefly Control. National Sustainable Agriculture Information Center (ATTRA), USA. [online] Available at: http://attra.ncat.org/attra-pub/PDF/ghwhitefly.pdf (Accessed: 26 April 2008).

Grewal, H. S. and Williams, R. (2002). Influence of potassium fertilization on leaf tostem ratio, nodulation, herbage yield, leaf drop and common leaf spot disease of alfalfa. *Journal of Plant Nutrition*, 25(4), p. 781-795.

Gupta, S. K. and Shyam, K. R. (1996). Antisporulant activity of some fungicides against Pseudoperonospora cubensis on cucumber. *Indian Journal of Mycology and Plant Pathology*, 26(3), p. 293-295.

Hatcher, P. E., Paul, N. D., Ayres, P. G. and Whittaker, J. B. (1997). Nitrogen fertilization affects interactions between the components of an insect-fungus-plant tripartite system. *Functional Ecology*, 11, p. 537-544.

Hesse, P. R. (1972). *A textbook of soil chemical analysis*. United States of America. First edition. Chemical publishing Co., Inc.

Hoffland, E., Jeger, M. J. and Beusichem, M. L. (2000). Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. *Plant and Soil*, 218(1-2), p. 239-247.

Horst, R. K. (1979). *Westcott's plant disease handbook*. Fourth edition. Van Nostrand Reinhold Company, New York.

Huber, D. M. (1980). The role of mineral nutrition in defence. In: *Plant disease-An Advanced Treatise*, edited by Academic press Inc., New York, p. 380-406.

Huber, D. M. and McCay-Buis, T. S. (1993). A multiple component analysis of the take-all disease of cereals. *Plant Disease,* 77, p. 437-447.

Huber, D. M. and Thompson, I. A. (2007). Nitrogen and plant disease. In: *Mineral nutrition and plant disease*, edited by L. E. Datnoff, W. H. Elmer, D. M. Huber, American Phytopathological Society, St. Paul, Minnesota, USA, p. 31- 44.

Huber, D. M. and Watson, R. D. (1974). Nitrogen Form and Plant Disease. *Annual Review of Phytopathology*, 12, p. 139-165.

Jarvis, W.R. (1992). *Cucumber diseases*. Ottawa, Canada.

Jones, J. B., Wolf, B. and Mills, A. H. (1991). Chapter 4: Preparation and Analysis. In: *Plant Analysis Handbook*. Micro-Macro Publishing, Inc., Georgia, USA, p. 23-26.

Keinath, A. P., Holmes, G. J., Everts, K. L., Egel, D. S. and Langston, D. B. Jr. (2007). Evaluation of combinations of chlorothalonil with azoxystrobin, harpin, and disease forecasting for control of downy mildew and gummy stem blight on melon. *Crop Protection*, 26, p. 83-88.

Kolota, E. and Osinska, M. (2001). Efficiency of foliar nutrition of field vegetables grown at different nitrogen rates. *Acta Horticulturae*, 563, p. 87-91.

Lamb, M. E., Shaw, L. N. and Cantliffe, J. D. (2001). Beit Alpha Cucumber : A new greenhouse crop for Florida. Horticulture Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. [online] Available at: http://www.hos.ufl.edu/protectedag/EDIS/CV27700.pdf (Accessed: 30 April 2008).

Latijnhouwers, M., Wit, P. J. G. M. and Govers, F. (2003). Oomycetes and fungi: similar weaponry to attack plants. *Trends in microbiology*, 11(10), 462-469.

Lebeda, A. (1999). *Pseudoperonospora cubensis* on *Cucumis* spp. and *Cucurbita* spp. – resistance breeding aspects. *Acta Horticulturae*, 492, p. 363-370.

Lebeda, A. and Prasil, J. (1994). Susceptibility of *Cucumis sativus* cultivars to *Pseudoperonospora cubensis*. *Acta Phytopathologica et Entomologica Hungarica*, 29(1-2), p. 89-94.

Lebeda, A. and Widrlechner, M. P. (2003a). Differentiation of *Pseudoperonospora cubensis* (Cucurbit Downy Mildew) Pathogenicity. In: *Abstracts of the 8th International Congerss of Plant Pathology*, Christchurch, New Zealand, 2-7 February. Abstract 634, Host/pathogen Interaction Session. (unpaged)

Lebeda, A. and Widrlechner, M. P. (2003b). A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *Journal of Plant Diseases and Protection*, 110(4), p. 337–349.

Leitch, M. H. and Jenkins, P.D. (1995). Influence of nitrogen on the development of *Septoria* epidemics in winter wheat. *Journal of Agriculture Science*, 124, p. 361-368.

Lerner, B. R. and Dana, N. M. (2001). Growing Cucumbers, Melons, Squash, Pumpkins and Gourds. Purdue University Cooperative Extension Service. [online]. Available at:http://www.hort.purdue.edu/ext/HO-8.pdf

(Accessed: 3 May 2008).

Leser, C. and Treutter, D. (2005). Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. *Physiologia Plantarum*, 123(1), p. 49-56.

Liang, Y. C., Sun, W. C., Si, J. and Romheld, V. (2005). Effects of foliar and root applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant Pathology*, 54, p. 678-685.

Lindenthal, M., Steiner, U., Dehne, H. W. and Oerke, E. C. (2005). Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. *Phytopathology*, 95(3), p. 233-240.

Long, D. H., Lee, F. N. and TeBeest, D. O. (2000). Effect of nitrogen fertilization on disease progress of rice blast on susceptible and resistant cultivars. *Plant Disease*, 84(4), p. 403-409.

Malathrakis, N. E. and Goumas, D. E. (1999). Fungal and bacterial diseases. In: *Integrated Pest and Disease Management in Greenhouse crops*, edited by R. Albajes, M. L. Gullino, J. C. van Lenteren, and Y. Elad, Kluwer Academic Publishers, Netherlands, p. 34-47.

Marr, W. C. (1995). Greenhouse cucumbers. Kansas State University, Agricultural Experiment Station and Cooperative Extension Service. [online]. Available at: http://www.oznet.ksu.edu/library/hort2/mf2075.pdf (Accessed: 4 May 2008).

Mau, F. L., Ronald and Jayma L. Martin Kessing, (1993). Crop Knowledge Master *Frankliniella occidentalis*. Extension Entomology and UH-CTAHR Integrated Pest Management Program. University of Hawaii. [online]. Available at: http://www.extento.hawaii.edu/kbase/Crop/Type/f_occide.htm (Accessed: 10 May 2008).

McDonald, B. A. and Linde, C. (2002). Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology*, 40, p. 349-379.

Mills, H. A. (2001). Cucumber. *Vegetable Crops*. Georgia, USA.

Nam, M. N., Jeong, S. K., Lee, Y. S., Choi, J. M. and Kim, H. G. (2006). Effects of nitrogen, phosphorus, potassium and calcium nutrition on strawberry anthracnose. *Plant pathology*, 55, p. 246-249.

Neykov, S. and Dobrev, D. (1988). Introduced cucumber cultivars relatively resistant to *Pseudoperonospora cubensis* in Bulgaria. *Acta horticulturae*, 220, p. 115-120.

Panagopoulos, G. X. (1995). *Ασθένειες κηπευτικών καλλιεργειών* (Vegetables crops diseases), Σταμούλη Α.Ε Αθήνα.

Panicker, S. and Gangadharan, K. (1999). Controlling downy mildew of maize caused by *Peronosclerospora sorghi* by foliar sprays of phosphonic acid compounds. *Crop Protection*, 18(2), p. 115-118.

Panstruga, R. 2003. Establishing compatibility between plants and obligate biotrophic pathogens. *Current Opinion in Plant Biology*. 6(4), p. 320-326.

Peirce, L. C. (1987). Vegetables Characteristics, Production and Marketing. J. Wiley and Sons.

Portz, D., Koch, E. and Slusarenko, A. (2008). Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *European Journal of Plant Pathology*, 122, p. 197-206.

Prabhu, A. S., Barbosa Filho, M. P., Filippi, M. C. and Zimmermann, F. J. P. (1999). Relationship between potassium fertilization and panicle blast severity in upland rice. *Pesquisa Agropecuaria Brasileira*, 34 (9), p. 1729-1732.

Prabhu, A. S., Fageria, N. K., Huber, D. M. and Rodrigues, F. A. (2007). Potassium and plant disease. In: *Mineral nutrition and plant disease*, edited by L. E. Datnoff, W. H. Elmer, D. M. Huber, American Phytopathological Society, St. Paul, Minnesota, USA, p. 57-78.

Reuveni, M., Agapov, V. and Reuveni, R. (1995). Induced systemic protection to powdery mildew in cucumber by phosphate and potassium fertilizers: effects of inoculum concentration and post-inoculation treatment. *Canadian Journal of Plant Pathology*, 17, p. 247-251.

Reuveni, M., Agapov, V. and Reuveni, R. (1996). Controlling powdery mildew caused by Sphaerotheca fuliginea in cucumber by foliar sprays of phosphate and potassium salts. *Crop Protection*, 15 (1), p. 49-53.

Reuveni, R. and Raviv, M. (1997). Control of downy mildew in greenhouse-grown cucumbers using blue photoselective polyethylene sheets. *Plant Disease*, 81(9), p. 999-1004.

Reuveni, R. and Reuveni, M. (1998). Foliar fertilizer therapy-a concept in integrated pest management. *Crop Protection*, 17(2), p. 111-118.

Reuveni, R. Dor, G. and Reuveni, M. (1998). Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. *Crop Protection*, 17,(9), p. 703-709.

Reuveni, R., Reuveni, M. and Agapov, V. (1996). Foliar sprays of NPK fertilizers induce systemic protection against Puccinia sorghi and Exserohilum turcicum and growth response in maize. *European Journal of Plant Pathology*, 102, p. 339-348.

Robak, J. (1995). Epidemiology and control of cucumber downy mildew *Pseudoperonospora cubensis*. Biuletyn, Warzywniczy. 43, p. 5-18.

Rodgers-Gray, B. S. and Shaw, M. W. (2000). Substantial reductions in winter wheat diseases caused by addition of straw but not manure to soil. *Plant pathology*, 49(5), p. 590-599.

Rotenberg, D., Cooperband, L. and Stone, A. (2005). Dynamic relationships between soil properties and foliar disease as affected by annual additions of organic amendment to a sandy soil vegetable production system. *Soil Biology and Biochemistry*, 37, p. 1343-1357.

Rouzet, J. and Jacquin, D. (2003). Development of overwintering oospores of *Plasmopara viticola* and severity of primary foci in relation to climate. *EPPO/OEPP Bulletin*, 33(3), p. 437-442.

Sanogo, S. and Yang, X. B. (2001). Relation of sand content, pH, and potassium and phosphorus nutrition to the development of sudden death syndrome in soybean. *Canadian Journal of Plant Pathology*, 23, p. 174-180.

Sherf, F. A. and Macnab, A. A. (1986). *Vegetables diseases and their control*. Second edition. New York, USA: John Wiley and Sons.

Shetty, N. V., Wehner, T. C., Thomas, C. E., Doruchowski, R. W. and Shetty, K. P. (2002). Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Scientia Horticulturae*, 94(3-4), p. 231-239.

Shi, Y., Li, B., Liu, X. (2005). Several infection factors of *Pseudoperonospora cubensis*. *Chinese Journal of Applied Ecology*, 16(2), p. 257-261.

Simoglou, K. B. (2004). *Οι επιδράσεις των θρεπτικών στοιχείων στην ανάπτυξη ασθενειών στα φυτά* (Effects of nutrients on the development of plant diseases). *Γεωργία-Κτηνοτροφία*, 4, p. 48-55.

Sivaprakasam, K., Pillayarsamy, K. and Rajagopalan, S. C. K. (1974). Influence of nitrogen on the incidence of downy mildew disease of pearl millet (*Pennisetum typhoides* Stapf and Hubb.). *Plant and Soil*, 41, p. 677-679.

Smith, J. P. (1977). *Vascular Plant Families*. Mad River Press, California.

Snowdon, A. L. (1990). A colour atlas of post harvest diseases and disorders of fruits and vegetables. *General Introduction and Fruits,* Vol. 1, Wolfe Scientific Ltd., London.

St. Amand, P.C. and Wehner, T. C. (1995). Greenhouse, detached-leaf and field testing methods to determine cucumber resistance to gummy stem blight. *Journal of the American Society for Horticultural Science*, 120 (4), p. 673-680.

Strengbom, J. and Reich, P. B. (2006). Elevated [CO_2] and increased N supply reduce leaf disease and related photosynthetic impacts on *Solidago rigida*. *Oecologia*, 149, p. 519-525.

Sweeney, D. W., Granade, G. V., Eversmeyer, M. G. and Whitney, D. A. (2000). Phosphorus, potassium, chloride and fungicide effects on wheat yield and leaf rust severity. *Journal of Plant Nutrition*, 23 (9), p. 1267-1281.

Tahvonen, R. (1985). Downy mildew of cucurbits found for the first time in Finland. Agricultural Research Centre, Institute of Plant Pathology Finland. [online]. Available at: http://chaos.bibul.slu.se/sll/slu/vaxtskyddsnotiser/VSN85-3/VSN85-3B.HTM. (Accessed: 30 December 2008).

Tanaka, S., Ito, T., Ochi, Y., Someya, Y. and Hirabayashi, T. (2000). Effect of nitrogen concentrations of nutrient solution on the occurrence and development of downy mildew in susceptible and resistant cucumber cultivars. *Journal of the Japanese society for horticultural science*, 69(3), p. 339-345.

Thakur, R. P. and Mathur, K. (2002). Downy mildews of India. *Crop Protection*, 21(4), p. 333-345.

Tian, S., Zhu, Z., Liu, B., Tian, S., Zhu, Z. and Liu, B. (1997). Effects of nitrogen and phosphorus on the occurrence of dark kernel of wheat. *Journal of Hebei Agricultural University*, 20(2), p. 33-35.

Tsikalas, P. (2003). *Αντικείμενα εργαστηρίου Θρέψης φυτών – Γονιμότητας εδαφών*. (Plant nutrition and soil fertility, laboratory lessons). Heraklion, Greece.

Tzamos, K. E. (2004). *Φυτοπαθολογία* (Fytopathology). Εκδόσεις Σταμούλη, Αθήνα.

Urban, J. and Lebeda, A. (2004). Resistance to fungicides in population of cucurbit downy mildew in the Czech republic. *Acta Fytotechnica et Zootechnica*, 7, p. 327-329.

Urban, J. and Lebeda, A. (2006). Fungicide resistance in cucurbit downy mildew – methodological, biological and population aspects. *Annals of Applied Biology*, 149, p. 63-75.

Urban, J. and Lebeda, A. (2007). Variation of fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. *Phytopathology*, 155, p. 143-151.

Van Der Meer, Q. P., Van Bennekom J. L. and Van Der Giessen, A. C. (1978). Gummy stem blight resistance of cucumbers (*Cucumis sativus* L .). *Euphytica*, 27, p. 861-864.

Vitug, A. (1997). Effect of nitrogen fertilization on sugarcane downy mildew infection. ARD Abstracts Researches, Sugar Regulatory Administration. [online]. Available at:
http://www.sra.gov.ph/abstract/absrde22%20(Sugarcane%20Disease%20-%20Control%20measures).html
(Accessed: 19 September 2008).

Walters, D. R. and Bingham, I. J. (2007). Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. *Annals of applied biology*, 151, p. 307-324.

Wang, J., Li, M., Zhang, P., Qiao, Z., Wang, J., Li, M., Zhang, P., Qiao, Z. (1998). Correlation between potassium content in apple bark in the spring and the occurrence of Valsa canker. *Acta Phytophylacica- Sinica*, 25(1), p. 61-64.

Wehner, T. C. (1996). Cucumber Breeding. North Carolina State University. [online]. Available at:

http://cuke.hort.ncsu.edu/cucurbit/cuke/cukehndbk/cukeoverview.html (Accessed: 10 May 2008).

Williams, H. P. and Palmer, M. J. (1982). *NC State and USDA Cucumber Disease Handbook*.

Wittwer, S. H. and Honma, S. (1979). Greenhouse Cucumbers. In: *Greenhouse Tomatoes, Lettuce, and Cucumbers*. East Lansing, Michigan State University Press.

Wyszogrodzka, A. J., Williams, P. H. and Peterson, C. E. (1986). Search for resistance to gummy stem blight (*Didymella bryoniae*) in cucumber (*Cucumis sativus* L.). *Euphytica*, 35, p. 603-613.

Wyszogrodzka, A. J., Williams, P. H. and Peterson, C. E. (1987). Multiple pathogen inoculation of cucumber (*Cucumis sativus*) seedlings. *Plant Disease*, 71(3), p. 275-280.

Yorinori, M. A., Klingelfuss, L. H., Paccola-Meirelles, L. D. and Yorinori, J. T. (2004). Effect of time of spraying of fungicide and foliar nutrient on soybean powdery mildew. Phytopathology, 152, p.129-132.

Zarboutis, B. G. and Gakni, I. A. (1992). *Γεωργικές καλλιέργειες* (Greenhouse crops). Εκδόσεις Ίων.

Zitter, T. A., Hopkins, D. L. and Thomas, C. E. (Eds.) (1996). *Compendium of cucurbit diseases*. The American Phytopathological Society. APS Press. St. Paul, Minnesota.

APPENDIX A

General Materials and Methods

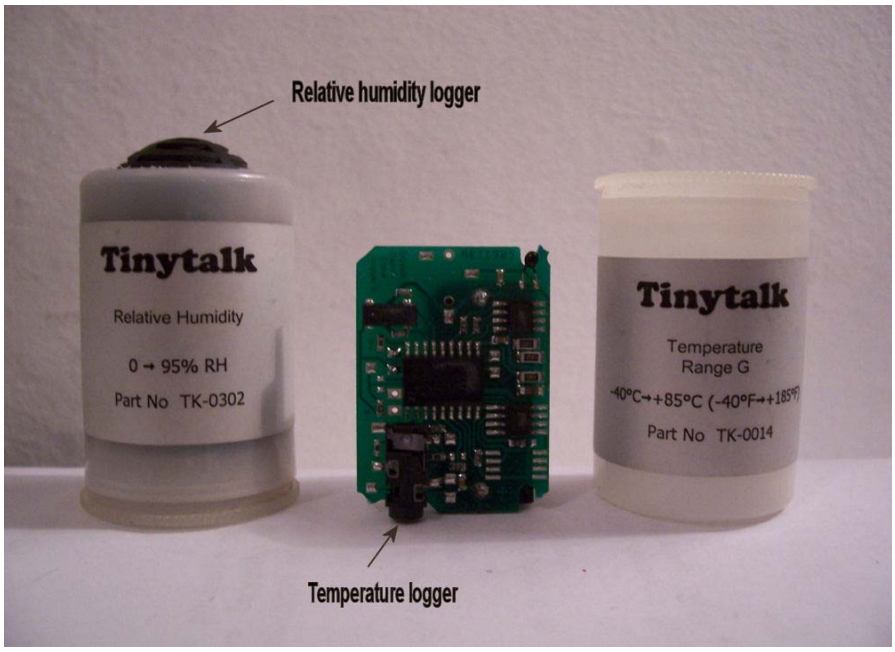

Plate A1. Tiny Talks monitored RH and temperature during each experiment.

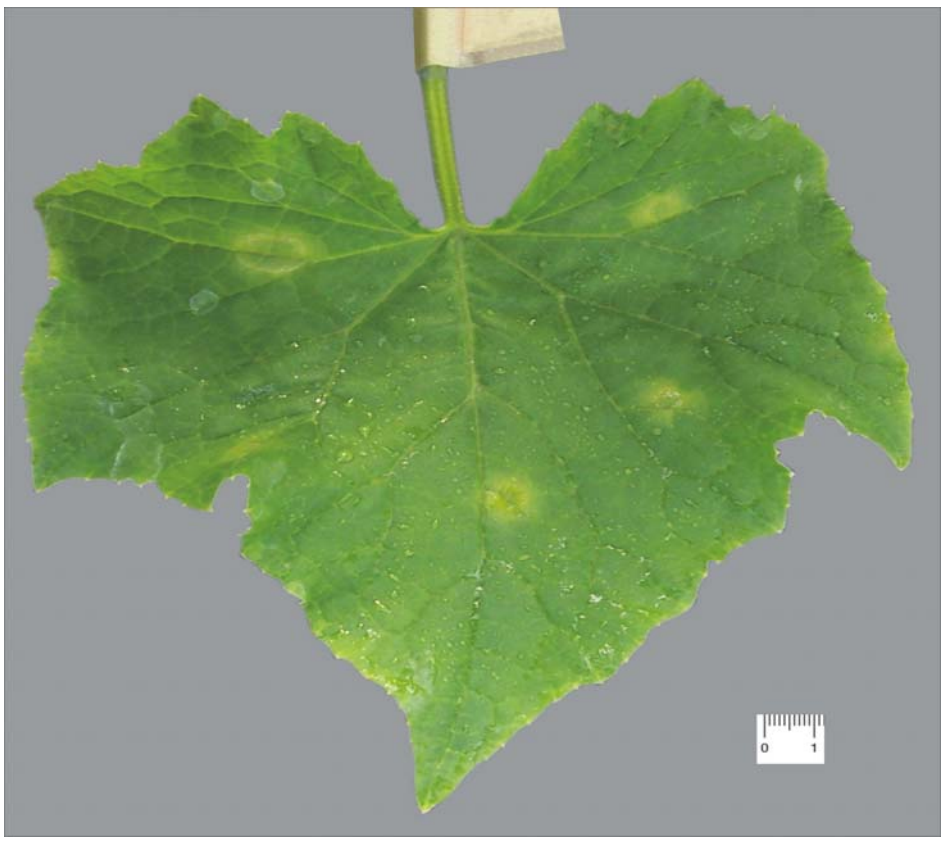

Plate A2. Symptoms of *P. cubensis* on cucumber leaf-first sampling.

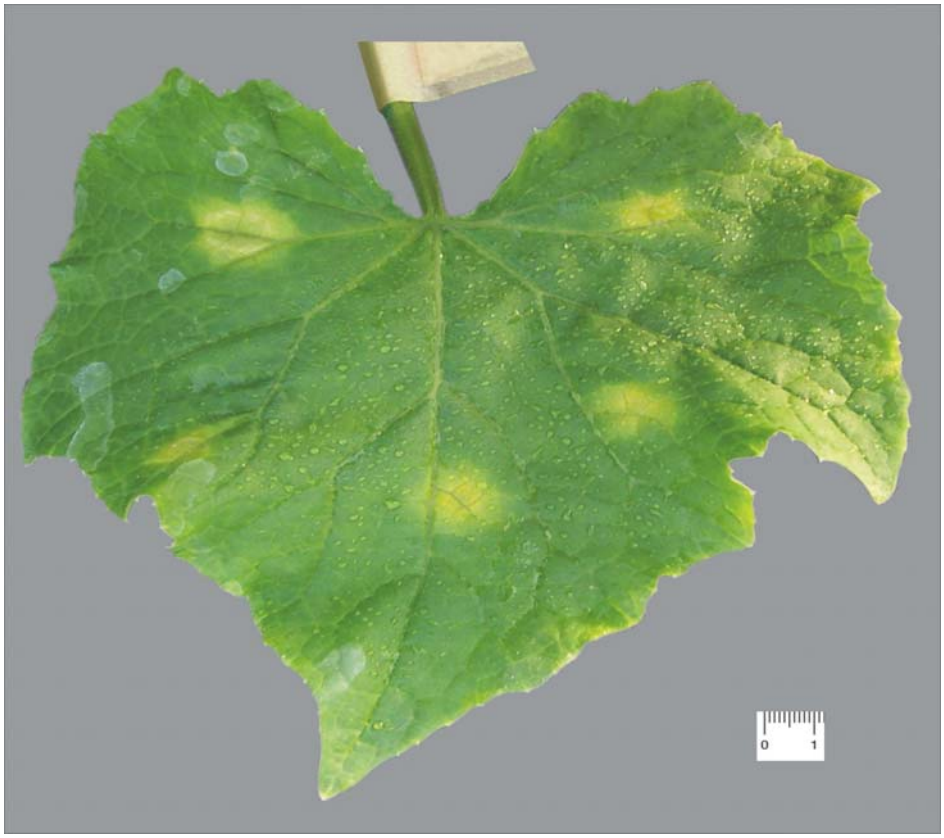

Plate A3. Symptoms of *P. cubensis* on cucumber leaf-second sampling.

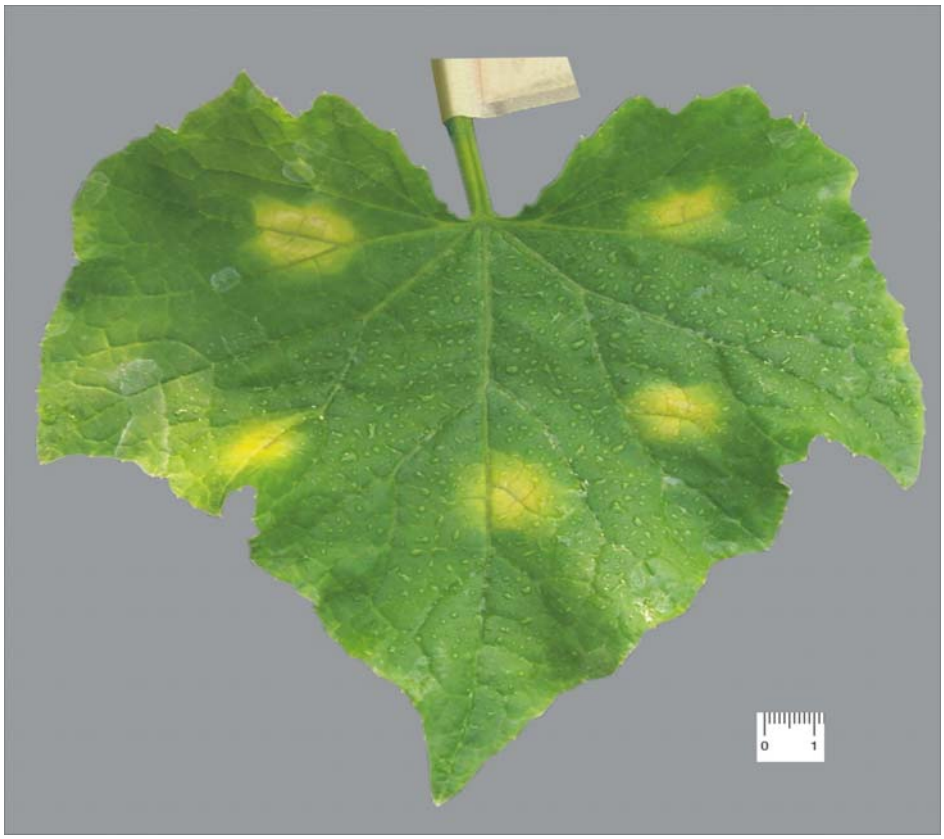

Plate A4. Symptoms of *P. cubensis* on cucumber leaf-third sampling.

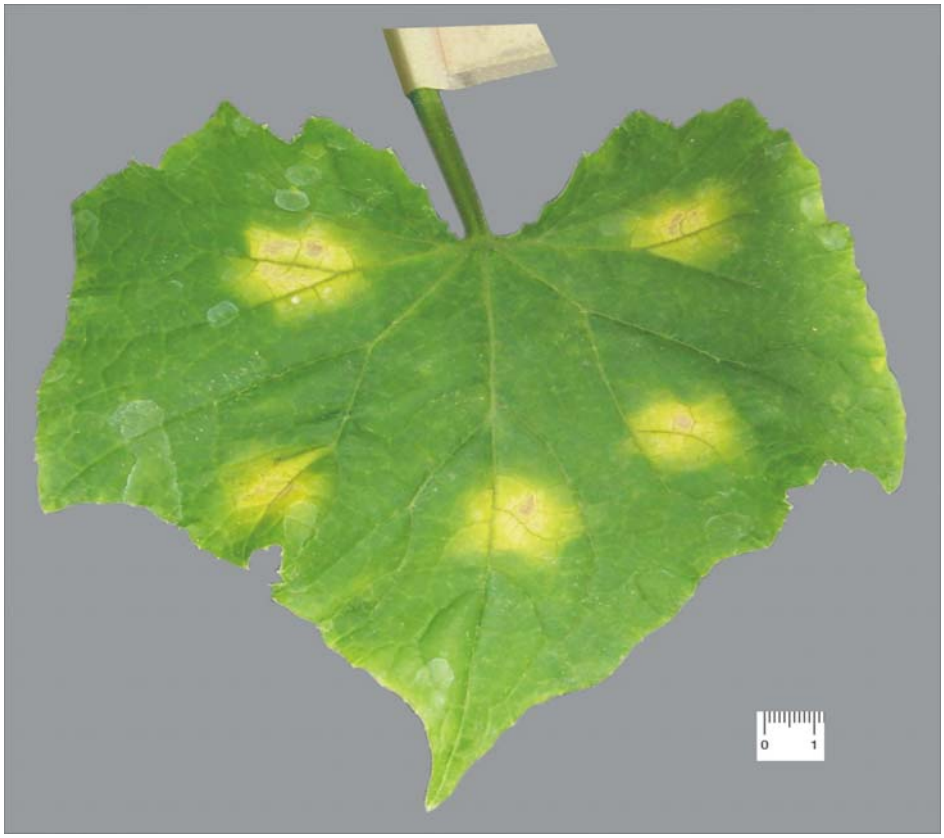

Plate A5. Symptoms of *P. cubensis* on cucumber leaf-fourth sampling.

Plate A6. Symptoms of *P. cubensis* on cucumber leaf-fifth sampling.

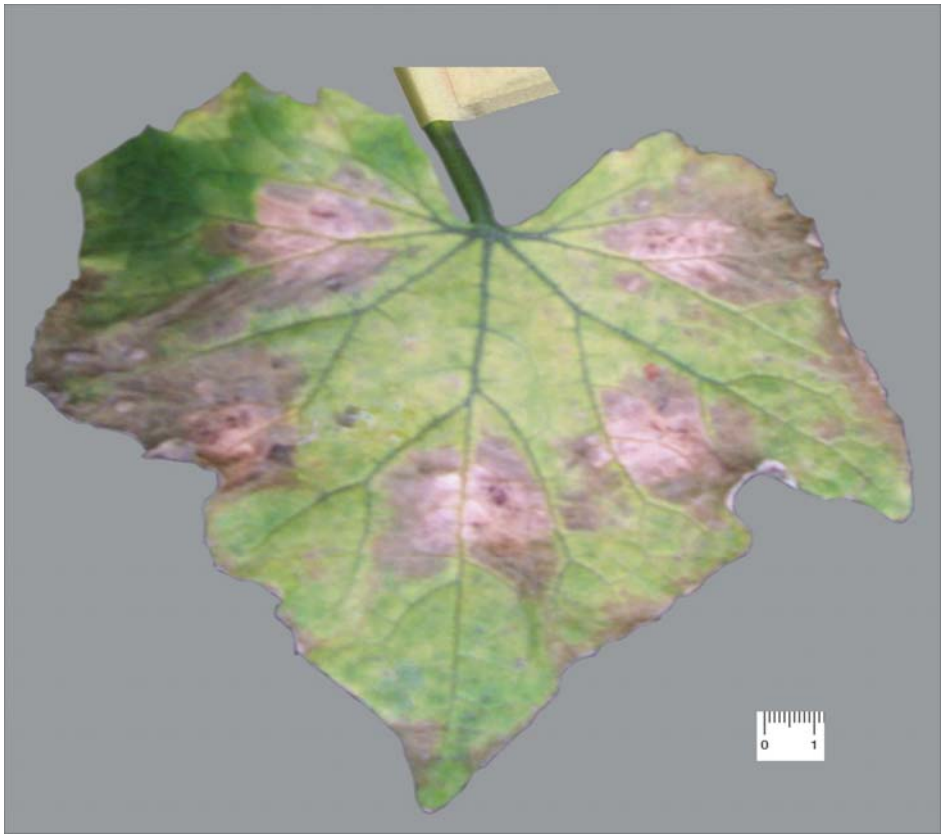

Plate A7. Symptoms of *P. cubensis* on cucumber leaf-sixth sampling.

Plate A8. Digestion of samples on a hot plate inside the fume cabinet

APPENDIX B

Fertilizer calculations about experiments

The Tables B1, B2 and B3 show the calculations made in order to determine the suitable quantities of the fertilizers used in each experiment. The columns N, P, K show the desirable concentrations reported in ppm in the fertilization solution. The next two columns concern the KNO_3 quantity per litre of water needed in order to create the desirable potassium concentration of each treatment and the corresponding N concentration given by this quantity of K fertilizer. The column 'remaining N ppm' results by subtracting acquired (from KNO_3) N concentration from the desirable N concentration. The column of NH_4NO_3 shows the quantities per litre needed for creating the 'remaining' N concentration. Volume of H_3PO_4 per litre with whom 50 ppm P achieved consist the next column. At last there are the final quantities/volumes of KNO_3, NH_4NO_3 and H_3PO_4 that were diluted in specific water volume in order to fertilize the cucumber plants, according to the treatments.

Table B.1. Calculations about the quantities of fertilizers needed for preparation of the fertilization solutions. (experiment Chapter Five: Effect of nitrogen fertilization on downy mildew development of cucumber.

Treatment	N ppm	P ppm	K ppm	KNO_3 g/L	N ppm (KNO_3)	remaining N ppm	NH_4NO_3 g/L	H_3PO_4 ml/L	KNO_3 fertilizer g/30L	NH_4NO_3 fertilizer g/30L	H_3PO_4 fertilizer ml/30L
1	100	50	200	0.52	70	30	0.09	0.11	15.66	2.68	3.24
2	200	50	200	0.52	70	130	0.39	0.11	15.66	11.64	3.24
3	300	50	200	0.52	70	230	0.69	0.11	15.66	20.60	3.24
4	400	50	200	0.52	70	330	0.99	0.11	15.66	29.56	3.24
5	500	50	200	0.52	70	430	1.28	0.11	15.66	38.5	3.24
6	600	50	200	0.52	70	530	1.58	0.11	15.66	47.46	3.24

Table B.2. Calculations about the quantities of fertilizers needed for preparation of the fertilization solutions. (experiment Chapter Six: Effect of potassium fertilization on downy mildew development of cucumber).

Treatment	N ppm	P ppm	K ppm	KNO_3 g/L	N ppm (KNO_3)	remaining N ppm	NH_4NO_3 g/L	H_3PO_4 ml/L	KNO_3 fertilizer g/30L	NH_4NO_3 fertilizer g/30L	H_3PO_4 fertilizer ml/30L
1	250	50	200	0.52	70	180	0.54	0.11	15.64	16.08	3.24
2	250	50	300	0.78	106	144	0.43	0.11	23.48	12.92	3.24
3	250	50	400	1.04	141	109	0.33	0.11	31.30	9.78	3.24
4	250	50	500	1.30	176	74	0.22	0.11	39.12	6.62	3.24
5	250	50	600	1.56	211	39	0.12	0.11	46.94	3.48	3.24
6	250	50	700	1.83	246	4	0.01	0.11	54.76	0.32	3.24

Table B.3. Calculations about the quantities of fertilizers needed for preparation of the fertilization solutions. (experiment Chapter Seven: Nitrogen, potassium and Fungicide effects on cucumber downy mildew).

Treatment	N ppm	P ppm	K ppm	KNO_3 g/L	N ppm (KNO_3)	remaining N ppm	NH_4NO_3 g/L	H_3PO_4 ml/L	KNO_3 fertilizer	NH_4NO_3 fertilizer	H_3PO_4 fertilizer
1	200	50	200	0.52	70	130	0.39	0.11	52.2 g/100L	38.8 g/100L	10.8 ml/100L
2	200	50	300	0.78	106	94	0.28	0.11	78g/100L	28g/100L	10.8 ml/100L
3	200	50	400	1.04	141	59	0.18	0.11	104g/100L	18g/100L	10.8 ml/100L
4	300	50	200	0.52	70	230	0.69	0.11	52.2 g/100L	68.6 g/100L	10.8 ml/100L
5	300	50	300	0.78	106	194	0.58	0.11	78g/100L	58g/100L	10.8 ml/100L
6	300	50	400	1.04	141	159	0.48	0.11	104g/100L	48g/100L	10.8 ml/100L
7	100	50	200	0.52	70	30	0.09	0.11	20.88g/40L	3.57g/40L	4.32ml/40L
8	100	50	200	0.52	70	30	0.09	0.11	20.88g/40L	3.57g/40L	4.32ml/40L

APPENDIX C

Relative Humidity and Temperature Records

Table C1. Relative Humidity and Temperature average of 24 hrs, three days before cucumber inoculation with *P.cubensis* until the last sampling during the first experiment (Chapter 4).

Day	Relative Humidity (%)	Temperature (°C)	Day	Relative Humidity (%)	Temperature (°C)
1	45.4	31.1	11	45.3	26.5
2	47.9	29.3	12	24.9	27.9
3	52.6	29.5	13	35.6	27.5
4	64.0	27.1	14	37.4	26.7
5	53.2	26.2	15	39.8	26.4
6	46.2	23.8	16	40.1	25.7
7	51.3	23.5	17	42.0	23.5
8	54.1	22.0	18	42.8	23.2
9	71.3	20.6	19	43.7	22.0
10	62.3	22.2	20	47.0	22.3

Table C2. Relative Humidity and Temperature average of 24 hrs, three days before cucumber inoculation with *P.cubensis* until the last sampling during the second experiment (Chapter 5).

Day	Relative Humidity (%)	Temperature (°C)
1	81.1	16.9
2	77.3	17.4
3	77.2	17.1
4	92.8	14.1
5	90.9	12.7
6	78.8	16.6
7	85.4	16.7
8	90.3	14.6
9	80.2	16.8
10	79.7	19.0
11	80.5	19.7
12	81.9	20.2
13	80.7	19.1
14	76.9	18.4
15	77.2	17.7
16	78.4	18.8
17	80.3	19.6
18	86.2	17.3
19	86.1	18.4
20	94.2	14.1
21	76.6	18.6

Table C3. Relative Humidity and Temperature average of 24 hrs, three days before cucumber inoculation with *P. cubensis* until the last sampling during the third experiment (Chapter 6).

Day	Relative Humidity (%)	Temperature (°C)
1	84.8	15.6
2	85.7	15.0
3	81.9	14.9
4	86.5	15.4
5	81.1	16.9
6	77.3	17.1
7	77.2	17.4
8	85.4	16.6
9	90.3	14.6
10	78.8	16.7
11	76.6	17.7
12	76.9	18.4
13	80.7	17.3
14	81.9	16.8
15	94.2	14.3
16	80.3	16.4
17	78.4	18.6
18	77.2	19.6
19	78.7	20.2
20	80.5	19.1
21	80.5	18.9

Table C4. Relative Humidity and Temperature average of 24 hrs, three days before cucumber inoculation with *P.cubensis* until the last sampling during the fourth experiment (Chapter 7).

Day	Relative Humidity (%)	Temperature (°C)	Day	Relative Humidity (%)	Temperature (°C)
1	29.1	16.3	14	28.6	14.8
2	29.3	15.9	15	24.4	14.5
3	20.4	16.2	16	24.5	12.6
4	17.7	14.3	17	22.6	12.6
5	17.2	13.4	18	25.8	12.0
6	14.1	13.4	19	25.5	11.7
7	20.7	14.5	20	25.8	12.4
8	17.0	15.3	21	22.3	13.8
9	21.6	15.2	22	22.8	14.1
10	28.3	16.4	23	28.1	14.8
11	11.2	14.3	24	32.1	14.9
12	31.6	14.5	25	21.2	15.2
13	29.2	14.2	26	26.5	16.1

APPENDIX D

Statistical Tables for Chapter Four

Tables D.1-D.16. ANOVA tables for leaf area, lesion area, relative leaf area and relative lesion area of cucumbers inoculated with three spore concentrations of *P. cubensis* on two aged leaves, in four samplings (Chapter 4, Figures 4.1, 4.2, 4.3).

Table D.1. Leaf area-first sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	1063.187	265.796	0.44	0.7765
Leaf age(A)	1	69584.592	69584.592	115.82	<.0001
Spore conc(B)	2	152.517	76.258	0.13	0.8815
AxB	2	5138.487	2569.243	4.28	0.0284
Error	20	12016.243	600.812		
Total	29	87955.027			

Table D.2. Lesion area-first sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	1.899	0.474	0.14	0.9639
Leaf age(A)	1	9.258	9.258	2.79	0.1104
Spore conc(B)	2	535.776	267.888	80.76	<.0001
AxB	2	14.163	7.081	2.13	0.1444
Error	20	66.343	3.317		
Total	29	627.442			

Table D.3. Relative leaf area-first sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	145.132	36.283	0.44	0.7765
Leaf age(A)	1	9498.779	9498.779	115.82	<.0001
Spore conc(B)	2	20.819	10.409	0.13	0.8815
AxB	2	701.439	350.719	4.28	0.0284
Error	20	1640.300	82.015		
Total	29	12006.471			

Table D.4. Relative lesion area-first sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	24.881	6.220	0.14	0.9639
Leaf age(A)	1	121.277	121.277	2.79	0.1104
Spore conc(B)	2	7018.142	3509.071	80.76	<.0001
AxB	2	185.531	92.765	2.13	0.1444
Error	20	869.031	43.451		
Total	29	8218.864			

Table D.5. Leaf area-second sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	1024.065	256.016	0.96	0.4504
Leaf age(A)	1	21460.225	21460.225	80.54	<.0001
Spore conc(B)	2	2505.320	1252.660	4.70	0.0212
AxB	2	384.426	192.213	0.72	0.4983
Error	20	5329.083	266.454		
Total	29	30703.120			

Table D.6. Lesion area-second sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	12.153	3.038	2.66	0.0628
Leaf age(A)	1	0.709	0.709	0.62	0.4399
Spore conc(B)	2	705.468	352.734	308.84	<.0001
AxB	2	11.559	5.779	5.06	0.0167
Error	20	22.842	1.142		
Total	29	752.733			

Table D.7. Relative leaf area-second sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	158.070	39.517	0.96	0.4504
Leaf age(A)	1	3312.509	3312.509	80.54	<.0001
Spore conc(B)	2	386.710	193.355	4.70	0.0212
AxB	2	59.338	29.669	0.72	0.4983
Error	20	822.574	41.128		
Total	29	4739.203			

Table D.8. Relative lesion area-second sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	142.445	35.611	2.66	0.0628
Leaf age(A)	1	8.313	8.313	0.62	0.4399
Spore conc(B)	2	8268.267	4134.133	308.84	<.0001
AxB	2	135.475	67.737	5.06	0.0167
Error	20	267.717	13.385		
Total	29	8822.219			

Table D.9. Leaf area-third sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	1969.686	492.421	1.06	0.4041
Leaf age(A)	1	25629.005	25629.005	54.93	<.0001
Spore conc(B)	2	22.284	11.142	0.02	0.9764
AxB	2	262.932	131.466	0.28	0.7574
Error	20	9331.923	466.596		
Total	29	37215.832			

Table D.10. Lesion area-third sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	496.762	124.190	0.87	0.5012
Leaf age(A)	1	166.102	166.102	1.16	0.2946
Spore conc(B)	2	862.100	431.050	3.01	0.0722
AxB	2	143.650	71.825	0.50	0.6133
Error	20	2867.390	143.369		
Total	29	4536.007			

Table D.11. Relative leaf area-third sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	297.636	74.409	1.06	0.4041
Leaf age(A)	1	3872.763	3872.763	54.93	<.0001
Spore conc(B)	2	3.367	1.683	0.02	0.9764
AxB	2	39.731	19.865	0.28	0.7574
Error	20	1410.134	70.506		
Total	29	5623.633			

Table D.12. Relative lesion area-third sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	456.967	114.241	1.06	0.4041
Leaf age(A)	1	5945.934	5945.934	54.93	<.0001
Spore conc(B)	2	5.170	2.585	0.02	0.9764
AxB	2	61.000	30.500	0.28	0.7574
Error	20	2165.008	108.250		
Total	29	8634.080			

Table D.13. Leaf area-fourth sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	530.724	132.681	0.46	0.7661
Leaf age(A)	1	19067.791	19067.791	65.71	<.0001
Spore conc(B)	2	8.459	4.229	0.01	0.9855
AxB	2	46.875	23.437	0.08	0.9227
Error	20	5803.408	290.170		
Total	29	25457.259			

Table D.14. Lesion area-fourth sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	171.405	42.851	0.58	0.6779
Leaf age(A)	1	59.614	59.614	0.81	0.3782
Spore conc(B)	2	2885.279	1442.639	19.66	<.0001
AxB	2	73.934	36.967	0.50	0.6117
Error	20	1467.788	73.389		
Total	29	4658.021			

Table D.15. Relative leaf area-fourth sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	72.212	18.053	0.46	0.7661
Leaf age(A)	1	2594.426	2594.426	65.71	<.0001
Spore conc(B)	2	1.151	0.575	0.01	0.9855
AxB	2	6.378	3.189	0.08	0.9227
Error	20	789.630	39.481		
Total	29	3463.798			

Table D.16. Relative lesion area-fourth sampling.

Source	d.f.	S.S	m.s	F	Sig.
Replicates	4	98.075	24.518	0.58	0.6779
Leaf age(A)	1	34.110	34.110	0.81	0.3782
Spore conc(B)	2	1650.915	825.457	19.66	<.0001
AxB	2	42.304	21.152	0.50	0.6117
Error	20	839.847	41.992		
Total	29	2665.254			

Tables D.17-D.29. ANOVA tables for soil and leaf nutrients of cucumbers inoculated with three spore concentrations of *P. cubensis* on two aged leaves (Chapter 4, Table 4.3).

Table D.17. Fe in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	26094.496	6523.624	2.95	0.0457
tr	5	149863.118	29972.623	13.55	<.0001
Error	20	44253.063	2212.653		
Total	29	220210.679			

Table D.18. Mn in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	2357.338	589.334	0.82	0.5251
tr	5	39693.035	7938.607	11.10	<.0001
Error	20	14299.677	714.983		
Total	29	56350.051			

Table D.19. Cu in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	2393.161	598.290	1.59	0.2151
tr	5	22018.389	4403.677	11.72	<.0001
Error	20	7513.881	375.694		
Total	29	31925.431			

Table D.20. Zn in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	375.097	93.774	2.34	0.0898
tr	5	2578.007	515.601	12.88	<.0001
Error	20	800.564	40.028		
Total	29	3753.669			

Table D.21. Ca in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	2.270	0.567	0.68	0.6161
tr	5	20.870	4.174	4.98	0.0040
Error	20	16.777	0.838		
Total	29	39.917			

Table D.22. Mg in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	0.106	0.026	1.80	0.1676
tr	5	0.305	0.061	4.15	0.0095
Error	20	0.294	0.014		
Total	29	0.706			

Table D.23. K in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	0.362	0.090	0.31	0.8699
tr	5	12.429	2.485	8.41	0.0002
Error	20	5.909	0.295		
Total	29	18.702			

Table D.24. P in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	0.005	0.001	0.52	0.7207
tr	5	0.095	0.019	7.45	0.0004
Error	20	0.051	0.002		
Total	29	0.152			

Table D.25. NH_4-N in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	426.411	106.602	1.36	0.2822
tr	5	11759.834	2351.966	30.06	<.0001
Error	20	1564.773	78.238		
Total	29	13751.019			

Table D.26. NO_3-N in cucumber leaves

Source	d.f.	S.S	m.s	F	Sig.
re	4	2272.688	568.172	0.89	0.4902
tr	5	17858.524	3571.704	5.57	0.0023
Error	20	12825.261	641.263		
Total	29	32956.475			

Table D.27. NH_4-N in soil

Source	d.f.	S.S	m.s	F	Sig.
re	4	12.505	3.126	1.66	0.1988
tr	5	72.596	14.519	7.71	0.0004
Error	20	37.684	1.884		
Total	29	122.786			

Table D.28. NO_3-N in soil

Source	d.f.	S.S	m.s	F	Sig.
re	4	64.712	16.178	1.11	0.3787
tr	5	100.747	20.149	1.38	0.2722
Error	20	291.124	14.556		
Total	29	456.584			

Table D.29. K in soil

Source	d.f.	S.S	m.s	F	Sig.
re	4	9727.973	2431.993	3.81	0.0185
tr	5	4451.794	890.358	1.39	0.2691
Error	20	12781.376	39.068		
Total	29	26961.143			

Tables D.30-D81. ANCOVA tables for leaf area, lesion area, relative leaf area and relative lesion area of cucumbers in the first sampling with soil and leaf nutrients, as covariates (Chapter 4).

Table D.30. Leaf area- leaf Fe

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	527.487	263.743	0.39	0.684
Fe	1	8.242	8.242	0.01	0.913
leaf	1	29.795	29.795	0.04	0.836
re	4	993.470	248.367	0.37	0.829
Fe*Conc	2	567.051	283.525	0.42	0.665
leaf*Conc	2	974.237	487.118	0.72	0.503
Fe*leaf	1	864.500	864.500	1.27	0.276
Error	16	10874.154	679.634		
Total	29	87955.027			

Table D.31. Lesion area- leaf Fe

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	1.060	0.530	0.20	0.821
Fe	1	15.407	15.407	5.77	0.028
leaf	1	3.329	3.329	1.25	0.280
re	4	5.287	1.321	0.50	0.739
Fe*Conc	2	3.224	1.612	0.60	0.558
leaf*Conc	2	34.866	17.433	6.53	0.008
Fe*leaf	1	1.474	1.474	0.55	0.468
Error	16	42.688	2.668		
Total	29	627.442			

Table D.32. Relative leaf area- leaf Fe

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	72.005	36.002	0.39	0.684
Fe	1	1.125	1.125	0.01	0.913
leaf	1	4.067	4.067	0.04	0.836
re	4	135.615	33.903	0.37	0.829
Fe*Conc	2	77.406	38.703	0.42	0.665
leaf*Conc	2	132.990	66.495	0.72	0.503
Fe*leaf	1	118.010	118.010	1.27	0.276
Error	16	1484.397	92.774		
Total	29	12006.471			

Table D.33. Relative lesion area- leaf Fe

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	13.896	6.948	0.20	0.821
Fe	1	201.817	201.817	5.77	0.028
leaf	1	43.614	43.614	1.25	0.280
re	4	69.257	17.314	0.50	0.739
Fe*Conc	2	42.237	21.118	0.60	0.558
leaf*Conc	2	456.713	228.356	6.53	0.008
Fe*leaf	1	19.308	19.308	0.55	0.468
Error	16	559.182	34.948		
Total	29	8218.864			

Table D.34. Leaf area- leaf Mn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	865.774	432.887	0.65	0.533
Mn	1	825.470	825.470	1.25	0.280
leaf	1	707.263	707.263	1.07	0.316
re	4	1025.751	256.437	0.39	0.814
Mn*Conc	2	777.696	388.848	0.59	0.567
leaf*Conc	2	1954.017	977.008	1.48	0.257
Mn*leaf	1	0.445	0.445	0.00	0.979
Error	16	10585.978	661.623		
Total	29	87955.027			

Table D.35. Lesion area- leaf Mn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	45.331	22.665	10.00	0.001
Mn	1	0.056	0.056	0.02	0.876
leaf	1	2.525	2.525	1.11	0.306
re	4	10.976	2.744	1.21	0.344
Mn*Conc	2	24.347	12.173	5.37	0.016
leaf*Conc	2	2.391	1.195	0.53	0.600
Mn*leaf	1	0.465	0.465	0.21	0.656
Error	16	36.261	2.266		
Total	29	627.442			

Table D.36. Relative leaf area- leaf Mn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	118.184	59.092	0.65	0.533
Mn	1	112.682	112.682	1.25	0.280
leaf	1	96.546	96.546	1.07	0.316
re	4	140.022	35.005	0.39	0.814
Mn*Conc	2	106.160	53.080	0.59	0.567
leaf*Conc	2	266.736	133.368	1.48	0.257
Mn*leaf	1	0.060	0.060	0.00	0.979
Error	16	1445.059	90.316		
Total	29	12006.471			

Table D.37. Relative lesion area- leaf Mn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	593.795	296.897	10.00	0.001
Mn	1	0.740	0.740	0.02	0.876
leaf	1	33.086	33.086	1.11	0.306
re	4	143.784	35.946	1.21	0.344
Mn*Conc	2	318.929	159.464	5.37	0.016
leaf*Conc	2	31.323	15.661	0.53	0.600
Mn*leaf	1	6.097	6.097	0.21	0.656
Error	16	474.985	29.686		
Total	29	8218.864			

Table D.38. Leaf area- leaf Cu

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	378.185	189.092	0.27	0.767
Cu	1	535.194	535.194	0.76	0.395
leaf	1	116.434	116.434	0.17	0.689
re	4	632.875	158.218	0.23	0.920
Cu*Conc	2	529.171	264.585	0.38	0.691
leaf*Conc	2	3374.977	1687.488	2.41	0.121
Cu*leaf	1	199.319	199.319	0.28	0.601
Error	16	11216.942	701.058		
Total	29	87955.027			

Table D.39. Lesion area- leaf Cu

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	2.946	1.473	0.47	0.635
Cu	1	10.355	10.355	3.28	0.088
leaf	1	10.220	10.220	3.24	0.090
re	4	5.035	1.258	0.40	0.806
Cu*Conc	2	3.756	1.878	0.60	0.563
leaf*Conc	2	8.847	4.423	1.40	0.274
Cu*leaf	1	7.791	7.791	2.47	0.135
Error	16	50.450	3.153		
Total	29	627.442			

Table D.40. Relative leaf area- leaf Cu

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	51.624	25.812	0.27	0.767
Cu	1	73.057	73.057	0.76	0.395
leaf	1	15.894	15.894	0.17	0.689
re	4	86.391	21.597	0.23	0.920
Cu*Conc	2	72.235	36.117	0.38	0.691
leaf*Conc	2	460.707	230.353	2.41	0.121
Cu*leaf	1	27.208	27.208	0.28	0.601
Error	16	1531.190	95.699		
Total	29	12006.471			

Table D.41. Relative lesion area- leaf Cu

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	38.598	19.299	0.47	0.635
Cu	1	135.642	135.642	3.28	0.088
leaf	1	133.874	133.874	3.24	0.090
re	4	65.959	16.489	0.40	0.806
Cu*Conc	2	49.201	24.600	0.60	0.563
leaf*Conc	2	115.899	57.949	1.40	0.274
Cu*leaf	1	102.066	102.066	2.47	0.135
Error	16	660.848	41.303		
Total	29	8218.864			

Table D.42. Leaf area- leaf Zn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	167.348	83.674	0.11	0.892
Zn	1	170.210	170.210	0.23	0.636
leaf	1	1030.145	1030.145	1.41	0.252
re	4	1173.465	293.366	0.40	0.805
Zn*Conc	2	224.480	112.240	0.15	0.859
leaf*Conc	2	858.172	429.086	0.59	0.567
Zn*leaf	1	51.727	51.727	0.07	0.793
Error	16	11710.454	731.903		
Total	29	87955.027			

Table D.43. Lesion area- leaf Zn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	17.981	8.990	6.07	0.010
Zn	1	11.427	11.427	7.71	0.013
leaf	1	0.972	0.972	0.66	0.429
re	4	7.445	1.861	1.26	0.327
Zn*Conc	2	15.332	7.666	5.17	0.018
leaf*Conc	2	10.589	5.294	3.57	0.052
Zn*leaf	1	0.410	0.410	0.28	0.606
Error	16	23.706	1.481		
Total	29	627.442			

Table D.44. Relative leaf area- leaf Zn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	22.844	11.422	0.11	0.892
Zn	1	23.234	23.234	0.23	0.636
leaf	1	140.621	140.621	1.41	0.252
re	4	160.186	40.046	0.40	0.805
Zn*Conc	2	30.643	15.321	0.15	0.859
leaf*Conc	2	117.146	58.573	0.59	0.567
Zn*leaf	1	7.061	7.061	0.07	0.793
Error	16	1598.558	99.909		
Total	29	12006.471			

Table D.45. Relative lesion area- leaf Zn

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	235.535	117.767	6.07	0.010
Zn	1	149.683	149.683	7.71	0.013
leaf	1	12.743	12.743	0.66	0.429
re	4	97.533	24.383	1.26	0.327
Zn*Conc	2	200.835	100.417	5.17	0.018
leaf*Conc	2	138.706	69.353	3.57	0.052
Zn*leaf	1	5.373	5.373	0.28	0.606
Error	16	310.532	19.408		
Total	29	8218.864			

Table D.46. Leaf area- leaf Ca

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	1008.892	504.446	0.91	0.421
Ca	1	412.190	412.190	0.75	0.400
leaf	1	158.677	158.677	0.29	0.599
re	4	373.474	93.368	0.17	0.951
Ca*Conc	2	869.481	434.740	0.79	0.472
leaf*Conc	2	3833.864	1916.932	3.47	0.056
Ca*leaf	1	1532.934	1532.934	2.77	0.115
Error	16	8844.568	552.785		
Total	29	87955.027			

Table D.47. Lesion area- leaf Ca

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	14.427	7.213	3.02	0.077
Ca	1	18.028	18.028	7.54	0.014
leaf	1	0.399	0.399	0.17	0.688
re	4	4.042	1.010	0.42	0.790
Ca*Conc	2	19.867	9.933	4.16	0.035
leaf*Conc	2	12.868	6.434	2.69	0.098
Ca*leaf	1	0.043	0.043	0.02	0.894
Error	16	38.250	2.390		
Total	29	627.442			

Table D.48. Relative leaf area- leaf Ca

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	137.720	68.860	0.91	0.421
Ca	1	56.266	56.266	0.75	0.400
leaf	1	21.660	21.660	0.29	0.599
re	4	50.981	12.745	0.17	0.951
Ca*Conc	2	118.690	59.345	0.79	0.472
leaf*Conc	2	523.349	261.674	3.47	0.056
Ca*leaf	1	209.256	209.256	2.77	0.115
Error	16	1207.345	75.459		
Total	29	12006.471			

Table D.49. Relative lesion area- leaf Ca

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	188.983	94.491	3.02	0.077
Ca	1	236.157	236.157	7.54	0.014
leaf	1	5.227	5.227	0.17	0.688
re	4	52.955	13.238	0.42	0.790
Ca*Conc	2	260.239	130.119	4.16	0.035
leaf*Conc	2	168.571	84.285	2.69	0.098
Ca*leaf	1	0.567	0.567	0.02	0.894
Error	16	501.036	31.314		
Total	29	8218.864			

Table D.50. Leaf area- leaf Mg

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	403.333	201.666	0.32	0.731
Mg	1	36.065	36.065	0.06	0.814
leaf	1	1.631	1.631	0.00	0.960
re	4	992.892	248.223	0.39	0.810
Mg*Conc	2	372.799	186.399	0.30	0.748
leaf*Conc	2	1693.127	846.563	1.34	0.289
Mg*leaf	1	525.507	525.507	0.83	0.375
Error	16	10102.685	631.417		
Total	29	87955.027			

Table D.51. Lesion area- leaf Mg

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	31.946	15.973	6.19	0.010
Mg	1	0.282	0.282	0.11	0.745
leaf	1	5.401	5.401	2.09	0.167
re	4	4.008	1.002	0.39	0.813
Mg*Conc	2	19.756	9.878	3.83	0.043
leaf*Conc	2	8.600	4.300	1.67	0.219
Mg*leaf	1	4.421	4.421	1.71	0.208
Error	16	41.268	2.579		
Total	29	627.442			

Table D.52. Relative leaf area- leaf Mg

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	55.057	27.528	0.32	0.731
Mg	1	4.923	4.923	0.06	0.814
leaf	1	0.222	0.222	0.00	0.960
re	4	135.536	33.884	0.39	0.810
Mg*Conc	2	50.889	25.444	0.30	0.748
leaf*Conc	2	231.123	115.561	1.34	0.289
Mg*leaf	1	71.735	71.735	0.83	0.375
Error	16	1379.086	86.192		
Total	29	12006.471			

Table D.53. Relative lesion area- leaf Mg

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	418.469	209.234	6.19	0.010
Mg	1	3.700	3.700	0.11	0.745
leaf	1	70.758	70.758	2.09	0.167
re	4	52.503	13.125	0.39	0.813
Mg*Conc	2	258.788	129.394	3.83	0.043
leaf*Conc	2	112.655	56.327	1.67	0.219
Mg*leaf	1	57.912	57.912	1.71	0.208
Error	16	540.56	33.785		
Total	29	8218.86			

Table D.54. Leaf area- leaf K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	435.238	217.619	0.32	0.729
K	1	815.934	815.934	1.21	0.288
leaf	1	657.705	657.705	0.97	0.338
re	4	1243.229	310.807	0.46	0.763
K*Conc	2	489.648	244.824	0.36	0.701
leaf*Conc	2	4183.657	2091.828	3.10	0.073
K*leaf	1	51.333	51.333	0.08	0.786
Error	16	10807.361	675.460		
Total	29	87955.027			

Table D.55. Lesion area- leaf K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	20.959	10.479	3.50	0.054
K	1	1.562	1.562	0.52	0.480
leaf	1	2.389	2.389	0.80	0.384
re	4	0.746	0.186	0.06	0.992
K*Conc	2	16.418	8.209	2.74	0.094
leaf*Conc	2	13.131	6.565	2.19	0.143
K*leaf	1	2.721	2.721	0.91	0.354
Error	16	47.885	2.992		
Total	29	627.442			

Table D.56. Relative leaf area- leaf K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	59.413	29.706	0.32	0.729
K	1	111.380	111.380	1.21	0.288
leaf	1	89.781	89.781	0.97	0.338
re	4	169.709	42.427	0.46	0.763
K*Conc	2	66.840	33.420	0.36	0.701
leaf*Conc	2	571.098	285.549	3.10	0.073
K*leaf	1	7.007	7.007	0.08	0.786
Error	16	1475.279	92.204		
Total	29	12006.471			

Table D.57. Relative lesion area- leaf K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	274.543	137.271	3.50	0.054
K	1	20.460	20.460	0.52	0.480
leaf	1	31.300	31.300	0.80	0.384
re	4	9.773	2.443	0.06	0.992
K*Conc	2	215.066	107.533	2.74	0.094
leaf*Conc	2	172.009	86.004	2.19	0.143
K*leaf	1	35.646	35.646	0.91	0.354
Error	16	627.250	39.203		
Total	29	8218.864			

Table D.58. Leaf area- leaf P

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	1124.018	562.009	0.84	0.448
P	1	285.997	285.997	0.43	0.521
leaf	1	157.107	157.107	0.24	0.633
re	4	1488.686	372.171	0.56	0.695
P*Conc	2	1030.179	515.089	0.77	0.477
leaf*Conc	2	1564.060	782.030	1.18	0.334
P*leaf	1	0.032	0.032	0.00	0.994
Error	16	10648.359	665.522		
Total	29	87955.027			

Table D.59. Lesion area- leaf P

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	6.148	3.074	0.86	0.441
P	1	5.126	5.126	1.44	0.248
leaf	1	0.374	0.374	0.10	0.750
re	4	1.184	0.296	0.08	0.986
P*Conc	2	3.183	1.591	0.45	0.647
leaf*Conc	2	8.789	4.394	1.23	0.318
P*leaf	1	0.178	0.178	0.05	0.825
Error	16	57.095	3.568		
Total	29	627.442			

Table D.60. Relative leaf area- leaf P

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	153.436	76.718	0.84	0.448
P	1	39.040	39.040	0.43	0.521
leaf	1	21.446	21.446	0.24	0.633
re	4	203.216	50.804	0.56	0.695
P*Conc	2	140.626	70.313	0.77	0.477
leaf*Conc	2	213.505	106.752	1.18	0.334
P*leaf	1	0.004	0.004	0.00	0.994
Error	16	1453.575	90.848		
Total	29	12006.471			

Table D.61. Relative lesion area- leaf P

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	80.542	40.271	0.86	0.441
P	1	67.148	67.148	1.44	0.248
leaf	1	4.903	4.903	0.10	0.750
re	4	15.521	3.880	0.08	0.986
P*Conc	2	41.702	20.851	0.45	0.647
leaf*Conc	2	115.127	57.563	1.23	0.318
P*leaf	1	2.340	2.340	0.05	0.825
Error	16	747.895	46.743		
Total	29	8218.864			

Table D.62. Leaf area- leaf NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	2952.998	1476.499	2.73	0.095
NH_4-N	1	578.000	578.000	1.07	0.316
leaf	1	378.792	378.792	0.70	0.414
re	4	2054.805	513.701	0.95	0.460
NH_4-N*Conc	2	3347.813	1673.906	3.10	0.072
leaf*Conc	2	4204.983	2102.491	3.89	0.041
NH_4-N*leaf	1	34.989	34.989	0.06	0.802
Error	16	8642.330	540.145		
Total	29	87955.027			

Table D.63. Lesion area- leaf NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	12.710	6.355	1.81	0.196
NH_4-N	1	0.741	0.741	0.21	0.652
leaf	1	6.370	6.370	1.81	0.197
re	4	6.444	1.611	0.46	0.765
NH_4-N*Conc	2	1.448	0.724	0.21	0.816
leaf*Conc	2	7.130	3.565	1.01	0.385
NH_4-N*leaf	1	5.639	5.639	1.60	0.223
Error	16	56.276	3.517		
Total	29	627.442			

Table D.64. Relative leaf area- leaf NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	403.104	201.552	2.73	0.095
NH_4-N	1	78.901	78.901	1.07	0.316
leaf	1	51.707	51.707	0.70	0.414
re	4	280.495	70.123	0.95	0.460
NH_4-N*Conc	2	456.999	228.499	3.10	0.072
leaf*Conc	2	574.009	287.004	3.89	0.041
NH_4-N*leaf	1	4.776	4.776	0.06	0.802
Error	16	1179.738	73.733		
Total	29	12006.471			

Table D.65. Relative lesion area- leaf NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	166.489	83.244	1.81	0.196
NH_4-N	1	9.717	9.717	0.21	0.652
leaf	1	83.442	83.442	1.81	0.197
re	4	84.420	21.105	0.46	0.765
NH_4-N*Conc	2	18.969	9.484	0.21	0.816
leaf*Conc	2	93.402	46.701	1.01	0.385
NH_4-N*leaf	1	73.869	73.869	1.60	0.223
Error	16	737.163	46.072		
Total	29	218.864			

Table D.66. Leaf area- leaf NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	729.249	364.624	0.64	0.540
NO_3-N	1	631.993	631.993	1.11	0.308
leaf	1	4988.805	4988.805	8.74	0.009
re	4	1001.603	250.400	0.44	0.778
NO_3-N*Conc	2	707.660	353.830	0.62	0.550
leaf*Conc	2	1859.082	929.541	1.63	0.227
NO_3-N*leaf	1	1052.942	1052.942	1.84	0.193
Error	16	9133.959	570.872		
Total	29	87955.027			

Table D.67. Lesion area- leaf NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	17.224	8.612	2.72	0.095
NO_3-N	1	3.939	3.939	1.25	0.280
leaf	1	3.478	3.478	1.10	0.309
re	4	6.229	1.557	0.49	0.741
NO_3-N*Conc	2	8.138	4.069	1.29	0.303
leaf*Conc	2	3.488	1.744	0.55	0.586
NO_3-N*leaf	1	0.590	0.590	0.19	0.671
Error	16	50.576	3.161		
Total	29	627.442			

Table D.68. Relative leaf area- leaf NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	99.547	49.773	0.64	0.540
NO_3-N	1	86.271	86.271	1.11	0.308
leaf	1	681.006	681.006	8.74	0.009
re	4	136.725	34.181	0.44	0.778
NO_3-N*Conc	2	96.600	48.300	0.62	0.550
leaf*Conc	2	253.777	126.888	1.63	0.227
NO_3-N*leaf	1	143.733	143.733	1.84	0.193
Error	16	1246.848	77.928		
Total	29	12006.471			

Table D.69. Relative lesion area-leaf NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	225.619	112.809	2.72	0.095
NO_3-N	1	51.604	51.604	1.25	0.280
leaf	1	45.570	45.570	1.10	0.309
re	4	81.597	20.399	0.49	0.741
NO_3-N*Conc	2	106.611	53.305	1.29	0.303
leaf*Conc	2	45.701	22.850	0.55	0.586
NO_3-N*leaf	1	7.736	7.736	0.19	0.671
Error	16	662.496	41.406		
Total	29	8218.864			

Table D.70. Leaf area-soil NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	1299.435	649.717	1.03	0.379
NH_4-N	1	24.668	24.668	0.04	0.845
leaf	1	4179.791	4179.791	6.62	0.020
re	4	1040.159	260.039	0.41	0.797
NH_4-N*Conc	2	1179.430	589.715	0.93	0.413
leaf*Conc	2	2885.205	1442.602	2.29	0.133
NH_4-N*leaf	1	527.043	527.043	0.84	0.374
Error	16	10096.398	631.024		
Total	29	87955.027			

Table D.71. Lesion area-soil NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	7.422	3.711	1.01	0.386
NH_4-N	1	1.581	1.581	0.43	0.521
leaf	1	1.125	1.125	0.31	0.587
re	4	6.662	1.665	0.45	0.768
NH_4-N*Conc	2	0.815	0.407	0.11	0.895
leaf*Conc	2	17.758	8.879	2.42	0.121
NH_4-N*leaf	1	2.749	2.749	0.75	0.399
Error	16	58.779	3.673		
Total	29	627.442			

Table D.72. Relative leaf area-soil NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	177.381	88.690	1.03	0.379
NH_4-N	1	3.367	3.367	0.04	0.845
leaf	1	570.570	570.570	6.62	0.020
re	4	141.989	35.497	0.41	0.797
NH_4-N*Conc	2	161.000	80.500	0.93	0.413
leaf*Conc	2	393.850	196.925	2.29	0.133
NH_4-N*leaf	1	71.945	71.945	0.84	0.374
Error	16	1378.228	86.139		
Total	29	12006.471			

Table D.73. Relative lesion area-soil NH_4-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	97.226	48.613	1.01	0.386
NH_4-N	1	20.720	20.720	0.43	0.521
leaf	1	14.742	14.742	0.31	0.587
re	4	87.268	21.817	0.45	0.768
NH_4-N*Conc	2	10.682	5.341	0.11	0.895
leaf*Conc	2	232.616	116.308	2.42	0.121
NH_4-N*leaf	1	36.009	36.009	0.75	0.399
Error	16	769.952	48.122		
Total	29	8218.864			

Table D.74. Leaf area-soil NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	462.667	231.333	0.38	0.690
NO_3-N	1	1312.008	1312.008	2.15	0.162
leaf	1	4864.847	4864.847	7.96	0.012
re	4	2483.503	620.875	1.02	0.428
NO_3-N*Conc	2	581.103	290.551	0.48	0.630
leaf*Conc	2	7193.674	3596.837	5.89	0.012
NO_3-N*leaf	1	456.217	456.217	0.75	0.400
Error	16	9775.229	610.951		
Total	29	87955.027			

Table D.75. Lesion area-soil NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	19.805	9.902	2.71	0.097
NO_3-N	1	0.591	0.591	0.16	0.692
leaf	1	2.916	2.916	0.80	0.385
re	4	1.613	0.403	0.11	0.977
NO_3-N*Conc	2	2.346	1.173	0.32	0.730
leaf*Conc	2	7.896	3.948	1.08	0.363
NO_3-N*leaf	1	5.367	5.367	1.47	0.243
Error	16	58.573	3.660		
Total	29	627.442			

Table D.76. Relative leaf area-soil NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	63.157	31.578	0.38	0.690
NO_3-N	1	179.098	179.098	2.15	0.162
leaf	1	664.085	664.085	7.96	0.012
re	4	339.015	84.753	1.02	0.428
NO_3-N*Conc	2	79.324	39.662	0.48	0.630
leaf*Conc	2	981.986	490.993	5.89	0.012
NO_3-N*leaf	1	62.276	62.276	0.75	0.400
Error	16	1334.386	83.399		
Total	29	12006.471			

Table D.77. Relative lesion area-soil NO_3-N

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	259.436	129.718	2.71	0.097
NO_3-N	1	7.752	7.752	0.16	0.692
leaf	1	38.199	38.199	0.80	0.385
re	4	21.131	5.282	0.11	0.977
NO_3-N*Conc	2	30.730	15.365	0.32	0.730
leaf*Conc	2	103.433	51.716	1.08	0.363
NO_3-N*leaf	1	70.305	70.305	1.47	0.243
Error	16	767.248	47.953		
Total	29	8218.864			

Table D.78. Leaf area-soil K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	466.528	233.264	0.42	0.663
K	1	84.230	84.230	0.15	0.701
leaf	1	1814.996	1814.996	3.28	0.088
re	4	1261.614	315.403	0.57	0.688
K*Conc	2	500.049	250.024	0.45	0.644
leaf*Conc	2	5735.399	2867.699	5.18	0.018
K*leaf	1	837.403	837.403	1.51	0.236
Error	16	8852.745	553.296		
Total	29	87955.027			

Table D.79. Lesion area-soil K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	7.606	3.803	0.97	0.398
K	1	0.224	0.224	0.06	0.813
leaf	1	3.955	3.955	1.01	0.329
re	4	2.848	0.712	0.18	0.944
K*Conc	2	2.448	1.224	0.31	0.735
leaf*Conc	2	10.390	5.195	1.33	0.291
K*leaf	1	3.344	3.344	0.86	0.368
Error	16	62.441	3.902		
Total	29	627.442			

Table D.80. Relative leaf area-soil K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	63.684	31.842	0.42	0.663
K	1	11.498	11.498	0.15	0.701
leaf	1	247.759	247.759	3.28	0.088
re	4	172.219	43.054	0.57	0.688
K*Conc	2	68.260	34.130	0.45	0.644
leaf*Conc	2	782.921	391.460	5.18	0.018
K*leaf	1	114.311	114.311	1.51	0.236
Error	16	1208.461	75.528		
Total	29	12006.471			

Table D.81. Relative lesion area-soil K

Source	d.f.	S.S	m.s	F	Sig.
Conc	2	99.639	49.819	0.97	0.398
K	1	2.937	2.937	0.06	0.813
leaf	1	51.815	51.815	1.01	0.329
re	4	37.314	9.328	0.18	0.944
K*Conc	2	32.071	16.035	0.31	0.735
leaf*Conc	2	136.105	68.052	1.33	0.291
K*leaf	1	43.814	43.814	0.86	0.368
Error	16	817.924	51.120		
Total	29	8218.864			

APPENDIX E

Statistical Tables for Chapter Five

Tables E.1-E.5. ANOVA tables for nutrients in leaves and soil of cucumbers treated with six nitrogen fertilization treatments (Chapter 5, section 5.3.1 First experiment).

Table E.1. Leaf NH_4-N.

Source	d.f.	s.s.	m.s.	F	Sig.
Replicates	3	646.441	215.480	0.40	0.7542
Treatments	5	11186.715	2237.343	4.17	0.0142
Error	15	8056.538	537.102		
Total	23	19889.694			

Table E.2. Leaf NO_3-N.

Source	d.f.	s.s.	m.s.	F	Sig.
Replicates	3	1471.717	490.572	1.91	0.1720
Treatments	5	24914.053	4982.810	19.36	<.0001
Error	15	3861.088	257.405		
Total	23	30246.859			

Table E.3. Soil NH_4-N.

Source	d.f.	s.s.	m.s.	F	Sig.
Replicates	3	1247.529	415.843	2.67	0.0852
Treatments	5	4135.308	827.061	5.31	0.0053
Error	15	2337.291	155.819		
Total	23	7720.128			

Table E.4. Soil NO_3-N.

Source	d.f.	s.s.	m.s.	F	Sig.
Replicates	3	2988.737	996.246	0.40	0.7527
Treatments	5	1508829.052	301765.810	122.17	<.0001
Error	15	37051.327	2470.088		
Total	23	1548869.116			

Table E.5. Soil K.

Source	d.f.	s.s.	m.s.	F	Sig.
Replicates	3	4530.413	1510.137	4.92	0.0141
Treatments	5	28392.453	5678.490	18.51	<.0001
Error	15	4601.628	306.775		
Total	23	37524.495			

Tables E.6-E.17. ANOVA tables for leaf and lesion area of cucumbers treated with six nitrogen fertilization treatments, in the six samplings (Chapter 5, section 5.3.2 Second experiment).

Table E.6. Leaf area-first sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	532.796	177.598	1.88	0.1757
treatments	5	15430.305	3086.061	32.74	<.0001
Error	15	1413.976	94.265		
Total	23	17377.078			

Table E.7. Leaf area-second sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	1009.669	336.556	1.99	0.1582
treatments	5	17228.424	3445.684	20.42	<.0001
Error	15	2531.276	168.751		
Total	23	20769.370			

Table E.8. Leaf area-third sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	610.970	203.656	1.77	0.1967
treatments	5	23616.253	4723.250	40.96	<.0001
Error	15	1729.597	115.306		
Total	23	25956.821			

Table E.9. Leaf area- fourth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	878.842	292.947	2.33	0.1158
treatments	5	30807.950	6161.590	48.99	<.0001
Error	15	1886.755	125.783		
Total	23	33573.548			

Table E.10. Leaf area- fifth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	217.825	72.608	0.53	0.6688
treatments	5	22756.847	4551.369	33.19	<.0001
Error	15	2056.744	137.116		
Total	23	25031.416			

Table E.11. Leaf area-sixth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	404.841	134.947	0.85	0.4883
treatments	5	25502.782	5100.556	32.10	<.0001
Error	15	2383.085	158.872		
Total	23	28290.709			

Table E.12. Lesion area-first sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	0.020	0.006	0.37	0.7792
treatments	5	1.896	0.379	20.08	<.0001
Error	15	0.283	0.018		
Total	23	2.200			

Table E.13. Lesion area-second sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	2.630	0.876	3.20	0.0540
treatments	5	14.320	2.864	10.44	0.0002
Error	15	4.116	0.274		
Total	23	21.067			

Table E.14. Lesion area-third sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	10.617	3.539	2.74	0.0803
treatments	5	64.698	12.939	10.00	0.0002
Error	15	19.404	1.293		
Total	23	94.720			

Table E.15. Lesion area-fourth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	21.720	7.240	4.40	0.0207
treatments	5	70.073	14.014	8.52	0.0005
Error	15	24.662	1.644		
Total	23	116.456			

Table E.16. Lesion area-fifth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	15.7048	5.234	1.07	0.3918
treatments	5	205.6732	41.134	8.40	0.0006
Error	15	73.4525	4.896		
Total	23	294.8306			

Table E.17. Lesion area-sixth sampling

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	2366.384	788.794	1.22	0.3378
treatments	5	12828.356	2565.671	3.96	0.0173
Error	15	9721.908	648.127		
Total	23	24916.649			

Tables E.18-E.43. ANCOVA tables for leaf and lesion area of cucumbers in the first sampling with soil and leaf nutrients, as covariates (Chapter 5, section 5.3.2 Second experiment).

Table E.18. Leaf area- leaf Fe

Source	d.f.	S.S.	m.s.	F	Sig.
Fe	1	7.643	7.643	0.08	0.7867
treatments	5	15413.351	3082.670	30.69	<.0001
replicates	3	308.304	102.768	1.02	0.4122
Error	14	1406.332	100.452		
Total	23	17377.078			

Table E.19. Leaf area- leaf Mn

Source	d.f.	S.S.	m.s.	F	Sig.
Mn	1	63.844	63.844	0.66	0.4295
treatments	5	4690.082	938.016	9.73	0.0004
replicates	3	586.430	195.476	2.03	0.1564
Error	14	1350.132	96.438		
Total	23	17377.078			

Table E.20. Leaf area- leaf Cu

Source	d.f.	S.S.	m.s.	F	Sig.
Cu	1	159.082	159.082	1.77	0.2041
treatments	5	15575.375	3115.075	34.75	<.0001
replicates	3	688.784	229.594	2.56	0.0966
Error	14	1254.893	89.635		
Total	23	17377.078			

Table E.21. Leaf area- leaf Zn

Source	d.f.	S.S.	m.s.	F	Sig.
Zn	1	35.708	35.708	0.36	0.5566
treatments	5	12461.938	2492.387	25.32	<.0001
replicates	3	274.172	91.390	0.93	0.4528
Error	14	1378.267	98.447		
Total	23	17377.078			

Table E.22. Leaf area- leaf Ca

Source	d.f.	S.S.	m.s.	F	Sig.
Ca	1	2.767	2.767	0.03	0.8708
treatments	5	12880.578	2576.115	25.56	<.0001
replicates	3	455.809	151.936	1.51	0.2560
Error	14	1411.208	100.800		
Total	23	17377.078			

Table E.23. Leaf area- leaf Mg

Source	d.f.	S.S.	m.s.	F	Sig.
Mg	1	8.321	8.321	0.08	0.7776
treatments	5	15434.063	3086.812	30.74	<.0001
replicates	3	493.250	164.416	1.64	0.2258
Error	14	1405.654	100.403		
Total	23	17377.078			

Table E.24. Leaf area- leaf P

Source	d.f.	S.S.	m.s.	F	Sig.
P	1	307.306	307.306	3.89	0.0687
treatments	5	14489.856	2897.971	36.66	<.0001
replicates	3	801.319	267.106	3.38	0.0486
Error	14	1106.669	79.047		
Total	23	17377.078			

Table E.25. Leaf area- leaf K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	20.585	20.585	0.21	0.6562
treatments	5	14914.989	2982.997	29.97	<.0001
replicates	3	240.002	80.000	0.80	0.5123
Error	14	1393.390	99.527		
Total	23	17377.07842			

Table E.26. Leaf area- leaf NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	436.800	436.800	6.26	0.0254
treatments	5	6967.886	1393.577	19.97	<.0001
replicates	3	729.176	243.058	3.48	0.0447
Error	14	977.175	69.798		
Total	23	17377.078			

Table E.27. Leaf area- leaf NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	23.006	23.006	0.22	0.6493
treatments	5	4865.914	973.182	9.16	0.0006
replicates	3	494.710	164.903	1.55	0.2482
Error	13	1380.621	106.201		
Total	22	13605.300			

Table E.28. Leaf area-soil K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	43.517	43.517	0.44	0.5158
treatments	5	5569.283	1113.856	11.38	0.0002
replicates	3	336.282	112.094	1.15	0.3652
Error	14	1370.459	97.889		
Total	23	17377.078			

Table E.29. Leaf area-soil NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	106.517	106.517	1.14	0.3036
treatments	5	12116.222	2423.244	25.95	<.0001
replicates	3	582.347	194.115	2.08	0.1491
Error	14	1307.458	93.389		
Total	23	17377.078			

Table E.30. Leaf area-soil NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	76.622	76.622	0.80	0.3856
treatments	5	7431.004	1486.200	15.56	<.0001
replicates	3	403.919	134.639	1.41	0.2815
Error	14	1337.353	95.525		
Total	23	17377.078			

Table E.31. Lesion area- leaf Fe

Source	d.f.	S.S.	m.s.	F	Sig.
Fe	1	0.006	0.006	0.30	0.5906
treatments	5	1.816	0.363	18.34	<.0001
replicates	3	0.006	0.002	0.10	0.9576
Error	14	0.277	0.019		
Total	23	2.200			

Table E.32. Lesion area- leaf Mn

Source	d.f.	S.S.	m.s.	F	Sig.
Mn	1	0.075	0.075	5.05	0.0412
treatments	5	1.665	0.333	22.40	<.0001
replicates	3	0.040	0.013	0.90	0.4644
Error	14	0.208	0.014		
Total	23	2.200			

Table E.33. Lesion area- leaf Cu

Source	d.f.	S.S.	m.s.	F	Sig.
Cu	1	0.000	0.000	0.00	0.9852
treatments	5	1.880	0.376	18.59	<.0001
replicates	3	0.019	0.006	0.32	0.8105
Error	14	0.283	0.020		
Total	23	2.200			

Table E.34. Lesion area- leaf Zn

Source	d.f.	S.S.	m.s.	F	Sig.
Zn	1	0.000	0.000	0.01	0.9177
treatments	5	1.671	0.334	16.53	<.0001
replicates	3	0.009	0.003	0.16	0.9246
Error	14	0.283	0.020		
Total	23	2.200			

Table E.35. Lesion area- leaf Ca

Source	d.f.	S.S.	m.s.	F	Sig.
Ca	1	0.006	0.006	0.34	0.5691
treatments	5	1.736	0.347	17.58	<.0001
replicates	3	0.026	0.008	0.45	0.7203
Error	14	0.276	0.019		
Total	23	2.200			

Table E.36. Lesion area- leaf Mg

Source	d.f.	S.S.	m.s.	F	Sig.
Mg	1	0.057	0.057	3.58	0.0792
treatments	5	1.699	0.339	21.10	<.0001
replicates	3	0.030	0.010	0.63	0.6072
Error	14	0.225	0.016		
Total	23	2.200			

Table E.37. Lesion area- leaf P

Source	d.f.	S.S.	m.s.	F	Sig.
P	1	0.002	0.002	0.14	0.7117
treatments	5	1.654	0.330	16.51	<.0001
replicates	3	0.022	0.007	0.37	0.7778
Error	14	0.280	0.020		
Total	23	2.200			

Table E.38. Lesion area-leaf K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	0.027	0.027	1.53	0.2362
treatments	5	1.918	0.383	21.03	<.0001
replicates	3	0.045	0.015	0.84	0.4943
Error	14	0.255	0.018		
Total	23	2.200			

Table E.39. Lesion area-leaf NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	0.008	0.008	0.42	0.5264
treatments	5	0.770	0.154	7.84	0.0011
replicates	3	0.026	0.008	0.45	0.7245
Error	14	0.275	0.019		
Total	23	2.200			

Table E.40. Lesion area-leaf NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	0.008	0.008	0.50	0.4908
treatments	5	0.799	0.159	8.97	0.0007
replicates	3	0.018	0.006	0.35	0.7917
Error	13	0.231	0.017		
Total	22	2.150			

Table E.41. Lesion area-soil K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	0.018	0.018	0.95	0.3457
treatments	5	1.719	0.343	18.14	<.0001
replicates	3	0.033	0.011	0.58	0.6364
Error	14	0.265	0.018		
Total	23	2.200			

Table E.42. Lesion area-soil NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	0.020	0.020	1.09	0.3141
treatments	5	1.559	0.311	16.61	<.0001
replicates	3	0.041	0.013	0.73	0.5514
Error	14	0.262	0.018		
Total	23	2.200			

Table E.43. Lesion area-soil NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	0.015	0.015	0.78	0.3911
treatments	5	0.635	0.127	6.63	0.0023
replicates	3	0.010	0.003	0.19	0.9031
Error	14	0.268	0.019		
Total	23	2.200			

APPENDIX F

Statistical Tables for Chapter Six

Tables F.1-F13. ANCOVA tables for lesion area of cucumbers in the first sampling with soil and leaf nutrients, as covariates (Chapter 6, Table 6.3).

Table F.1. Lesion area-leaf Fe

Source	d.f.	S.S.	m.s.	F	Sig.
Fe	1	0.000	0.000	0.00	0.9892
treatments	5	0.158	0.031	6.10	0.0034
replicates	3	0.024	0.008	1.55	0.2451
Error	14	0.072	0.005		
Total	23	0.282			

Table F.2. Lesion area-leaf Mn

Source	d.f.	S.S.	m.s.	F	Sig.
Mn	1	0.000	0.000	0.00	0.9549
treatments	5	0.149	0.029	5.77	0.0043
replicates	3	0.034	0.011	2.20	0.1339
Error	14	0.072	0.005		
Total	23	0.282			

Table F.3. Lesion area- leaf Cu

Source	d.f.	S.S.	m.s.	F	Sig.
Cu	1	0.034	0.034	12.58	0.0032
treatments	5	0.118	0.023	8.67	0.0006
replicates	3	0.066	0.022	8.04	0.0023
Error	14	0.038	0.002		
Total	23	0.282			

Table F.4. Lesion area- leaf Zn

Source	d.f.	S.S.	m.s.	F	Sig.
Zn	1	0.006	0.006	1.44	0.2500
treatments	5	0.168	0.033	7.14	0.0016
replicates	3	0.017	0.005	1.23	0.3362
Error	14	0.065	0.004		
Total	23	0.282			

Table F.5. Lesion area- leaf Ca

Source	d.f.	S.S.	m.s.	F	Sig.
Ca	1	0.012	0.012	3.00	0.1053
treatments	5	0.184	0.036	8.61	0.0007
replicates	3	0.034	0.011	2.66	0.0889
Error	14	0.059	0.004		
Total	23	0.282			

Table F.6. Lesion area- leaf Mg

Source	d.f.	S.S.	m.s.	F	Sig.
Mg	1	0.003	0.003	0.68	0.4250
treatments	5	0.173	0.034	7.02	0.0018
replicates	3	0.040	0.013	2.72	0.0845
Error	14	0.069	0.004		
Total	23	0.282			

Table F.7. Lesion area- leaf P

Source	d.f.	S.S.	m.s.	F	Sig.
P	1	0.000	0.000	0.11	0.7505
treatments	5	0.160	0.032	6.23	0.0031
replicates	3	0.018	0.006	1.21	0.3408
Error	14	0.072	0.005		
Total	23	0.282			

Table F.8. Lesion area- leaf K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	0.006	0.006	1.29	0.2748
treatments	5	0.178	0.035	7.49	0.0013
replicates	3	0.039	0.013	2.76	0.0810
Error	14	0.066	0.004		
Total	23	0.282			

Table F.9. Lesion area- leaf NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	0.000	0.000	0.08	0.7810
treatments	5	0.129	0.025	5.00	0.0078
replicates	3	0.024	0.008	1.55	0.2455
Error	14	0.072	0.005		
Total	23	0.282			

Table F.10. Lesion area- leaf NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	0.011	0.011	2.61	0.1286
treatments	5	0.178	0.035	8.17	0.0009
replicates	3	0.048	0.016	3.67	0.0385
Error	14	0.061	0.004		
Total	23	0.282			

Table F.11. Lesion area-soil K

Source	d.f.	S.S.	m.s.	F	Sig.
K	1	0.000	0.000	0.07	0.7887
treatments	5	0.129	0.025	5.01	0.0077
replicates	3	0.014	0.004	0.96	0.4385
Error	14	0.072	0.005		
Total	23	0.282			

Table F.12. Lesion area-soil NH_4N

Source	d.f.	S.S.	m.s.	F	Sig.
NH_4N	1	0.007	0.007	1.68	0.2159
treatments	5	0.106	0.021	4.60	0.0108
replicates	3	0.042	0.014	3.04	0.0643
Error	14	0.064	0.004		
Total	23	0.282			

Table F.13. Lesion area-soil NO_3N

Source	d.f.	S.S.	m.s.	F	Sig.
NO_3N	1	0.002	0.002	0.40	0.5352
treatments	5	0.172	0.034	6.83	0.0020
replicates	3	0.034	0.011	2.28	0.1240
Error	14	0.070	0.005		
Total	23	0.282			

Tables F.14-F.26. ANOVA tables for nutrients in leaves and soil of cucumbers treated with six potassium fertilization treatments (Chapter 6, section 6.3.1).

Table F.14. Leaf Fe

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	305.091	61.018	0.71	0.6277
replicates	3	2424.916	808.305	9.36	0.0010
Error	15	1295.936	86.395		
Total	23	4025.944			

Table F.15. Leaf Mn

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	93.671	18.734	4.45	0.0110
replicates	3	119.537	39.845	9.46	0.0009
Error	15	63.169	4.211		
Total	23	276.378			

Table F.16. Leaf Cu

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	6.929	1.385	1.76	0.1822
replicates	3	20.210	6.736	8.55	0.0015
Error	15	11.823	0.788		
Total	23	38.963			

Table F.17. Leaf Zn

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	7.007	1.401	0.60	0.6982
replicates	3	126.103	42.034	18.11	<.0001
Error	15	34.818	2.321		
Total	23	167.929			

Table F.18. Leaf Ca

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	1.846	0.369	1.60	0.2208
replicates	3	0.443	0.147	0.64	0.6011
Error	15	3.467	0.231		
Total	23	5.757			

Table F.19. Leaf Mg

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	0.053	0.010	2.32	0.0950
replicates	3	0.033	0.011	2.41	0.1076
Error	15	0.069	0.004		
Total	23	0.156			

Table F.20. Leaf K

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	0.867	0.173	1.74	0.1873
replicates	3	1.081	0.360	3.61	0.0385
Error	15	1.500	0.100		
Total	23	3.450			

Table F.21. Leaf P

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	0.008	0.001	1.04	0.4281
replicates	3	0.046	0.015	9.28	0.0010
Error	15	0.025	0.001		
Total	23	0.080			

Table F.22. Leaf NH_4N

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	1466.504	293.300	2.10	0.1218
replicates	3	2399.959	799.986	5.73	0.0081
Error	15	2093.851	139.590		
Total	23	5960.315			

Table F.23. Leaf NO_3N

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	27368.668	5473.733	4.64	0.0093
replicates	3	10718.332	3572.777	3.03	0.0623
Error	15	17705.996	1180.399		
Total	23	55792.997			

Table F.24. Soil K

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	329350.774	65870.154	37.64	<.0001
replicates	3	32053.832	10684.610	6.10	0.0063
Error	15	26253.363	1750.224		
Total	23	387657.969			

Table F.25. Soil NH_4N

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	1142.954	228.590	5.35	0.0051
replicates	3	374.791	124.930	2.92	0.0681
Error	15	640.766	42.717		
Total	23	2158.512			

Table F.26. Soil NO_3N

Source	d.f.	S.S.	m.s.	F	Sig.
treatments	5	79.463	15.892	0.31	0.9022
replicates	3	30.826	10.275	0.20	0.8966
Error	15	781.165	52.077		
Total	23	891.455			

APPENDIX G

Statistical Tables for Chapter Seven

Tables G.1-G.8. ANOVA tables for lesion and leaf area of cucumbers treated with two nitrogen and three potassium levels in four samplings (Chapter 7).

Table G.1. Lesion area – first sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	0.094	0.031	0.63	0.6095
K	2	1.637	0.818	16.32	0.0002
N	1	0.814	0.814	16.24	0.0011
K*N	2	42.029	21.014	418.88	<.0001
Error	15	0.752	0.050		
Total	23	45.327			

Table G.2. Lesion area – second sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	5.050	1.683	0.44	0.7285
K	2	263.377	131.688	34.34	<.0001
N	1	8.867	8.867	2.31	0.1492
K*N	2	925.610	462.805	120.68	<.0001
Error	15	57.524	3.834		
Total	23	1260.429			

Table G.3. Lesion area – third sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	24.781	8.260	0.84	0.4940
K	2	54.446	27.223	2.76	0.0953
N	1	1616.743	1616.743	163.96	<.0001
K*N	2	5086.601	2543.300	257.93	<.0001
Error	15	147.908	9.860		
Total	23	6930.480			

Table G.4. Lesion area – fourth sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	102.344	34.114	1.17	0.3533
K	2	589.366	294.683	10.12	0.0016
N	1	6954.774	6954.774	238.95	<.0001
K*N	2	12601.518	6300.759	216.48	<.0001
Error	15	436.575	29.105		
Total	23	20684.579			

Table G.5. Leaf area–first sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	2538.675	846.225	6.47	0.0050
K	2	1979.154	989.577	7.57	0.0053
N	1	1119.750	1119.750	8.57	0.0104
K*N	2	444.700	222.350	1.70	0.2158
Error	15	1960.600	130.706		
Total	23	8042.881			

Table G.6. Leaf area–second sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	139.587	46.529	0.86	0.4822
K	2	3386.557	1693.278	31.37	<.0001
N	1	78.498	78.498	1.45	0.2465
K*N	2	1231.193	615.596	11.40	0.0010
Error	15	809.739	53.982		
Total	23	5645.576			

Table G.7. Leaf area–third sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	184.381	61.460	1.82	0.1863
K	2	4691.094	2345.547	69.56	<.0001
N	1	2766.751	2766.751	82.05	<.0001
K*N	2	383.203	191.601	5.68	0.0146
Error	15	505.785	33.719		
Total	23	8531.215			

Table G.8. Leaf area–fourth sampling.

Source	d.f.	S.S	m.s.	F	Sig.
replicates	3	333.660	111.220	0.73	0.5479
K	2	6678.492	3339.246	22.03	<.0001
N	1	9281.375	9281.375	61.23	<.0001
K*N	2	4075.741	2037.870	13.45	0.0005
Error	15	2273.562	151.570		
Total	23	22642.831			

Tables G.9-G.21. ANOVA tables for soil and leaf nutrients of cucumbers treated with two nitrogen and three potassium levels (Chapter 7).

Table G.9. Leaf Fe

Source	d.f	S.S.	m.s.	F	Sig.
replicates	3	519.649	173.216	0.44	0.7301
K	2	2391.564	1195.782	3.01	0.0794
N	1	655.169	655.169	1.65	0.2183
K*N	2	30282.551	15141.275	38.16	<.0001
Error	15	5951.845	396.789		
Total	23	39800.780			

Table G.10. Leaf Mn

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	97.289	32.429	0.86	0.4831
K	2	169.243	84.621	2.24	0.1404
N	1	91.615	91.615	2.43	0.1399
K*N	2	346.696	173.348	4.60	0.0277
Error	15	565.589	37.705		
Total	23	1270.433			

Table G.11. Leaf Cu

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	347.193	115.731	2.79	0.0763
K	2	1434.621	717.310	17.31	0.0001
N	1	2554.133	2554.133	61.65	<.0001
K*N	2	1749.759	874.879	21.12	<.0001
Error	15	621.437	41.429		
Total	23	6707.145			

Table G.12. Leaf Zn

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	56.277	18.7593	2.98	0.0648
K	2	48.283	24.1419	3.84	0.0451
N	1	3.367	3.3672	0.54	0.4757
K*N	2	54.551	27.2756	4.33	0.0327
Error	15	94.384	6.292		
Total	23	256.862			

Table G.13. Leaf Ca

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	0.169	0.056	1.27	0.3203
K	2	0.053	0.026	0.60	0.5589
N	1	0.861	0.861	19.35	0.0005
K*N	2	4.940	2.470	55.50	<.0001
Error	15	0.667	0.044		
Total	23	6.692			

Table G.14. Leaf Mg

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	0.006	0.002	1.74	0.2016
K	2	0.005	0.002	2.15	0.1510
N	1	0.002	0.002	2.14	0.1644
K*N	2	0.007	0.003	3.09	0.0753
Error	15	0.019	0.001		
Total	23	0.041			

Table G.15. Leaf K

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	0.907	0.302	1.59	0.2336
N	1	0.000	0.000	0.00	0.9741
K	2	0.728	0.364	1.92	0.1817
N*K	2	0.805	0.402	2.12	0.1550
Error	15	2.854	0.190		
Total	23	5.297			

Table G.16. Leaf P

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	0.015	0.005	3.48	0.0426
K	2	0.003	0.001	1.04	0.3775
N	1	0.041	0.041	28.08	<.0001
K*N	2	0.010	0.005	3.46	0.0581
Error	15	0.022	0.001		
Total	23	0.0921			

Table G.17. Leaf NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	639.572	213.190	1.73	0.2044
K	2	363.696	181.848	1.47	0.2606
N	1	570.449	570.449	4.62	0.0483
K*N	2	1366.483	683.241	5.53	0.0159
Error	15	1852.218	123.481		
Total	23	4792.421			

Table G.18. Leaf NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	2690.316	896.772	11.49	0.0004
K	2	555.930	277.965	3.56	0.0543
N	1	2761.776	2761.776	35.38	<.0001
K*N	2	1159.025	579.512	7.42	0.0057
Error	15	1171.041	78.069		
Total	23	8338.091			

Table G.19. Soil NH_4-N

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	67.724	22.574	3.66	0.0368
K	2	71.179	35.589	5.77	0.0138
N	1	16.947	16.947	2.75	0.1181
K*N	2	14.073	7.036	1.14	0.3456
Error	15	92.472	6.164		
Total	23	262.397			

Table G.20. Soil NO_3-N

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	184.453	61.484	5.03	0.0131
K	2	97.813	48.906	4.00	0.0404
N	1	1194.173	1194.173	97.75	<.0001
K*N	2	20.684	10.342	0.85	0.4484
Error	15	183.245	12.216		
Total	23	1680.369			

Table G.21. Soil K

Source	d.f.	S.S.	m.s.	F	Sig.
replicates	3	6683.445	2227.815	2.79	0.0767
K	2	43792.480	21896.240	27.40	<.0001
N	1	348.221	348.221	0.44	0.5192
K*N	2	551.980	275.990	0.35	0.7134
Error	15	11985.470	799.031		
Total	23	63361.598			

APPENDIX H

ABSTRACT SUBMITTED TO THE EUROPEAN JOURNAL OF PLANT PATHOLOGY

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Manuscript Number: EJPP1166

Title: Effect of nitrogen fertilization on cucumber downy mildew disease

Article Type: Original Research (full papers)

Keywords: inoculation; lesion area; nutrients; *Pseudoperonospora cubensis*

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Abstract:

The objective of this study was to examine the effect of six nitrogen fertilization treatments on the downy mildew development on cucumber plants. The treatments were 100-600 ppm nitrogen in the irrigation solution, which was given to plants grown in pots in a greenhouse. The cucumber leaves were inoculated with a zoospore suspension of the pathogen Pseudoperonospora cubensis. The experimental data indicate that 300 ppm nitrogen in the irrigation solution had a positive effect on the leaf area and negative effect on the lesion area. This might be an indication that this concentration has a suppressive effect on disease development. The results also indicated that the disease progress for downy mildew, regardless of N treatments, followed a cubic curve. Furthermore, the NH4-N and NO3-N in leaves and soil produced statistical significant differences due to treatments. One interesting observation was that P concentration in leaves in the 300 ppm N treatment had the highest internal concentration and was significantly different from all other treatments.