CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

DIPLOMA THESIS

2008 **Mrs. Alya Duzan, Bc.**

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

INSTITUTE OF TROPICS AND SUBTROPICS

Thesis Title: **LATE BLIGHT ON TOMATO**

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

INSTITUTE OF TROPICS AND SUBTROPICS

Department: Crop Production and Agro-Forestry **Academic year: 2005/06**

SETTING M.Sc. THESIS

For Mr. /Mrs.

Mrs Alya H.B. Duzan, Bc.

Course: Agriculture in Tropics and Subtropics

This thesis is required by the Head of Department as laid down in the rules of the Master studies at CZU.

Thesis Title:

Late Blight on Tomato

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Code for compiling this M.Sc. diploma thesis

1. Introduction
1.1. Importance of the tomato
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Extent of the Thesis report: 40 – 60 pages

Bibliography:

CIPOTATO: On-line manual for *Phytophthora* i*nfestans* laboratory work. GILLB, International Potato Centre (CIP), 1996 – 2002, last modified: 5.4. 2002-04-07. http://www.cipato.org/gilb/Pubs/Manual GF- Pdf.
ERWIN, D.C., RIBEIRO, K., Phytophthora Diseases Worldwide. APS PRESS, 1996,

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Declaration

I declare, that this M.Sc. Thesis work out my self according rules of the Master studies at CZU after Code for compiling this diploma thesis under guidance of supervisors.

Name of author: .Mrs Alya H.B. Duzan, BSc. Signature:……

Prague 26.04.2008

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To my parents

To my husband

To My children

CONTENTS

Late Blight on Tomato

1. Introduction

1.1. Importance of the tomato

The tomato plants can be grown in a fairly wide range of soil and climatic conditions, and they are also suitable for container growing. The cultivated tomato belongs to the gnus *Solanum* (*Solanum lycopersicum*) and *Lycopersicon* (*Lycopersicon esculentum* Mill.) is recently adopted food crop that has achieved prominence and popularity largely in the past centure. Tomato is one of the most widely produced fruit vegetable from the family Solanaceae.

Fruit is valued for its high nutrition, and the crop is grown throughout the world. Tomato originated in South America, mountainous region of the Andes in Peru, Ecuador and Chile. Domestication and cultivation of the tomato outside its center of origin appear to have first occurred in early civilization of Mexico. Field-grown tomatoes are widely grown in warm temperate and tropical climates, and glasshouse tomatoes are produced in many additional regions. There is a little commercial, outdoor production in northern Europe, although tomatoes are grown outdoors in gardens. A wide diversity of fruits types is available, including large red fruit “beefsteak”, small cherry, yellow pear, and others. Tomato is grown for fresh market produce as well as a range of processed products such as tomato juice, canned peeled tomatoes, tomato purée and sauce, ketchup, soups, and sun-dried tomatoes (Koike et al. 2007).

World tomato production and consumption has risen dramatically in the past four decades to more than 60 million metric tons in 1985. World production tomatoes according (Jones, Jr., 2008) tomatoes remains a minor crop of luxury status in many areas of the world, and substantial opportunity exists to further increase its contributions to human nutrition and well-being (Table 1).

1.2. Importance of Late blight Tomato

Late blight is a highly destructive disease affecting both tomato and potato. This disease was first described on potato in 1845 and on tomato in 1847. The disease is responsible for the infamous Irish potato famine of 1840s. Late blight can be a very serious disease on tomatoes, particularly when the weather is consistently cool and rainy. Patogen attacks all aboveground parts of the tomato plant. Late blight is caused by *Phytophthora infestans*

((Mont) de Bary from the Kingdom Chromista contains a large group Oomycota of major plant pathogens. Disease control requires constant plant observation and evaluation, as new strains appear from introduction of disease organisms from outside sources. For example, an outbreak of a new strain of the late blight pathogen (*P. infestans*) introduced from Mexico, posed, a serious problem for the tomato industry in any place of the world, and required specific control measures by growers (Fry et al 1993).

The cultivated tomato (*Lycopersicon esculentum* Mill.) is a recently adopted food crop that have achieved prominence and popularity largely in the 20s century. Its versatility in fresh or processed form and its adaptability have plaid major roles in its rapid and widespread use. Word tomato production and consumption has risen dramatically in the past three decades to more than 60 million metric tons in 1985 (Table 1).

Tab 1. World Production of Tomatoes[a]

	Area (10^3 ha)	Production (10^6 t)	Yield (t/ha)	Consumption per Capita (kg)
World	2,588	60.8	23.5	12.6
Africa	445	6.0	13.6	10.8
North and Central America	311	10.8	34.8	26.9
South America	133	3.4	25.7	12.7
Asia	798	15.2	19.0	5.4
Europe	506	18.1	35.8	36.8
Czech Republic[b]	0.7	0.024	30.7	27.2
Oceania	15	0.3	23.5	15.0
USSR or Russia	380	6.9	18.1	24.6
Developed countries	1,108	35.3	31.9	29.2
Developing countries	1,480	25.5	17.2	7.0

[a]Data from Food and Agriculture Organization, 1985 Agriculture Yearbook, United Nations, Rome. Data[b] 2005.

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posed, a serious problem for the tomato industry in any place of the world, and required specific control measures by growers.

1.3. Aim of MS diploma thesis

The aim of this study was isolation of causal agent of late blight of tomato/potato *Phytophthora infestans* in pure culture, determine mating types A1, A2 and test a seed-born of the causal agent by the seeds of tomato their origin are from tomato fruit with the severity symptoms of the late blight disease.

2. Literature

2.1. Origin of tomato

The tomato and its close relatives have their center of origin in a narrow, elongated, mountainous region of the Andes in Peru, Ecuador, and Chile. These primitive relatives of the tomato occupy many diverse and distinct environments and represent an almost in exhaustible gene pool for the improvement of the species.

2.1.1. Plant characteristics and biology of tomato

Cultivated forms of the tomato are self-pollinating, tender, herbaceous perennials almost universally cultivated as an annual crop. Under suitable growing conditions the perennial forms a deep-branching taproot, which may forage the soil profile to depths of 1.2m or more. It is commonly classified as a tender, warm-season vegetable crop, with an optimum mean temperature for growth in the range of 21-23ºC. Growth and development stop at temperature below 10ºC. The tomato flower is perfect, with functional male and female parts. Cultivated varieties form a tight, protective another cone surrounding the stigma, which leads predominantly to self-fertilization. After pollination and fertilization, fruit growth occurs through cell division followed by cell enlargement. Several (usually 4-8) flowers are borne on each cluster “compound inflorescence”, and a single indeterminate plant may produce, under greenhouse conditions, as many as 20 clusters during its cropping cycle. The period of time from pollination to fruit ripening varies from less than 6 weeks to more than one week, depending upon the variety and temperatures.

A tomato fruit consists of 94-95 % water; the remaining 5-6% is a complex mixture of predominantly organic constituents, sugars and organic acids are the primary determinants of tomato flavor; however, fruit texture and other complex flavor. The growing environment may markedly influence growth rate, fruit-set, yield, and fruit quality. Tomato improvement efforts of the past four decades have resulted in *cultivars* for a variety of environments,

methods of production, and fruit use. A major focus of this effort has been the development of cultivars resistant to prevalent diseases (Jones et al. 1991). Jones, Jr. (2008) presented that, has been made considerable progress in breeding disease resistance or tolerance to the more commonly occurring tomato plant diseases and viruses, such as the *Verticillium* and *Fusarium* wilts, tobacco mosaic virus, tomato mosaic virus, *Alternaria* stem canker, *Stemphylium* gray spot, *Septoria* leaf spot, and bacterial speck (*Pseudomonas*).

Breeding over the past 50 years has substantially changed the tomato plant and its fruit characteristics. Varieties available today for use by both the commercial and home gardener have a vide range of plant characteristics. They are resistant to many of the blight and wilt diseases that affect tomatoes. They are specifically adapted to a particular set of growing conditions, such as tropical temperatures, field and greenhouse conditions, and fresh market versus processing tomato-type fruit. Days to maturity range from about 60 to more than 95 days, although several 45 days determine varieties have been introduced for use in northern latitudes. Fruit size, color, texture, and acidity can be selected by variety, whether adapted to field or greenhouse conditions, and for long- and short-day suitability. Genetic engineering techniques conditions applied to tomato breeding have been used to produce fruit with a long shelf life, resistance to bruising, and high lycopene content.

The commercial production of tomatoes in the tropics offers a unique challenge in terms of varieties that can withstand high temperatures and disease and insect pressures. The Asian Vegetable Research and Development Centre (AVRDC) have an active breeding programme to select cultivars that are adapted to the tropical environment (http:/www.awrdc.org).

2.1.2. Botany systems of the tomato

Tomato is a member of the economically important botanical family Solanaceae. This group includes several widely cultivated plants (potato, tomato, tobacco, pepper and eggplant), which have had a long and interesting association with humankind. Taxonomic classification has been the subject of much recent discussion, as new information and collections of the vast diversity in the genus have forced a reassessment of earlier taxonomic treatments. The cultivated tomato is most commonly referred to as *Lycopersicon esculentum* Mill.; however alternative names (*Solanum lycopersicon* L. and *L. esculentum* (L.) Karsten) have also been used. The current scientific classification for tomato is:

Kingdom: Plantae

Subkingdom: Tracheobionia

Division Magnoliopsida

Subclass: Asteridae

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *Solanum lycopersicum*

Binomial name: *Solanum lycopersicon*

Genus: *Lycopersicon esculentum,* tom*ato; Lycopersicon pimpinellifolium,* currant tomato; *Lycopersicon esculentum* var. *cerasiforme,* cherry tomato; *Lycopersicon esculentum* var. *pyriforme,* pear tomato; *Lycopersicion esculentum* var. *grandifium,* potato-leaved tomato; *Lycopersicon esculentum* var. *validum,* upright tomato.

The botanical classification of the tomato has had an interesting history, first being placed in the genus *Solanum,* along with the potato, and being identified as *Solanum lycopersicon.* However, this designation was changed to *Lycopersicon esculentum*, its simple meaning being "edible". Although there are similar characteristics between potato and tomato plants, it is the flower color (yellow for tomato and mostly white or violet for potato) and particularly the shape and opening of the pollen-bearing structures that are the characteristics that separate the two plants.

Nomenclature debate results from a departure from the rules of International Code of Botanical Nomenclature in arriving at the widely use name, *L. esculentum,* proposed by Miller in 1768 to replace the Linnean name, *S. lycopersicon.* According to convention, the species name *lycopersicum* should have been retained following acceptance of the new genus *Lycopersicon*; however, in order avoid further confusion in nomenclature, many systematists have argued for tradition rather than strict convention in naming the cultivated tomato and have proposed the adoption of the long-used nomenclature, *L. esculentum*. Current evidence suggests the genus *Lycopersicon* consists of eight species differing in degree of relatedness to the cultivated tomato.

2.1.3. Tomato products

The United Nations Food and Agriculture Organization (FAO) reported that a good commercial yield of tomatoes under irrigation ranges between 45 and 65 tons/ha. Since

tomato fruit statistics are quickly out of date, their publication in book form seems questionable and therefore of relatively little value. Based on the FAO world data for 2004 (FAO Statistical Yearbook, Rome Italy), tomato fruit both fresh market and processing data are being reported by 144 countries. The total acreage is approximately 2.8 million ha with yearly worldwide fruit production of 84.7 million metric tons.

Table 2. The 10 leading fruit-producing countries

Country	**Area harvested (ha)**	**Fruit production (metric tons**
China	1 255 100	30 142 000
India	549 000	7 600 000
Turkey	220 000	8 000 000
Egypt	191 000	6 780 000
United States	172 810	12 766 000
Italy	135 000	7 496 997
Iran	130 000	4 200 000
Nigeria	127 000	889 000
Spain	70 500	4 366 500
Mexico	67 084	2 148 130

Source: FAO Statistical (FAO Stat) Database results (http://faostat.fao.org/).

2.1.3.1. Consumer consumption and preferences

Consumers today are more educated, demanding high quality products. Consumer preference criteria when selecting a particular tomato fruit can be divided into four categories: quality, variety, size, and package type. The four fruit categories for tomatoes are gourmet, premium, mainstream, and bargain (Jones et al. 1991).

2.1.3.2. Tomato production: field-versus greenhouse-grown fruit

The standard fresh market tomato is the “mature green fruit” fruit that is picked in the field at this stage of maturity and either allowed to naturally ripen while in transit to the market or ripened by exposure to ethylene (C_2H_4) gas. Field-grown fruit is normally marketed in bulk and is almost entirely beefsteak varieties.

For greenhouse-grown fruit, the fruit can be picked at the vine-ripe stage and normally packaged three to four fruit per pack in clam shells. There are many tomato fruit types-beefsteak (fewer are being produced), grape, cherry, plum, and tomato-on-the-vine (TOV), frequently referred to as cluster tomatoes. There are two primary fruit colors: red, the major color, and yellow; more yellow fruit is beginning to enter the marketplace, particularly for TOV varieties. Roma varieties are also gaining in popularity among consumers, both field and greenhouse grown. It has been determined that the smaller fruit types have grater flavor, thus explaining their increased consumer preference in the marketplace.

The competition between field and greenhouse-grown fruit continues, and the majority of fresh market fruit is being produced some distance from the point of sale. The question of quality between field- and greenhouse-grown fruit is of major importance for the future of the greenhouse tomato industry.

Fruit from soil-grown field plants can be coated with soil or dust particles, which although removed by washing prior to placement in the market, can affect shelf life. Normally the shelf life of greenhouse-grown fruit is better than that of field-grown fruit, which may be due to the fact that some soil residue remains of the fruit. The degree of bruising of the fruit in harvesting and handling also contributes to shelf life.

2.1.4. Cultural systems

The tomato plant can be grown successfully in a range of environmental settings and rooting systems both in the field and in greenhouses. Tomato plants can grown equally well in soil, modified soil, soilless mixes, organic substrates, and hydroponically in troughs, or in bags or bucket of sand, gravel, perlite, pine bark, or rockwool slabs (Jones,Jr. 2005).

2.1.4.1. Field production

Tomato plants grow best in well-drained, fertile, slightly acid (pH 6 to 6.8) loam type soils. The tomato plant will not grow well in soils that are either very acid (pH less than 5.6) or alkaline (pH greater than 7.5), or soils that are not adequately fertilized, as tomato is considered to have a “medium” to “high” nutrient. For the production of quality fruit, both nitrogen and potassium fertilization must be carefully managed. Tomato plants do not grow-well in either heavy textured soils, in sandy soils unless well irrigated, or in shallow surface soils (tomato plant roots penetrate the soil to a depth of 1,5m when unrestricted).

The tomato plants grow best in those climatic zones where air temperatures range between (18.3˚C to 32.2˚C) during the growing season. The tomato plant cannot tolerate frost and blossom drop will occur when the air temperature, particularly at night, drops below 12.7˚C. In addition, high air temperatures, greater than 35˚C, reduce fruit set and inhibits development of normal fruit color. The growing site should be open for easy air best site position is one in which there is air movement. The tomato plant tends to do better in relative dry air conditions, as high relative humidity tends to be associated with both insect and disease problems.

The tomato plant has some degree tolerance to soil salinity, which is a growing problem world vide, resulting from over-fertilization or use of saline (brackish) irrigation water. A soil salinity measurement (EC) of less 2,5 (dS/m) will not affect plant growth. The greatest impaction, to keep the plants fully turgid at all times. Irrigation water that has an EC greater than 3.0 dS/m is considered unacceptable for irrigation vegetable crops, tomato included (Jones, Jr. 2008).

The ideal moisture condition is one in which plant receive sufficient water, either by rainfall or irrigation, to keep the plants fully turgid at all times. Water used measured as evapotranspiration under optimum growing conditions is between 5 and 6 mm/day. The tomato plant cannot tolerate water-saturated soil condition.

2.1.4.2. Greenhouse production

Keeping a greenhouse tomato plant in profitable production over an extended period of time requires considerable management skill. This includes:

The growing of tomato plants in partially or completely enclosed shelters is widely practiced. Greenhouse tomato production in precisely controlled environments is increasing in many parts of the world (Jensen and Malter, 1995). In these controlled environments, hydroponics is the primary means used for supplying the tomato plant with its water and nutrient needs (Jones, Jr., 2005, Resh, 2001).

Various systems of plant culture in the greenhouse are in the testing and evaluation stage. In one system, for example, after the setting of four to five fruit clusters, the plant is topped to stop any further stem growth. By topping, all the generated photosynthetic goes to the fruit already set, which results in large fruit. Giacomelli et al. (1993) describe a single truss system in which the plant is topped after the first fruit cluster is set and the plant is replaced after the fruit is harvested. Currently the success of the single truss system is based on the ability of each plant to produce at least 900 – 1000g of fruit. This system of fruit production allows for a unique design in terms of the growing method (flood and drain hydroponics) on moving

trays of plants, trays that are brought to the workers on an automated tray movement systems. With the systems, greater utilization of the greenhouse growing space is obtained. Other types of cluster systems are described by Logenfra et al. (2001).

In the greenhouse, the tomato plant can be kept productive for 6 to 9 months or even longer.

- Training the plant up a support wire.
- Prompt removal of leaf axial suckers and vegetative stems from the fruiting truss.
- Leaf pruning of lower leaves.
- Flower pollination.
- Fruit thinning on the truss.
- Lowering the growing plant.

By training the plant up a vertical supporting twine, removing older leaves as the lower fruit clusters are harvested, and lowering the main plant stem that keeps the whole plant upright within easy reach of workers, a continuous production of fruit can be maintained. This process can be sustained as long as the plant is kept actively growing and free from disease and other stress. In the greenhouse, it is possible to control the environment and those factors that affect the plant´s well being, thus keeping tomato plant productive over a long period of time.

2.1.4.3. Home gardening

The number of garden vegetable is tomato, with some home gardeners growing just a few tomato plants to produce sufficient fruit to eat or can during the growing season (vegetation period). The home gardener has several options for growing tomato plants: a) in a soil garden plot, b) among flowers or other unwegetable plants, or c) in some kind of container. Some may even grow them hydroponically, as has been described by a hobby grower (Schneider, 1998, 2000).

Most home gardeners tend to select older standard varieties and are somewhat reluctant to brink never varieties into their gardens. One of the errors made by home gardeners is to select a variety not well adapted to their growing conditions. Designated heirloom varieties are attracting considerable attention based on a wider selection of fruit characteristics, including high fruit flavor. The only exception would be the Roma or paste-type tomato for making sauce and salsa, and fruit that comes in various shapes (oxhart, plum, pear, long pear, pepper, lemon, and colors (red, red/orange, and yellow) (Jones, Jr. 2008). One of the common errors made by gardeners is to follow advice given for a particular climatic zone or environmental

condition when their garden is not in the same climatic zone or environment. Although the tomato plant has some degree of tolerance to a range of growing conditions, transferring instructions suited to a particular set of growing conditions to another can result in poor performance and a disappointing crop. Home garden tomato success is based on several important factors: the site selected, soil preparation including liming, fertilization, the tomato variety selected, and proper management of the plants during the growing season.

2.1.5. Seed and seedling production

2.1.5.1. Seed characteristics

The tomato seed is 3 to 5mm in size, silky in appearance, flat and light cream to brown in color. It contains a large coiled embryo surrounded by a small amount of endosperm. The weight of an individual seed varies considerably with 300 – 350 seeds weighing 1 gram. Tomato seeds are not mature, and therefore viable, until the tomato fruit is mature. For the home gardener, seed recovered from mature fruit may not come true to the variety due to cross pollination. Mature seed can remain viable for up to 4 years in hermetically sealed containers at seed moisture content of 5.5%. Tomato seeds had passed through the human digestive system and then withstood the sewage treatment processing procedures.

Tomato seed has been produced and sold by seed companies for over 100 years. At first the main function was to increase open-pollinated selections made by tomato growers. Later, during 1950s, were developed new hybrids tomatoes by seed producers for home and market gardeners. Because of the demand for consistently high yields, fruit quality, and disease resistance, large commercial producers also began to use hybrid tomato cultivars, and by 1988 hybrids accounted for an estimated 85% of North American fresh-market tomato production. The production of hybrid tomato seed is a very labor-intensive process. Hand emasculation is performed of each flower by using tweezers to remove the anther cone and petals. Pollen collected from fresh dried flowers of male parent lines is applied to the exposed stigmatic surface of the emasculated female parent flower. Two or three sepals are removed from each pollinated flower to ensure proper identification when the fruit is harvested. As the fruit ripens it is picked and crushed and the seed extracted by hand. In the seed production of open-pollinated tomato cultivars the crop is grown much like a commercial field crop. The seed are carefully inspected and rouged for of any off-types. When the fruit is matured, it is harvested by either hand or machine. Processed tomato cultivars frequently go to a tomato cannery, where the fruit is cleaned, sorted, and crushed and the seed extracted. Fresh-market cultivars are usually field-trashed and brought directly to the seed washer. A short fermentation of 8-24

hr, depending on the temperature, is separate the seed from the surrounding gelatinous matrix. The pulp, skins, undeveloped seed, and other tomato material float off by means of a flume system. Until1970, a longer fermentation of 72-96 hr was as the standard recommendation for control of seed-borne diseases, such as bacterial canker. However, because of premature seed sprouting and continued disease problems, this practice has gradually been replaced by a short acid bath. The seed is then collected and either centrifuged or screened to removed the acid. Sun drying is possible in arid climates; however, artificial heating is more commonly required to reduce the seed moisture content to acceptable levels for packaging. The seed is normally dried to optimum moisture content of 6-8%. After drying, the seed passes through a series of shakers and screens that remove tomato skins, dirt, and undeveloped seeds. At the same time, seed doubles are separated and seeds are sized. A “debearding” machine is used to remove the soft hairs that remain attached to the seed coat. In this way, the final product flows easily through the planters. A gravity table, air columns, or the equipment can be used to further separate the seed into more uniform fractions before packaging. Seed treatments are applied at this stage. Seed samples are taken randomly from each seed lot and evaluated for germination and physical purity. Further testing for seedling vigor, hybrid purity, and seed-borne pathogens is sometimes done. The finished seed is then weighed and packaged into cans, paper, or hermetically sealed foil containers. A label identifying the crop name, cultivars, seed lot, purity, germination percent, and date performed is applied to each package. After information on seed treatments and appropriate warnings are affixed, the seed ready for distribution to growers.

2.1.5.2. Seed production

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on the temperature, is required to separate the seed from the surrounding gelatinous matrix. The denser, mature tomato seed sinks and is collected. Until 1970, a longer fermentation of 72-96 hr was the standard recommendation for control of seed borne diseases, such a bacterial canker. However, because of premature seed sprouting and continued disease problems, this practice has gradually been replaced by a short acid bath. The seed is then collected and either centrifuged or screened to remove the acid. Sun drying is possible in arid climates; however, artificially heating is more commonly required to reduce the seed moisture content to acceptable levels for packaging. The seed is normally dried to optimum moisture content of 6-8 %.

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2.1.5.3. Field transplant production

Tomato seed is sown from February to late April, and plants are produced for shipments within 65 days, depending on cultivar, seedling date, environmental conditions, and management practices. Transplants usually follow a winter cover crop of rye or other crop, which is harrowed and deep-turned with a moldboard plow. The land is prepared by forming raised plant beds with a rotary cultivator and bed shaper so that the beds are 1.5-2.0m wide. Recommended herbicides are pre-plant-incorporated for weed control. Fungicides may be incorporated into the soil for control of Pythim-induced damping-off. Coated tomato seeds are planted with a precision seeder so that they are spaced approximately 1cm apart in double zigzag rows, with four of five double rows per bed. The seeds are planted only a few millimeters deep. Depending upon conditions, the fields can be irrigated three or four times

per day to wet the crust of the soil to ensure that the seedlings emerge properly. Fertilizers are incorporated as recommended (Jones et al. 1991).

The plants are harwested when they attain marketable size, but to maximize plant yield and reduce labor costs, they are clipped several times during the course of the season to even plant growth; in this way, they can be harvested at one time rather than in the multiple harvests. Clipping to increase the uniformity of transplant size also increass the fruit yields in production fields; the greater uniformity allows maximum yields from once-over machine–harvested fields. Clipping results in the production of harder plants with thicker and stronger stems, which may survive transplant shock better than unclipped plants. Clipping allso will deblossom early-flowering plants and promote lateral branching, which again improves plant uniformity and increased yields. Clipping plants fit adequately into the packing crate, so that stem breakage is minimal. The major disadvatage of clipping is the dissemination of plant pahogens. It is an especially serious problem with several of the bacterial pathgens. In general, clipping is started when the plants reach a heigh of 19-20cm. Transplants should be harvested when the foliage is dry and the soil is not too wet. Prior to harvest, the beds are undecut with a steel bar to loosen the roots. The plants are pulled by hand and the soil is removed from the roots, usually by vigor shaking of the plants or slapping of the root against the puller´s leg. They are packed loose, with bare roots, in woodn crates. The bundling of plants and wrapping of roots in wet moos has been discontinued because loosely packed plants separate easily and survive well. In general, the pest control measures used in transplant beds are similar to those used for routine tomato culture. where possible, crop rotation is recommended, but it is often impractical. Standard recommended bactericides, fungicides, insecticides, and nematicides are used for controlling pests.

All transplants produced in this program must be grown from seed that has been test and certified free of plant pathogens. In addition, plants cannot be produces on land following a crop that is a host to the soybean cyst nematode. Once planted, each seed lot is assigned a card identifying its location within the field. Upon emergence, is examined periodically every plant bed by a state inspector until harvest. In general, the inspectors examine overall plant quality and horticultural characteristics (including cultivars identity) and look for any disease symptoms. If disease symptoms are observed and cannot be properly diagnosed in the field, specimens are brought into the laboratory, where trained personnel make a diagnosis. If the plants are deep to be afflicted with a disease or infested with nematodes, they lose their certification.

2.1.5.4. Containerized transplant production

Containerized tomato transplants are an alternative to field-produced transplants and direct seeding for crop establishment. They are generally more expensive than field-produced transplants or direct seeding. In spite of grater initial costs, growers of high-value fresh-market tomatoes tend to use containerized transplants because their rate of survival in the field is a higher production time shorter than that of field-seeded tomatoes. For example: As a broad generalization, direct seeding is used primary for the establishment of processing tomatoes in California, field – grown transplants for processing tomatoes in the eastern and middle-western states for example USA, and containerized transplants for fresh-market tomato crops. The conditions and procedures for the production of high quality containerized tomato transplants are outlined in the following sections (Jones et al. 1991).

Seed

To qualify for effective use of containerized transplants in production, tomato seed must have a high germination potential and rate. Radical emergence should occur within 3 days at 25° C, and germination should be complete in 7 days. In addition, the seed must be disease free and of high purity.

A seed protectant fungicides or disinfestant, such as thiram, captan, or calcium hypochlorite (or a mixture of them), is universally applied by the seed producer. Various seed coatings, generally of diatomaceous earth or ground vermiculite as well as other proprietary constituents, are applied to the seed in a 4:1 density coating. Coating is necessary for unitization in the seeding process.

Media

Plant growing mixes of different sorts are always used fore containerized-transplant production. The most commonly used mixes have various proportions of high-grade Canadient´s peat and no. 2 horticultural vermiculate or without 2 horticultural perlit. The proportion of the peat may vary from 50 to 85 %, depending upon the season of the year and grower preference. The basic mix is amended with lime, superphosphate, calcium nitrate, micronutrients, and a wetting agent to produce the final growing mix. Strict sanitation practices, including the use of only intact original containers, are followed throughout the preparation of the mix.

Containers

Various sizes of expanded or hard polystyrene master containers are used for transplant production. Individual cells within the container usually have an inverted cone or pyramid-shape and open bottom to permit air pruning of roots. The cell size for tomato transplants

varies from 1.6 to 5cm in diameter and 3.8 to 7.6cm deep. Probably the most commonly used cell size is 2.8cm in diameter and 7.2cm deep. Used containers are meticulously steam pasteurized or chlorine-treated prior to reuse.

Seeding

The rotary-drum vacuum type seeder is the device most commonly used for the seeding operation. At least three alternative seeding procedure may be used: direct seeding to a final stand, which requires exceptionally high-quality seed and precision in all phases of the operation; direct over-seeding, followed by thinning to a single plant per cell; and seeding into 1.3cm plug-type trays, followed by transplanting into growing trays 2 or 3 weeks later.

Scheduling

It is imperative that accurate schedules be developed to ensure the orderly movement of the product through the transplant operation for delivery on a specific date. Each grower must develop a workable schedule to fit the circumstance of production. A growing schedule for example: Florida, would require 4 weeks in summer and 7 weeks in winter for direct seeding, 4.5 weeks in summer and 7.5 weeks in winter for direct seeding with thinning, and 5 weeks in summer and 8 weeks in winter for seeding into plug-type trays and transplanting into containers.

2.1.6. Culture and management

Sanitation in and around growing houses is essential to the production of high-quality transplants. Anything that may come in contact with the growing trays is treated with chlorine, bromine, or formalin solutions between crops. Weeds in and around growing houses are mechanically or chemically controlled. Smoking in growing houses is universally prohibited, and people are commonly required to rinse their hands with isopropyl alcohol before entering.

The watering volume and frequency is influenced by the season of production and the cell size. The initial watering after seeding and seed covering consists of spraying with a hollow-cone nozzle to settle the covering, followed by a thorough watering with flat fan nozzles in a watering tunnel. The trays are not watered against UN till they are placed in the growing house after 3 days in the seed germination chamber. Water stress should be avoided during the early seedling development stage. During the growth stage, the watering frequency increases from two or three to five or six times a day. During the finishing stage, through watering is reduced to once per day, supplemented by misting as needed.

The temperature in the germination chamber is maintained at 25° C for 3 days. Thereafter, the trays are placed in the growing house, open side walls, ventilators, shading, and fans are used to reduce growing house temperatures. In cooler weather, night temperatures are maintained at 15° C for 1 week after seeding or transplanting, followed by 13° C thereafter. During the last two weeks, night temperatures are allowed to fall as low as 2° C if permit ambient condition.

Fertilizer added to the growing mix is sufficient to carry the plants to the cotyledon expansion stage. Overhead liquid fertilization with a mixture of Ca $(NO_3)_2$ and KNO_3 with nitrogen at 50ppm then applied on a 3-day schedule. Later, the nitrogen concentration is raised to 200ppm and the frequency of application is increased to every other day. Fertilization is reduced during finishing stage.

Sanitation is paramount for successful pest management in transplant production an in practiced in every phase of the operation. Preventive applications of crop protection chemicals are applied on a regular basis for plant diseases, such as bacterial spot and speck, early and late blight, and target spot. Insecticides are applied as needed for leaf miner and warms. The selection of crop protection chemicals for use in growing houses is challenging (because many are not specifically registered for greenhouse use) and complicated (because labels sometimes do not explicit state that the material can or can be used in greenhouses).

Hardening

The preparation of transplants for field planting is accomplished by reducing the nitrogen concentration in the fertilizers to 50ppm for two weeks in the winter and for 1 week before delivery data in the summer. Water application is reduced concomitantly to one thorough watering per day, so plants are slightly water-stressed. In the winter, gradually lowered temperature assists in the hardening process.

Postproduction handling

For plants to be pulled, a thorough watering is done the afternoon before the shipment date. For plant sold locally in returnable trays, thorough watering is delayed until the morning of the shipping date. In both cases, a commercial antitranspirant is applied prior to shipment. Plants designated for distant locations are pulled and placed in well ventilated, corrugated, waxed-cartons. In the summer, the plants are pre-cooled to 10° C before shipment. Truck shipments should be at the same temperature and not in mixed loads with ethylene-generating fruits. Air shipments should be on direct flights whenever possible.

The ultimate goal in the production of containerized transplants is to produce disease- and insect-free plants that are able to resume growth with favorable conditions shortly after transplanting in the field.

2.1.7. Agricultural practices, tillage and cultivations

As is the case with any agricultural crop, cultural practices in tomato production have been developed to optimally provide the essentials for crop growth in order to achieve a desire production level. These practices must be compatible with maintaining a higher degree of fruit quality. Hence, cultural practices are intended to provide the producer with a much control as possible over outside influences to protect the crop from adversity. These practices are not universal to all growing situations; they are usually particular to the location where the tomatoes are grown. They include methods for field and bed preparation, irrigation and drainage, fertilization, seeding or transplant use, staking or trellising, and the use of row covers or tunnels.

2.1.7.1. Field and bed preparation

Commercial production of tomatoes occurs in area where suitable climatic, soil and market conditions exist. Field melioration procedures are very site-dependent and must be taken into account the drainage requirements, irrigation method, crop nutritional requirements, and pH control. Liming material is used to adjust the pH to near 6.5 and can provide the crops calcium and magnesium requirements. Control of the pH is important for the avoidance of certain diseases, such as Fusarium wilt disease and gray mold (which are favored by low soil pH) and Verticillium wilt disease (which are favored by high soil pH) (Jones et al. 1991).

Although tomatoes can be grown in many ways, production fields are commonly prepared by forming beds that may or may not be covered with plastic mulch. The raised bed allows a more defined root zone and improves drainage control during high rainfall periods. These beds can be spaced according to irrigation, drainage, and harvesting requirements and configurations may vary considerable. Plastic mulch serves many purposes; it facilitates soil fumigation for disease and nematode control, provides weed control, reduced evaporation from the cropped area, minimizes the leaching of fertilizer during heavy rains and, if staking is not used, provides a protective barrier that prevents direct contact between fruit and soil and subsequent fruit rots.

2.1.7.2. Irrigation and drainage

Since tomato production requires a major financial investment, irrigation is almost always a necessity to ensure desired production levels. The choice of irrigation system is depended on soil conditions, availability of water, climate, economics, and personal preference. Systems that are commonly used include overhead sprinkler, furrow, micro-irrigation, level-basin, and sub-irrigation systems.

The quality of irrigation water can be significant factor, affecting both tomato plant and the long-term composition of the soil or other rooting media. Both organic and inorganic substances in irrigation water need to be known to assess the impact that is use will have on the tomato plant.

Irrigation of tomatoes grown in soils where a slope and infiltration rates are excessive is often achieved by using overhead sprinkler systems. Besides applying water required for crop growth, overhead sprinkler irrigation can improve transplant establishment, apply fertilizer, cool the crop, and in some situation, provide frost or freeze protection in. The major drawback of overhead sprinkler systems is the increased potential of foliar diseases due to increased exposure of leaves surfaces to wet conditions.

Production areas where soils have sufficient water-holding capacities to support crop growth for several days after a single irrigation event can use surface irrigation, such as furrow or level-basin systems, if the slope of the field is slight. Even though these systems can be very effective, they can be somewhat inefficient in applying more water than the crop needs.

In production areas with a particularly high water table is sub-irrigation systems may be used. This systems operate by raising the naturally high water table to a level at which water moves by capillary action into the production bed, thereby maintaining optimum soil moisture for crop growth. Water is conveyed in the field through surface ditches or buried drainpipe and is applied continuously or for several hours daily to maintain a high, stable water table. This process prevents the loss of applied fertilizer in the bed and the development of a deep root system, which is susceptible to flood damage. This system has low-intensity management, but it can in application efficiency.

In areas where water availability is limited “semi desert”, micro-irrigation is being used. These systems operates by applying water through small-diameter polyethylene tubing with emission ports designed and spaced according to the water-holding and transmission characteristics of the soil and the desired application rate. With a tube installed in each bed, that system allows precise application of water and nutrients in the crops rooting zone. Micro-

irrigation systems can be very high in application efficiency; however, because so many operation aspects require special attention, their management intensity is higher than most other irrigation systems.

As important as irrigation is for tomato production, one must not overlook the need for proper of drainage, especially in areas where periods of heavy rainfall can occur or where soil have very poor internal drainage. Although tomato plants can tolerate high soil moisture, inundation with water can cause severe crop loses by oxygen depletion in the rooting zone or by encouraging disease.

2.1.7.3. Fertilization

Maintaining proper nutritional condition for tomato production is critical not only for obtaining a desired production level, but also for achieving a high level of fruit quality. Nutritional conditions in the soil can play a role in preventing or causing certain disease problems (as calcium does in blossom-end rot). Fertilizer can be applied banded, broadcast, or injected (as in micro-irrigation). The primary concern is to provide to proper levels of nutrients required at any stage of plant development. At the same time, there is a need to use fertilization procedures that minimize the potential for leaching losses from the production area, which may cause not only profit losses, but also an environmental problem. Conscientious irrigation management can greatly aid in avoiding leaching losses.

2.1.7.4. Planting methods

Tomatoes can be planted by direct, plug, and gel seeding or as bare-rooted or containerized transplants. In direct seeding, after seed germination plants are thinned to the desired population. Plug seeding is a method in which seed are mixed in a peat-vermiculate medium and planted as plugs at desired interval in a row. Gel seeding involves the use of a gelatinous medium to germinate the tomato seeds prior to planting. The gel and pre-germinated seeds are then inserted at the desired location in the bed. Both plug and gel seeding may require thinning after the plants become established.

Containerized transplants are use very extensively in commercial tomato production, because the transplant offers a more uniform, vigorous plant, normally grown under controlled condition to avoid diseases, such as damping-off and Fusarium crown rot. It use allows a tomato producer the flexibility of having more control over the time between planting and harvesting. Bare-rooted transplants are a lower-cost alternative, but there may be a higher incidence of transplant shock when planted in the field, resulting in less control over the uniformity of the plant stand. Transplants are generally more costly then other planting methods, but the advantage usually outweigh the increased costs incurred.

2.1.7.5. Additionally agricultural practice

Staking or trellising can be used when mechanical harvesting is not implemented. Although they involve increased material and labor costs, these practices can improve overall production and fruit quality, because they give more control over the canopy structure, resulting in improved pesticide-spray coverage. Keeping the canopy upright allows more air movement through it, which minimize amount of time that the foliage remains wet (from dew or rainfall), and therefore lowers the potential for diseases requiring high humidity. Also these practices make hand harvesting easier and more efficient, since the laborer-spend less time locating the fruit.

Synthetic tunnels or row covers are often used in cooler climates to create an artificially warmer environment, which give young seedlings an early start at the beginning of the growing season and provide some protection from damaging could periods.

In summer, cultural practices are used that allow the producer as much control as possible over the development and protection of the potato crop. Since market conditions can be extremely volatile, a producer must make the outcome of the crop as predictable as possible. Still, the tomato producer must ensure that the cultural practices that are used have minimal negative impact on environment.

2.2. Diseases on Tomato

2.2.1. Bacterial Diseases on Tomato

2.2.1.1. BACTERIAL CANCER *Clavibacter michiganensis* subsp.*michiganensis*

Introduction and significance

Today is still an important tomato disease found throughout the world. Periodic outbreaks can cause significant damage to tomato crops in numerous geographic regions.

Symptoms and diagnostic features

Initial symptoms are after primary, systemic infection of the lower leaves as a curling of leaflets, wilting, chlorosis and brown necrosis and shriveling of leaf tissue. These symptoms sometimes develop on only one side of the leaf, with the other leaf side appearing normal. Internal vascular tissue begins to turn light yellow to tan in color. If disease is developed the vascular tissue turns darker brown to red-brown. Secondary fruit symptoms are very characteristic and are a useful diagnostic feature. Spots occur on green fruit and are at first small, round, and white or yellow (up to 3-4mm in diameter) and develop raised, brown centers that remain encircled by white to cream-colored spots.

Causal agent

This pathogen is *Clavibacter michiganensis* subsp.*michiganensis* aerobic, non-spore-forming Gram + bacterium.

Disease cycle

Infested seed is a particularly important a source of inoculums because bacteria can spread and disease can develop when plants are grown under greenhouse conditions. A 1% seed transmission rate is sufficient to give 100% disease. The practices of clipping or mowing transplants and using overhead sprinkler irrigation can significantly spread the pathogen, though young, diseased transplants may show few or no symptoms of bacterial canker until later their growth. During transplanting, the diseased or contaminated plants result in bacterial spread to equipment, workers´ hands and previously cleaned transplants. Bacteria enter the tomato host via stoma's, small wounds, and brakes trachoma's. After entering through these openings, the pathogen can become systemic and will colonize and move through the xylem tissue. The disease development is favorable by warm condition (24-32° C) and factors that create succulent growth of the tomato (Chang et al. 1991, Gleason et al. 1991).

Control

Monitor tomato seed fields regularly so tat bacterial cancer problems can be detected at an early stage and managed appropriately. Obtain and plant high-quality seed that does not have detectable, economically important levels of *C. michiganensis* subsp.*michiganensis.* Use a hot water seed treatment or treat seed with hydrochloric acid, calcium hypochlorite, or other

recommended materials. Hot water treatments can reduce seed viability and germination percentages. Seed health testing and certification programs help regulate the availability and cleanliness of such seed. Such seed tests usually involve the washing of a 10.000 seed sample and subsequent planting of the liquid onto semi-selective medium. Discard heavily infested seed. Use resistant cultivars if such are available.

-Sanitize benches that hold transplants, transplants trays, and equipment that come into contact with plants.

-Lower the water pressure in irrigation equipment to reduce damage to leaves.

-Consider applying preventive fungicides (copper-based materials) for protecting transplants.

-Realize that the practice of moving transplants to regulate transplant height can readily spread the pathogen. Instead, implement growing practices that involve air movement or differential temperature management.

-Minimize damage to plants during transplanting, transplant only when foliage is dry, and periodically sanitize transplanting equipment.

-Avoid using overhead sprinkler irrigation in the field.

-In the field, use new wooden stakes that have been steamed or treated with chlorine.

-With an appropriate disinfectant, periodically and regularly sanitize tools such as clippers and pruning shears.

-Do not allow equipment or workers to pass through fields when foliage is wet.

-Copper sprays may provide some disease control.

-Once the tomato crop is finished, incorporate the crop residues to enhance plant Decomposition and the dissipation of bacteria.

-Rotate to a non-host crop before returning to tomato and do not allow volunteer tomato or weed hosts to survive.

2.2.1.2. BACTERIALS SPOT *Xanthomonas campestris* pv. *vesicatoria*

Introduction and significance

While bacterial spot occurs throughout the world, this disease is most serious in tropical and sub-tropical tomato growing areas, where both high humidity and rainfall are present.

Symptoms and diagnostic features

Initial foliar symptoms consist of circular to irregularly shaped, water-soaked spots on leaves. These spots later turn dark brown and usually remain smaller than 5mm in diameter.

As disease progresses, the spots may coalesce and result in leaves having significant necrotic areas. Severely infected seedlings can become defoliated. Dark streaks may develop on petioles and stems. Early symptoms on green fruit consist of small (less than 3mm in diameter) raised blisters. These diseased spots enlarge into brown, rough scabs that have 5-8mm in diameter.

Control

Use seed that does not have detectable, economically important levels of the pathogen. Regularly monitor tomato seed fields so that early outbreaks can be controlled. The control measures are almost the same as in the case bacterial cancer (Goode and Sasser, M. 1980).

2.2.2 Fungal Disease

2.2.2.1 BLACK MOLD *Alternaria alternata*

Introduction and significance

Pathogen *A. alternata* affect only ripe tomato fruit. Significant fruit losses can occur if the environmental conditions favor disease development.

Symptoms and diagnostic features

On ripe tomato fruit the initial symptoms consist of an irregularly shaped flecks and stains on the cuticle. The affect areas are small and tan to brown. With suitable environmental conditions, the tinny infected areas expand into large, sunken-circular to oval-shaped lesions that can extend deep into the fruit. The black, velvety growth of the pathogen covers the surface of the lesions. Lesions break down and the fruit will rot. The large infected areas can also support the growth of other decay fungi such as *Stemphylium* species, which are considered by some researchers to be weak tomato fruit pathogens, and *Cladosporium* and *Aspergillus* species, which are saprophytes. Postharvest spread of the disease can occur if infected fruit are stored for a long time.

Causal agent

Black mold is caused by fungus *Alternaria alternata*. The pathogen can be isolated on standard microbiological media such as potato dextrose agar, and colonies are usually dark green to black and have limited aerial mycelium. Conidia are pale to gold brown, smooth or verruculose, measure 20-63 x 9-18µm, and have tree to five cross septa and occasional longitudinal septa. Conidia are produced in long chains that sometimes branch. The pathogen *A. alternata* is not host specific to tomato, and it is an active saprophyte and can colonize damage tissues of other plants and readily grows on dead organic matter. Source *A. alternata* is commonly found on decaying plant material in and around fields, including senescent and dead tomato leaves of the current crop. Fruit damaged by sunburn or blossom end rot (a

calcium deficiency) are particularly susceptible to colonization by *A. alternata*. Disease development is most rapid at 24-28°.

Control

Harvest fruit in a timely manner so that ripe fruit are not left in the field longer than necessary. Do not irrigate with overhead sprinklers. Apply protectant fungicides 4 to 6 weeks before harvest (Pearson and Hall 1975).

2.2.2.2. EARLY BLIGHT *Alternaria solani*

Introduction and significance

Early blight occurs on tomato throughout the world and can be an important disease.

Symptoms and significance

The disease begins a small, brown to black, circular lesions on mature leaves. Such lesions may be surrounded by chlorotic tissue. As disease dev elops, the spots enlarge and can be 8 to 10mm or larger in diameter. At this later stage the leaf spots contain characteristic concentric rings. With severe infections, plants can become defoliated and the exposed fruit are subject to sunburn damage. Infections on stems initially consist of small, brown, sunken lesions. Stem lesions expanded and become elongated or oval lesions with concentric rings. Stem lesions can eventually girdle the stem and result in stem or plant death. Seedlings can show stem lesions et the soil level when they are less than 3 weeks old. Fruit infection, either the green or ripe stage, evaluate consist of sunken, dark brown to black, circular spots that also contain concentric rings. Early blight green fruit and stem symptoms may resemble fruit and stem infections caused by the Alternaria stem canker pathogen.

Causal agent

Early blight caused the fungus *A. solani*. The pathogen can be isolated on standard microbiological media. Cultures appear gray-brown in color and usually produce a yellow or red diffusible pigment. Sporulation in culture is depended by exposing colonies to light. Conidia are olivaceous brown to dark brown and are obclavate to obpyriform, having seven to eight cross septa, and occasional longitudinal septa. Conidia usually have long-beaked apical cells that can be the same as or exceed the length of the spore body. Conidia are produced singly or in chains of two and measure 150-300 x 15-19µm. *Alternaria solani* produces resilient structures called chlamydospores, which enable the pathogen to survive in soil for a period of time. This pathogen is seedborne in tomato and can result in diseased seedlings or transplants. Affected seedlings may develop collar rot in which the base of the plant is girdled due to a stem canker. Plants with collar rot can become stunted, wilted and dead. This

pathogen also affects other solanaceous host such as potato and aubergine (eggplant). Alternaria patogens on tomato and potato, historically both designated as *A. solani,* may be distinct species. Morphologically and genetically the early blight isolates from tomato are distinct from those from potato. Tomato isolate are more virulent to on tomato, and the potato isolates are more aggressive on potato. The tomato isolates apparently do not produce spores very well in culture, while the potato isolates sporulate extensively. The tomato early blight pathogen is proposed to be *A. tomatophila,* while the potato pathogen remain the *A. solani.*

Disease cycle

Initial inoculum of *A. solani* is present on infested tomato crop debris and tomato seed. In addition, the pathogen can overwinter on volunteer tomato plants and on other solanaceous plant such as aubergine (eggplant), potato, and the weeds black nightshade (*Solanum nigrum*) and horsenettle (*S. carolinense*). Conidia are blown onto plants via winds and splashing water. Wet, mild conditions (with a temperature range of (24-29° C) are favor disease development. In Europe, early blight is most important when summer temperatures are high.

Control

Used seed that does not have significant levels of the pathogen. Treat the infested seed lots with hot water or fungicides. Inspect and discard infected, symptomatic transplants. Multiple applications of fungicides are usually required to control this disease. Some forecasting programs, such as TOMCAST, are used to assist with fungicide timing for early blight control. Practice crop rotation so that the selected field did not have tomato in the previous year. Remove volunteer tomato and host weed species.

2.2.2.3. SOUTHERN BLIGHT *Athelia rolfsii,* anamorph = *Sclerotium rolfsi*

Introduction and significance

Southern blight, or Sclerotium stem rot, occurs on a large number of vegetable and ornamental especially in warmer regions of the world. This disease is found in many tomato growing regions in the world.

Symptoms and diagnostic features

On the tomato, the early symptoms consist of a water-soaked lesion on crown and lower stem tissue that is in contact with the soil. These infection sites turn light to dark brown and can rapidly girdle the entire crown. Crown tissue is often crocket or split. Above–ground symptoms consist of wilting and a quick collapse of all foliage. If soil moisture conditions are suitable, the patogen will form thick, white mycelium or layer on crown, lower stem, even on the surrounding soil around the crown. Sclerotia are small (1-2mm in diameter), spherical, tan

to light brown forma profusely on and in this white growth. Sclerotia are characterized by having an outer, differentiated, pigment rind. Fruit in contact with infested soil can also become infected, developing sunken, water-soaked lesions that later support the typical white mycelium growth and sclerotia.

Causal agent

Southern blight is caused by *Sclerotium rolfsii,* which is an imperfect fungus in the mycelia-sterilia category and produces no asexual spores (according older references list the fungus as *Corticium rolfsii*). *Sclerotium rolfsii* has a broad spectrum of host plant and forms a basidiomycetes with perfect stage (*Athelia rolfsii*), though it is unknown whether this stage is involved in the disease.

Disease cycles

Because of its resilient sclerotia, the pathogen can survive in the soil and in crop debris for many years. The fungus is favorable by high temperatures above 30° C. In the soil, sclerotia near host tissue will germinate, from infective mycelium, and directly penetrate root and crown tissue of tomato.

Control

Rotate with non-host plants so that soil inoculum levels are reduced. Deep plowing of fields prior to planting, which inverts the soil profile, may help reduced inoculum levels. Pre-plant treatment of soil with effective fumigants will give short-term control; however, such treatment may be too expensive to implement for processing tomato crops (Bulluck and Ristaino, 2002).

2.2.2.4. GRAY MOLD *Botryotinia fuckeliana* (teleomorp) asexual form *Botrytis cinerea*

Introduction and significance

Gray mold is a commonly found disease of tomato and is manifested in several ways. Gray mold can be particularly damaging in greenhouse environments, due to the elevated humidity in such structures.

Symptoms and diagnostic features

Petiole and stem become infected and develop tan to darker brown lesions. The developing lesions can eventually girdle the entire petiole or stem and show concentric rings due to the sporulation of the pathogen and coloration of the lesion. Leaves may also have brown lesions with sporulation. The gray mold pathogen often infects petioles, stems, and leaves that are damage or senescing. Senescing petals are also subject to gray mold infections; if such petals are in contact with the developing fruit, the fungus can grow from the petal and into the fruit

tissue. Infection on green or ripe fruit resulted in a soft decayed, circular rot that can eventually envelop the entire fruit. The fungus usually produces spores “sporulates” on the fruit calyx or in the center of the fruit lesion where the epidermis has split. A distinct fruit infection, called “ghost spots”, occurs when the pathogen invades the fruit but then dies prior causing decay. The resulting symptom is a white to yellow ring that can range from 3-10mm in diameter. On green house tomatoes, stem infections are the most prominent problem. Cracked or wounded stems and cut stubs remaining after fruit are harvested are primary location for gray mold infections.

Causal agent

Gray molds, is caused by asexual fungus *Botrytis cinerea.* The sexual stage, *Botryotinia fuckeliana,* is rarely found on the crop. Conidia are clustered at the branch tips and are singe celled, pale brown, ellipsoid to obovoid, and measure 6-18 x 4-11 µm. Some isolates of pathogen produce spores poorly in culture unless incubated under lights (12 h light/12 h dark). If form, sclerotia are black, oblong or dome-shaped, and measure 4-10mm. The fungus grows best at 18-23° C but is inhibited at warm temperature above 32° C.

Disease cycles

B. cinerea survives in and around fields as a saprophyte on crop debris, as a pathogen on numerous crops and weed plants, and as sclerotia in the soil. Conidia develop from these sources and become windborne. When conidia land on senescent or damaged tomato tissue, they will germinate if free moisture is available and rapidly colonize this food base. Once established, the pathogen will grow into adjacent healthy stems and leaves, resulting in disease symptoms and the production of additional conidia. Cool temperature, free water moisture, and high humidity favor the development of the disease. Tomato tissues that are damaged from other diseases can become colonized by *B. cinerea* that act as a secondary decay organism.

Disease control

Reduce plant wetness by avoiding or reducing sprinkler irrigation. Adequate ventilate greenhouses, or heat them to reduce overall humidity. Fungicides may be useful in protecting fruit from gray mold. It is important to use a diversity of fungicides with different modes of action because *B. cinerea* commonly develops resistance to such materials. Dichlofluanid is useful for control of the ghost spot symptom as it is one of the few fungicides that prevent spore germination (Morgan, 1984).

2.2.2.5. ANTHRACNOSE *Colletotrichum coccodes*, *C. gleosporioides, C. dematium*

Introduction and significance

Anthracnose disease occurs in various parts of the world where tomato is grown. The disease is usually of minor importance unless environmental condition favor for development of the pathogen.

Symptoms and diagnostic features

The disease primary affects the fruit. Young, green fruit may be infected, but disease symptoms are not expressed until fruit begin to ripen. Ripe fruit initially show small, circular, depressed lesions. Lesions can then become quit large (12-15mm in diameter), sunken, and contain concentric rings. Lesion centers are usually tan, but become black as fungal structures (microsclerotia and acervuli) form in the tissues. If humid, wet weather occurs, the fruiting bodies in the lesions will release pink-colored spore masses, harvested fruit infected with anthracnose will not ship or store well, and are very susceptible to secondary fruit decay organisms. On leaves develop small, circular, and tan to brown spots that often are ringed with yellow halos. Roots initially show brown lesions and later rot. As root cortex tissue break down, the black microsclerotia of the pathogen form profusely, giving this phase of the disease the name black dot root rot. Black dot root rot is part of the brown root rot disease complex that occurs on greenhouse-grown tomato in Europe.

Causal agents

Antracnose is caused by several species of the fungus *Colletotrichum*: *C. coccodes, C. gleosporiodes,* and *C. dematium. C. coccodes* is the species most frequently associated with the fruit disease and appears to be the only causal agent of black dot root rot. The minute (about 0.3mm in diameter), cup-shape acervuli fruiting bodies are usually present in fruit lesions. In acervuli release single-celled, hyaline conidia that are cylindrical with obtuse ends. Conidia measure 16-24 x 2-5µm. Long, brown, septa setae are usually present in the acervuli. The pathogen forms small (0.2-0.4mm), irregularly shaped survival structures called microsclerotia. *C. coccodes* has a broad host range and can infect a number of other plants such as cucurbits, legumes, potato, and weeds.

Disease cycles

The fungus survives in the soil in the form of microsclerotia or as acervuli and microsclerotia on dried plant residue. The fungus can be seedborne. The pathogen is splashed from the soil onto tomato foliage and fruit and initiates infections. In addition, the fruit that are in contact with the soil become infected by soilborne inoculum. Ripe fruit are particularly susceptible to infection. The root phase of anthracnose disease is often found in infested

greenhouse situations due to high concentrations of inoculum and favorable conditions for disease development. Optimum temperatures for disease development are 20-24° C. When wet, humid weather just com, acervuli and conidia are produced and conidia are spread by splashing water.

Control

Rotate crops so that non-host is grown at least every other year. Stake plants or use mulch materials to reduce the number of fruit in contact with soil. Avoid sprinkler irrigation which spreads the conidia. Apply fungicides as necessary and use disease forecasting programs such as TOMCAST to schedule applications. Harvest fruit in a timely manner so that they are not overly ripe. Researchers are attempting to develop resistant cultivars (Batson and Roy, 1982, Dillard, 1989).

2.2.2.6. FUSARIUM WILT *Fusarium oxysporum f.sp. lycopersici*

Introduction and significance

Fusarium wilt occurs on tomato throughout the world and can be an important disease.

Symptoms and diagnostic

If young seedlings are infected, plant can be stunted and exhibit poor growth. Commonly Fusarium wilt occurs on older plant. Initial symptoms on such plants are chlorosis of lower leaves followed by wilting of that foliage. Characteristically, the yellow to yellow-gold discolorations and wilting symptoms often first occur on only one side of the plant. A disease progresses, the entire plant will turn chlorose, wilt, and then collapse and dry up. The vascular tissue is discolored and turns brown with the discoloration extending into the upper stems. The extensive brown is a helpful feature in distinguishing this disease from Fusarium crown and root rot, in which vascular browning is found only in the lover stem.

Causal agent

Fusarium wilt is caused by the fungus *Fusarium oxysporum* f.sp. *lycopersici.* The pathogen morphology and colony characteristics are similar to other *F. oxysporum* fungi.

Disease cyclus

Like other Fusarium wilt pathogens, *F. oxysporum* f.sp. *lycopersici* is a soil inhabitant that can survive in the soil for idenfinite periods of time due to the production of overwintering chlamydospores. The fungus is favorable by temperatures, and optimum wilt development takes place at 28° C. Researchers find that Fusarium wilt may be more severe if plants are grown under certain nutrient conditions. For example, low nitrogen, low phosphorus, high potassium, and ammoniacal forms of nitrogen may enhance disease.

Control

Grower should be use resistant cultivars. Use nitrate-base fertilizers instead of ammoniacal ones. Do not plant tomato in fields having high populations of root knot nematode (*Meloodogyne* species) as this nematode can cause the plants Fusarium wilt resistant to be overcome. Practice good field sanitation so than infested soil and mud are not spread to infested fields (Borrero, Triallas, Ordovas, Tello and Aviles, 2004).

2.2.2.7. FUSARIUM CROWN & ROOT ROT *F. oxysporum* f.sp. *radicis- lycopersici*

Introduction and significance

Fusarium crown and root rot is found in many parts of the world on both field and greenhouse grown tomatoes. The disease can be particularly severe in greenhouse production environments.

Symptoms and diagnostic features

The initial symptoms chlorosis is on the lover leaves. Such leaves later become necrotic and then wither**.** In many causes, successively younger leaves develop chlorosis and necrosis until only the upper part of the plant has healthy, functional foliage. Infected plants can be stunted and not productive. In other cases plants decline more rapidly and collapse completely. A tan to brown discoloration develops in the vascular tissue of the root and extends into the adjacent tissues of the lover stem, and does not extend beyond 10-30cm above the soil line. This limited, lover discoloration is a helpful feature in distinguishing this disease from Fusarium wilt, in which the vascular browning can extend far into the upper stems. Examination of the outside surface of plant crowns and lover stems may reveal the presence of large, irregual, brown, necrotic cankers. On occasion orange spore deposits may form on these cankers.

Causal agent

Fusarium crown and root rotate caused by the fungus *Fusarium oxysporum* f.sp. *radicis-lycopersici*. The pathogen morphology and colony characteristics are similar to other *Fusarium oxysporum* fungi. The fungus forms one-or two-celled, oval to kidney shaped microconidia on monophialides, and four to six-celled fusiform curved macroconidia. are usually produced in cushion-shape structures called sporodochia and appear orange-colored in culture or in infected stem cankers. Chlamydospres are also formed. The pathogen is usually readily isolated from symptomatic vascular tissue.

Disease cycles

Like Fusarium wilt pathogens, *Fusarium oxysporum* f.sp. *radicis-lycopersici* is a soil inhabitant that can survive in the soil for indefinite periods of time due to the production of overwintering chlamydospores. In addition to soil inoculum, the pathogen can also grow saprophytically and produced conidia on decaying organic matter. In greenhouse, microconidia can reach tomato plants by becoming airborne or by being transported by fungus gnats. Optimum disease development takes place at temperatures between 20-22° C

Control

In greenhouses, steam the soil and then apply fungicides prior to transplanting tomato. For infested outdoor fields, no control measures have been developed. In such cases, use crop rotations that not include host plants. Some resistant cultivars are being developed for this disease (Rove and Farley, 1981, Rekah, Shtienberg, and Katan, 2001).

2.2.2.8. POWDERY MILDEW Levellula taurica (anamorph = Oidiopsis taurica *Oidium neolycopersici, O. lycopersici*

Introduction and significance

Powdery mildew disease can significantly limit tomato production in various parts of the world. Severe disease can cause early plant senescence and reduce yield.

Symptoms and diagnostic features

There are two types of powdery mildew fungi that infect tomato. *Level Lula taurica* (anamorph = *Oidiopsis taurica* initially causes light green, irregular shape leaf spot. Spots can have diffuse margins, but often tend to appear angular and vein-delimited. As the spots age, the tissue becomes chlorose and then necrotic. *L. taurica* usually infects the older leaves; the younger leaves escape infection until they mature. The other powdery mildew on tomato is caused by on or more species of Oidium. The powdery mildew disease produces in white colonies on upper and on stems and petioles. The infected, underlying tissue may initially turn purple but later becomes chlorose and necrotic. Severe infections cause leaves to be twisted and deformed.

Causal agents

For *L. taurica,* the asexsual (anamorph) stage is named *O. taurica.* Conidiophores develop only from endofytic mycelium and emerge through stomata in the ower leaf epidermis. In contrast to *L. taurica, Oidium* species produce epiphytic, conidiophore is bearing by mycelium that grows superficially on host surfaces.

Disease cycle

Powdery mildew species are obligate pathogens and survive on overwintering tomato, alternate hosts, or possible as cleistothecia from their respective perfect stages. The asexual conidia of these fungi are dispersed by winds. Powdery mildew development favorable under mild temperatures below 30° C. Interestingly, in greenhouses where humidity is extremely high (95 % RH), powdery mildew caused by *O. lycopersici O. lycopersici* may be suppressed.

Control

Fungicides, such as sulfur, may be used if the severe disease become. Irrigation systems may influence disease severity; studie indicate that furrow irrigated tomato may have more severe powdery mildew than sprinkler irrigated plantings. No other control option is available (Palti, J. 1971).

2.2.2.9. PHYTOPHTHORA ROOT ROT *Phytophthora capsici, P. cryptogea, P. drechsleri, P. parasitica* (=*P. nicotianae* var. *parasitica*)

Introduction and significance

There are several soilborne *Phytophthora* species that cause diseases on tomato. Different tomato-producing regions throughout the world may have different species involved in this disease.

Symptoms and diagnostic features

Diseases caused by *Phytophthora* sp. are manifested as seed decays, seedling damping-off, root rots and fruit rots. Symptoms of Phytophthora root rot initially consist of water-soaked root lesions that later turn dark gray to brown. The discoloration can occur on both the fine feeder and larger taproots. As lesions expand, individual roots become girdled or entirely rotted. The discoloration will affect both vascular and stele tissues of the root and can move up the main taproot and into the plant crown and lower main stem. In advanced stages, the root will be soft and decayed. The plant crown can show surface and internal discoloration. Above ground symptoms consist of foliage that first turns dull gray-green, then later wilts. The entire plant canopy can rapidly collapse and die.

Phytophthora fruit disease is called buckeye rot. Buckeye rot almost always occurs on fruit that are touching infested soil. Green and ripe fruit can show similar symptoms. The disease start as small, brown spots on fruit surfaces in contact wit soil. Spots grow into large circular or irregularly oblong lesions that can covered more than half of the fruit. The lesions are characterized by concentric rings of alternating light and dark brown discoloration. Diseased fruit are initially firm, but will later become soft and rotted. The white mycelium of the pathogen can sometimes be observed when the lesion breaks open and rots. The early, firm lesion symptoms on the fruit may resemble the fruit infections caused by the late blight pathogen.

Causal agents

Phytophthora root rot is caused by several species including *Phytophthora capsicum, P. cryptogea, P. drechsleri, P. parasitica* (=*P. nicotianae* var. *parasitica*). Buckeye rot is caused by *P. capsici, P. drechsleri, and P. parasitica.* All three species are oomycetes, soil inhabitants and can persist in soils for extended periods of time.

Disease cycle

Phytophthora spores are diseminate by surface vater and movement of infested soil (Café-Filho and Daniway 1995). Infection process on both root and fruit require wet soil conditions.

Control

Plant tomato in the fields, that having very well drain soils that drain well. Prepare soil so that drainage is enhanced and law areas are avoided. Carefully manage irrigation so that excess soil water is reduced. Stake plants to keep fruit off the ground, or use plastic mulches on bed tops. Some fungicides may help manage both root and fruit infections (Neher, D.A., McKeen and Duniway, 1993).

2.2.2.10. LATE BLIGHT *Phytophthora infestans*

Introduction and significance

Late blight disease of potato is one of the world`s most well known plant diseases because of its historic significance and role in the Irish potato famine of the 1840s. Late blight is also important tomato disease. Worldwide, the late blight reemerged in the 1990s through early 2000s as a very serious disease, that concern for both potato and tomato crop, and devastating epidemics has been chronicled on various continents (Caten, C.E., Jinks, J.L., 1968, Cohen et al., 1998).

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Symptoms and diagnostic feature

Initial late blight symptoms on tomato foliage are irregularly shaped, pale green, water-soaked spots and areas on leaves. These areas rapidly expand into large brown to gray leaf lesions that in severe cases can kill the entire leaf. If environmental conditions are suitable, high humidity, low temperature 14-20º C, the leaf undersides can be covered with the velvet white growth of the pathogen. Stems and petioles can also develop the water-soaked and later brown to gray lesions. Stems lesions caused by late blight of tomato and potato (Photo 1.).
The pathogen can rapidly colonize tomato foliage and cause entire plants to turn brown, shrivel and die. Infected green fruit develop circular to oval to irregularly shaped lesions that are green-brown to dark brown in color (Photo 2. Fruit infections caused by *P. infestans* late blight disease of tomato). Such lesions can become quite large, sometimes covering the entire fruit. Characteristically these green fruit lesions are very firm and do not break down and rot unless the infections are old and secondary decay organisms are present in the fruit.

Causal agent

Late blight is caused by the Oomycetes *Phytophthora infestans*. Causal agent of Late blight disease *Phytophthora infestans* that is heterothallic and requires two distinct mating types (A1 and A2) to undergo sexual reproduction and produce the sexual spore, the oospores. The presence and importance of oospores appears to differ depending on where the crops (tomato and potato) are grown. In parts of the USA including California, even if both mating types are present in one area, it appears that sexual recombination is not common; the oospores are rarely found in host plant tissue in these areas. In Canada (British Columbia), Central Mexico, and parts of Europe (Netherlands, Poland, Russia), the oospores is more readily found too, and genetic evidence indicates grater diversity is present in populations, which presupposed sexual recombination.

The causal agent is sometimes difficult to maintain in culture, though cultures can be grown on V-8 juice, pea or rye B agar, and other media. Incubating the symptomatic tissue is produced at low temperatures (below 20° C) and with high humidity will also encourage the causal organisms *P. infestans* to grow and sporulate after only one to a few days. Mycelium is coenocytes (lacking cell cross walls) and produced ellipsoid to ovoid, hyaline, semi-papillae sporangia that measure 21-38 x 12-23µm. Sporangia are deciduous, have pedicels measuring up to 3µm long, and are distributed by winds and splashing water. Sporangia can germinate directly and infect tomato tissues, or can produce swimming zoospores (3-8in one sporangium) that are realized and after penetration in a plant tissue infect host plant. Optimum

temperature for growth of the causal agent is 20° C. Tomato and potato are the main hosts, though other plants in the Solanaceae can also support the pathogen.

The recent increase in late blight severity coincides with worldwide changes in population genetics of *P. infestans*. Recent advance in molecular methods and DNA technology have enabled researchers to precisely characterized isolates and obtain some insight into reasons for this increased severity. Research reveals that *P. infestans* is a very complex organism. While all *P. infestans* isolates belong to one of two mating types, all individuals also simultaneously belong to one of serious distinct clonally lineages, which are asexual descendants from single genotypes. Prior to the 1980s, the various regions of the world were primarily populated by *P. infestans* isolates that were of the A1 mating type and overwhelmingly belong to the US-12 clonally lineage group that is sensitive to the widely used fungicide mentality.

However, the recent serious outbreaks were often caused by novel lineages. In Nord America, four such lines caused significant damage to crops (potato and tomato): UD-6 MR (A1 mating type; resistant to metalaxyl; highly virulent to tomato), US-7 (A2 mating type; resistant to metalaxyl; highly virulent to tomato); US-8 (A2 mating type; resistant to metalaxyl; highly virulent to potato), and US-11 (A1 mating type; resistant to metalaxyl; highly virulent to potato and tomato). The occurrence and spread of these new pathogen lines is most likely due to the shipment of potato seed tubers, tomato fruit, and tomato transplants between areas and continents.

The population genetics are in flux, because new clone lineages replace older ones a few seasons. Significant changes in *P. infestans* population structures have also been documented in Europe, South America, and Asia. These new aggressive isolates can reproduce more rapidly than older genotypes, thereby shortening the disease cycle to 5-7 days under favorable condition. Additional changes in virulence and fungicide insensitive could occur more frequently in the future because sexual reproduction will likely be more common now that both mating types are widely distributed.

One additional parameter of *P. infestans* diversity is aggressiveness of isolates is highly virulent on potato and tomato. The available evidence indicates that the never genotypes are pathologically more fit than populations made up of the older isolates (discussed by Spielman (1991) and Spielman et al. (19931). These and the studies summarized by Fry et al. (1993) and Fry and Goodwin (1995) confirm that *P. infestans* is highly variable and because of its ability to become adapted to different hosts and different situation remains a major treat to world food production. Therefore, *P. infestans* can be further divided up into tomato-

aggressive and tomato non-aggressive isolates. This factor is not as an important management consideration; if tomato non-aggressive isolates is present in a field-tomato, than crop loss due to late blight is likely to be limited and fungicides may be needed less frequently. There is some evidence that A2 mating type occurs more frequently on tomato than on potato (Erwin and Ribeiro 1996).

Disease cycle

In contrast to most other *Phytophthora* species, *P. infestans* usually is not considered a soil-borne pathogens, though this aspect might change if both mating types are present and oospores are formed that can persist in the soil for several years. The causal agent over-winters in volunteer host plants, potato cull piles, and residential gardens. Potato plants and cull tubers may be an important source of inoculum for tomato fields. Optimum growth of the pathogen is at 18-22° C. A striking feature of late blight is the speed of disease development and spread. If conditions are suitable for the pathogen, entire fields can become infected after only few days.

Photo 1. Primary and stem symptoms of potato late blight

Control

Fungicides are key tools for managing late blight. Apply protectant fungicides prior to late blight infection. The pathogen has developed resistance to some fungicide products such as metalaxyl. Late blight forecasting systems may be helpful in deciding when fungicides should best be applied. Several forecasting systems are used for identifying late blight infection periods in potato crops. In the UK, Smith periodes define when whether conditioned is favorable for blight infection. A full Smith period requires relative humidity greater than 90% for a minimum of 11 hours on two consecutive days and temperature above 10° C.

Avoid or reduce the use of sprinklers for irrigation. Destroy old tomato fields after harvest is completed. Eliminate pathogen reservoir sources such as potato tuber or tomato fruit cull piles and volunteer potato or tomato plants. Because disease can develop on transplants inside greenhouses, carefully inspect tomato transplants and removed suspect plants and trays. Some resistant cultivars are available.

2.2.2.11. DAMPING-OFF, FRUIT ROOTS *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*

Introduction and significance

Several soilborne pathogens cause seed, seedling, and transplant diseases of tomato. Pathogens include species of *Phytophthora, Pythim,* and *Rhizoctonia.* These pathogens can also infect tomato fruit and cause field and post harvest fruit rots.

Symptoms and diagnostic features

These diseases have various phases. Tomato seeds can be infected prior to germination and result in seed death. Newly germinated seedlings can be infected to such a degree that plants do not emerge above the soil (pre-emergence damping-off. Seedlings might emerge from the ground but become diseased after soil emergence (post-emergence damping-off). Finally, transplants placed in the ground can develop root rots of stem lesions from these same pathogens. Initial symptoms of post-emergence damping-off occur on seedlings stem contact with the soil. These symptoms consist of shriveled stems that have discolored, tan to dark brown lesions. With time the lower stem collapses, roots decay, and the cotyledons and leaves wilt. Such plants usually bend over. Damping-off diseases often result in death of the seedling and subsequent reduction of plant stands. However, even if plants do not succumb to these pathogens, the surviving plant may be stunted and delayed in development. These three pathogens can also infect roots and crowns of older plants as well as fruit. Phytophthora fruit infections are called buckey rot and can be caused by several species of *Phytophthora.*

Pythium fruit infections are called watery rot and usually infect ripe tomato fruit. Symptoms consist of irregular, water soaked lesions on fruit that are in contact with the soil. Once infected, the fruit is rapidly colonized by the *Pythium* pathogen, the epidermis breaks, and the fruit becomes soft and watery, White mycelia growth can be observed on the rotted tissues. *Rhizoctonia* fruit rot can affect immature green fruit but is mostly a problem on a ripe fruit. Dark brown, circular to oblong lesions develop on ripe fruit that are touching the soil. These lesions enlarge and break down into a mushy, soft rot. *Rhizoctonia solani* will form characteristic brown, persistant hyphae on the fruit surface.

Causal agents

Phytophthora and *Pythium* species are in oomycete group. *Phytophthora cappsici* and *P. parasitica* usually infect roots of older plants, but can cause damping-off. A number of *Pythium* species cause damping-off: *P. aphanidermatum, P. arrhenomanes, P. debaryanum, P. myriotylum, P. ultimum.* All this organisms survive in the soil as saprophytes and are favored by wet soil conditions. With the exception of *P. ultimum,* these pathogens usually produce zoospores that swim to and infect susceptible tissues. Sexual structures-antheridia, oogonia, and zoospores-are produced by all species. In addition to tomato, these pathogens can infect numerous other plants.

R. solani is a soilborne fungus with a very brad host range. *R. solani* has no asexual spores, but produce characteristically coarse, brown, approximately right-angle branching hyphae. The hyphae are distinctly constricted at branch points, and cross walls with dolipore septa are deposited just after the branching. Hyphae cells with multi-nucleate are small, tan to brown loosely aggregate clumps of mycelia function as sclerotia. This fungus can survive by infecting and thriving on a grate number of plant hosts, besides tomato, and can also persist in the soils as a saprophyte. The teleomorph stage, *Thanatephorus cucumis*, is not commonly observed.

Disease cycle

Most of these soilborne pathogens survive in fields for indefinite amounts of time. Wet soil conditions and cool temperatures (15-20° C) generally favor these organisms and after ability to grow and their ability to grow and infect hosts. However, for pathogen such a *P. aphanidermatum* and *P. myriotylum* warmer soil conditions (32-37° C) are favorable. The seedling stage is most susceptible to infection, though these pathogens can infect the feeder roots of mature plants.

Control

The disease damping-off is primarily controlled by creating conditions that are not favorable for the pathogens, which are causal agents. Plant on raised beds and in soil, drain well so that overly wet soil conditions are reduced. Carefully manage irrigation and do not apply excess water. Plant seed, that has been treated with fungicides, and avoid planting seed too deeply, which delays seedling emergence and increases the chance of infection. Post-plant fungicides may provide control for some of these pathogens. Avoid planting too soon into fields that still have extensive crop residua in the soil. Rotate crops, as consecutive tomato plantings will increase the populations of the soilborne pathogens. For transplants, prepare good quality beds and do not plant too deep (Golden, and Van Gundy 1975).

To avoid fruit rots, keep bed tops dry by carefully managing the irrigation or by using buried drip irrigation. Prepare soil to enhance drainage and avoid law areas. Stake plants or use plastic mulches on the bed tops to keep fruit off the ground. Some fungicides may help manage both root and fruit infection.

2.2.2.12. CORKY ROOT ROT *Pyrenochaeta lycopersici*

Introduction and significance

Corky root rot, or brown rot, was first described from Europe but now also occurs in North America. Corky root rot is important where tomato crops are grown repeatedly in the same soil.

Symptoms and diagnostic features

Initial symptoms consist of plants that show poor vigor, are sunken, and begin to wilt. Leaves show chlorose color and later fall of the plant. The most characteristic symptoms occur on large roots and consist of brown lesions that have a rough corky or wrinkle texture. Such lesion appears often as horizontal bands across the length of the root; lesions are dry and have cracks that run lengthwise along the root. Smaller feeder roots may either show the brown, rough lesions or may be completely rotted. Internal tissues of the large roots do not exhibit discoloration or symptoms. Affected plants rarely collapse and die, but can experience a reduction in yield.

Causal agent

The Patogen *Pyrochaeta lycopersici* cause the disease corky root. This pathogen is a slow-growing fungus that forms gray colonies on standard microbiological media but is difficult to isolate without using semi-selective media. In culture the pathogen forms brown to black pycnidia that measure 150-300 µm in diameter. Pycnidia released spores through a circular

pore (ostiolum) that is ringed with three to twelve light brown setae. Single-celled, cylindrical to allantoid, hyaline conidia measure 4-8 x 1.5-2µm and are borne on conidiophores within the pycnidial body. The fungus forms microsclerotia 63.5 x 448µm (Campbell at al. 1995, Grove and Campbell 1987).

Disease cycle

Pyrenochaeta lycopersici is soilborne fungus and can survive for long periods of time as microsclerotia in soil or on old tomato roots. The fungus prefers cool conditions and optimum disease development takes place at 15-20° C, though the range is from 8-32° C. The pathogen can also infect aubergine (eggplant), melon, pepper, sunflower, spinach, and squash. *Pyrenochaeta lycopersici* often co-infects tomato roots with the back dot pathogen (*Colletotrichum coccodes*).

Control

Apply fumigants to field soil, and steam or fumigants to greenhouse planting areas. Rotate away from tomato to avoid buildup of inoculum. Delay planting material until later in the spring when soil is warmer. Some European tomato cultivars are resistant to this pathogen. Additional control measures including grafting to resistant rootstocks and mounding soil around the stem base to allow new adventious roots to grow.

2.2.2.13. WHITE MOLD, SCLEROTINIA ROT *Sclerotinia minor, S. sclerotiorum*

Introduction and significance

White mold, or Sclerotinia rot, is an occasional tomato problem. Both pathogens occur on tomato throughout the world. Disease caused by the *S. sclerotiorum* species is the more important *Sclerotinia* disease.

Symptoms and diagnostic features

The type of symptoms are seen on tomato depend on which species of *Sclerotinia* is involved. *Sclerotinia minor* infects tomato tissues that are in contact with the soil *Sclerotinia minor* causes a water-soaked lesion to develop at the crown and a lower stem. The lesion enlarges and can girdle the plant, resulting in the collapse of the canopy and foliage. With time, the crown and stem lesions turn light tan to off-white in color. White mycelium and small (3-5mm), black, irregularly shaped sclerotia form around and within the decayed crown (Purdy, 1979).

Sclerotinia sclerotiorum is the other species that can attact tomato. Because *S. sclerotiorum* has an airborne spore stage, infection can occur throughout the tomato canopy. These aboveground infections are usually occurred on damaged stems or petioles, or where a

nutrients source, such as a senescent flower petal, falls onto stems or petioles. These infections are water-soaked lesions that gradually enlarge and encircle the stems. Older infections turn off-white to white-gray in color. White mycelium can be observed on infected lesions if conditions are favorable. The large, black sclerotia can grow on the outer surface or in the central cavity of stem. *S. sclerotiorum* can also infect tomato crowns and lower stems. Fruit can become infected and develop a soft, watery rot (Lobo, Lopes and Silva, 2000). This pathogen produces white mycelium and black, oblong or dome-shaped sclerotia. Sclerotia are significantly large (5-10 mm long) than those of *S. minor*.

Causal agent

For detail, sclerotium of *S. sclerotiorum* germinates carpogenically by forming apothecia that are cup-shaped and stalked. Optimum conditions for producing apothecia are soil temperature 15° C and soil water potentials between –0.03 and –0.07 Map. Mature apothecia contain cylindrical to clavate asci. Each ascus contains eight ascospores that are single-celled, hyaline, elliptical, and measure 9-13 x 4-5 µm. Synchrony of sclerotial germination and ascospore production within flovering of tomato flowers is an iomportant aspect of white mold disease epidemiology (Abawi, and Grogan, 1979).

Contamination occurs in two ways:

- Through mycelium from sclerotia, at soil level.
- Through ascospores from apothecia (organ for sexual reproduction of the fungus formed from sclerotia) which contaminate aerial the part of the tomato and disseminate the disease over several hundred meters.

Environments encouraging development: this fungus encouraged by relatively low temperatures between 15˚ C and 18˚ C and high relative humidity either at soil level or within the crop canopy. Light soil, rich in humus, is most suitable for its development.

Control

During cultivation remove dead plants carrying scleroses. Reduce humidity in greenhouses by ventilating as much as possible. Avoid overhead irrigation. Apply a solution containing one of the active ingredients of the following fungicides* to the neck of the plants: benomyl, thiophanat methyl, iprodione, vinclozoline, procimidone. If air-borne contamination appears to be occurring, spray with iprodione + neutral oil. At the end of cultivation, remove and destroy the affected plants and their sclerotia.

Important note: *Approval of fungicides may vary in different countries and local labels should be consulted before use.

2.2.2.14 VERTICILLIUM WILT *Verticillium dahlie*

Introduction and significance

Verticillium wilt is a well-known disease that affects hundreds of different crops and is an important tomato production factor throughout the world. The closely related pepper and aubergine (eggplant) are also subject to this disease.

Symptoms and diagnostic feathers

On tomato, early consist of the chlorose of leaf margins and tips of older, lower leaves; these yellowed areas are sometimes angular in shape and interveinal. The chlorotic section later turn necrotic and die. Shoot tips and foliage wilt, especially during the warmer times of the day, and recover at night. Internal vascular tissue discolors to a tan to light brown color. This coloring is most evident in the main stems closer to the crown; such discoloration may not be evident in the upper, smaller stems. Verticillium wilt vascular discoloration tends to be lighter and subtler than the vascular discoloration caused by Fusarium wilt, though this distinction is not always clear. Disease symptoms can be accentuated if the infected plant is bearing a heavy load fruit or is stressed by some other factor. Even if diseased plants do not collapse completely, plant growth and yields can be significantly reduced, sometimes by over 20%. The overall symptoms are similar to those caused by Fusarium wilt; hence disease confirmation will require laboratory analysis. On tomato, Verticillium wilt tends to develop more slowly than Fusarium wilt.

Causal agent

The causal agent is *Verticillium dahlie.* The pathogen can be isolated on standard microbiological media, though semi-selective media such as NP-10 can be useful for isolation. On general purpose media, the pathogen forms the characteristic hyaline, verticilliate conidiophores bearing three or four phialides at each node, and hyaline, single celled, ellipsoidal conidia that measure 2-8 x 1-3µm. Cultures alder form dark-brown to black torulose microsclerotia that consist of groups of swollen cells formed by repeated budding. Microslerotia size varies greatly and is in the range of 15-100µm in diameter. Microsclerotia enable the pathogen to survive in the soil for extend periods of time (up to 8 to 10 years). *Verticillium dahliae* has an extensive host range of crops and weeds. Two distict tomato races have been documented.

Disease cycles

The patogen survives in the soil as dormant microsclerotia, but can also persist as epiphytes on non-host roots. Cool to moderate weather conditions favor the pathogen, end disease is

enhanced at temperatures between 20-24° C. The fungus enters host roots through wounds, and later systemically infects tomato vascular tissue.

Control

Growing of plant resistant or tolerant cultivar is the one of the best method for control. The plant with Ve gene is resistant to tomato race 1; however, resistance has not yet been identified for tomato race 2. It seems likely that new races of *V. dahliae* will continue to emerge and overcome the currently available genetic resistance. Pre-plant treatment of soil with effective fumigants will give short-term control but will not eradicate the pathogen from fields. For greenhouse production, steaming of soil can also provide short-term control. Rotate crops so that tomato is not planted in fields having a history of the problem. Rotation with non-host crops, such as small grains and corn, can lower inoculum levels in the soil but will not eradicate the pathogen. Minimize spread of infested soil to other, uninfected areas (Ashworth, Huisman, Harper, and Stromberg, 1979, Harrington, and Robinson, 2000).

2.2.3 Virus diseases

2.2.3.1 ALFALFA MOSAIC Alfalfa mosaic virus

Introduction and significance

Alfalfa mosaic virus (AMV) is present throughout the world but is a serious production concern in only certain regions.

Symptoms and diagnostic features

Tomato plants that are infected early in their development may die. Leaves will develop irregularly shaped, bright yellow patches and a bronze discoloration. Foliage of severely diseased plants will curl downward. A red to red-brown discoloration occurs in the vascular tissue of the lower main stem. Symptomatic fruit can be distorted and exhibit irregularly, sunken, dark brown, necrotic spots, rings shaped, and patches.

Causal agens,

AMV is the only member of the alfamovirus group and has particles that are bacilliform and measure 30-56x18nm. The particles contain three single-stranded genomic RNA and a fourth subgenomic RNA. AMV is transmitted by a number of aphid vectors and can be seedborne in tomato.

Disease cycle

Disease is usually most severe when tomato fields are planted close to infected alfalfa plantings. AMV is also seedborne in tomato.

Control

According general suggests, use seed that does not have significant levels of the pathogen for its transmission in the tomato crops.

2.2.3.2 BEET CURLY TOP Beet curly top virus

Introduction and significance

Beet curly top virus (BCTV) occurs in North and South America, Asia, the Middle East, and the Mediterranean region. This virus is an important pathogen of many crops such as tomato, pepper, and *Chenopodium* plants (Soto and Gilbertson, 2003).

Symptoms and diagnostic features

If infected early in development, tomato plants will die. Plants, that are infected later remain extremely stunted and become very chlorosis with a bronze or purple tinge. Leaves become thick and brittle in texture, chlorosis with purple veins, and rolls upwards. Fruit ripen prematurely and are small, wrinkled, and red.

Causal agent

BCTV is a geminivirus with isometric particles that measure 18-22 in diameter and which occur singly or in pairs. The BCTV genome is a single-stranded circular DNA. BCTV is vectored in a persistent manner by the leafhopper (*Circulifer tenelus*). *C. opacipennis*is is a vector in Mediterranean region. In the plant, BCTV is restricted to the phloem tissue. On a molecular level, researchers have compared strains of BCTV from North America and the Middle East and found them to be similar, providing evidence, that these various strains share a common origin.

Disease cycle

This virus infected many weed and crop hosts. Recent research on curly top disease as it occurs on Amaranthaceae, Fabaceae, Solanaceae, and other crops indicates that the viral agent may differ depending upon the host being considered. Beet curly top as a disease may actually be caused by one of four different curly top virus species: *Beet curly top virus* (BCTV), *Beet mild curly top virus* (BMCTV), *Beet severe curly top virus* (BSCTV), and *Spinach curly top virus* (SCTV). Research is ongoing to further determine the relationships of these various viruses.

Control

According general suggests for managing virus diseases.

2.2.3.3 CUCUMBER MOSAIC VIRUS Cucumber mosaic virus

Introduction and significance

Cucumber mosaic virus (CMV) is commonly found throughout the world and can cause disease on over 800 crops and weed hosts, including tomato and many other vegetable crops. CMV is most prevalent in temperate regions.

Symptoms and diagnostic features

CMV severely stunts tomato plant growth and early infections results in small, yellow, bushy plants. Leaves may show a mottled pattern. A most striking symptom occurs when leaf blades do not develop and the leaves take on an elongated, filiform shape known as “shoestring” or “strap-leaf”. Infected plants may not produce many fruit, or fruit that do develop are small and slow to mature.

Causal agent and disease cycle

CMV is a cucumovirus with particles that are isometric in shape (29nm in diameter) and contain three single-stranded (ssRNA). Many CMV strains have been documented, an on tomato alone many different strains have been found. CMV is transmitted in a non-persistent manner by several aphid vectors.

Control

CMV is difficult to control because of its extremely wide host range. According general suggests for managing virus diseases. Use seed that does not have significant levels of the pathogen (Palukaitis, Roossinck, Dietzgen and Francki, 1992).

2.2.3.4. POTATO VIRUS Y Potato virus Y

Introduction and significance

Potato virus Y (PVY) is a patogen of solanaceous plants around the world and is of major economic importance.

Symptoms and diagnostic features

Symptoms can vary greatly but generally consist of veinbunding, in which dark green bands form along leaf veins, and a downward rolling of the leaves. Mottle and mosaic patterns and some leaf distortions can also occur. A disease develops; leaves can show brown, necrotic interveinal lesions. Fruit usually do not exhibit symptoms.

Causal agent and disease cycle

PVY is a potyvirus with long (730 x 11nm) flexous rods that contain ssRNA. It is vectored in a non-persistent manner by several aphids, with *Myzus persicae* being particularly important. This virus is also readily transmitted mechanically.

Control

According general suggests for managing virus diseases.

2.2.3.5. TOBACCO MOSAIC Tobacco mosaic virus
TOMATO MOSAIC *Tomato mosaic virus*

Introduction and significance

Tobacco mosaic virus (TMV) and *Tomato mosaic virus* (ToMV) *viruses* are two closely related virus pathogens. Both viruses can be infected tomato and other solanaceous plants.

Symptoms and diagnostic features

TMV and ToMV both can infect tomato and cause light and dark green mottling or mosaic on foliage. Leaflets may be deformed and narrow, giving the leaf a fern-like appearance. Fruit set may be reduced, and fruit may ripen unevenly.

Causal agents and disease cycle

TMV and ToMV TMV and ToMV are viriuses in the tobamovirus group. These viruses have straight rod particles that measure 300 x 18nm and contain single-stranded linear RNA genom. TMV has no known vector and is readily transmitted mechanically. ToMV also lack a known vector, is transmit mechanically, and can be seed-borne in tomato.

Control

According general suggests for managing virus diseases. Because ToMV is seed-borne in tomato, use seed that does not have significant levels of the pathogen. Seed infested with ToMV can also treated with dry heat (70° C for 2 to 4 days) or within trisodium phosphate (10% for 15 minutes) (Broadbent, L. 1976).

2.2.3.6. TOMATO SPOTTED WILLT Tomato spotted wilt virus

Introduction and significance

In some regions, this virus is very common on tomato, pepper and many other crops.

Symptoms and diagnostic futures

*Tomato spotted wilt virus (*TSWV) causes on leaves spots that have irregularly shape, and are small (3-8mm in diameter), circular with black color. Stem and shoots may have a streaks or lesion on the epidermis. Severally affected plants may wilt or be stunted. Symptomatic fruit will develop chlorosis rings, patches and lesions.

Causal agent

TSWV is a tospovirus and has isometric particles, measuring approximately 80-110 nm, which are surrounded with membranes. TSWV has a genom consisting of three linear single stranded RNA is vectored by several species of trips; at least eight species are found to be vectors. The western flower (*Frankliniella occidentalis*) and tobacco (*F. fusca*) thrips are probably by the most important vectors.

Disease cycle

TSWV has one of the most extensive host ranges of any known plant virus and can infect over 900 different cultivated and weedy plant species. Therefore initial inoculum can come from any number of landscape plants, weeds, and other plants. Thrips insects vector, the virus disseminated into the tomato crops. It is well documented that thrips insects can only acquire the virus as larvae that feed on diseased plants; after acquiring the virus, the insects carry the virus for the rest of their lives.

Control

According general suggests for managing virus diseases. The broad host range of TSWV and the difficulty in controlling thrips makes this disease particularly difficult to manage (Momol, Olson, Funderburk, Stavisky, and Marois,2004).

2.3.3.7. TOMATO YELLOW LEAF CURL Tomato yellow leaf curl

Introduction and significance

Tomato yellow leaf curl (TYLCV) is one of the most damaging tomato virus diseases in tropical and sub-tropical areas, where entire tomato plantings can become infected if vector population is high. The disease has become widely distributed and has been reported in the Mediterranean region (particularly in southern Europe), The Middle East (Israel), Africa, and recently in North America (Caribbean area, southeastern USA, and Mexico).

Symptoms and diagnostic features

If infected at a young stage, tomato plants can be severely stunted and will not produce fruit. Foliage shows an upright or erect growth habit, leaves curl upwards and may be crumpled. Interveinal chlorosis is also observed in the leaves.

Causal agent and disease cycle

TYLCV is a Geminivirus that consists of twinned icosahedral particles and single-stranded ssDNA genoms. Been, pepper, and weeds are also hosts. TYLCV is vectored in a persisted manner by the sweet potato whitefly (*Bemisia tabaci*). This virus is apparently not seedborne in tomato. Researchers in Europe believe two distinct species of this virus exist: *Tomato*

yellow leaf curl-sardinia (TYLCV-Sar), *Tomato yellow leaf curl –Israel* (TYLCV-Is.) (Accotto et al. 2000, Dellate, Dalmon and 2003).

Control

According general suggests for managing virus diseases.

2.2.4. Disordies caused by abiotic factors and physiological conditions

2.2.4.1 BLOSSOM END ROT

Symptoms and diagnostics features

Blossom end rot is a physiological disorder of tomato fruit. Initial symptoms can occur on green fruit and consist of small, light brown flecks and lesion that are usually clustered on the blossom end of the developing fruit. At the disorder worsens, the blossom end has a circular to oblong lesion that is dark brown to black in color, firm in texture, and sunken. Secondary decay organisms may colonize the lesion and result in a fruit rot.

Causal agent

Blossom end rot is caused by a calcium deficiency in the tissue at the end of the fruit. Calcium is not particularly mobile in plant tissues, so the distal end of the fruit can experience shortages of this nutrient. Plants that are growing rapidly, subject to water stress, and fertilized with nitrogen rates may be more susceptible. Uneven watering practices contribute to the development of the disorder. Pepper and squash fruit can also develop similar symptoms due to calcium deficiencies.

Control

To minimize damage from this disorder, do not over fertilize with high-nitrogen fertilizers. Irrigate crops regularly and with appropriate amounts of water. Calcium fertilizer supplements may help in some cases, but these treatments are generally ineffective. Choose cultivars that tend to develop this problem less frequently than others.

3. Materials and Methods

3.1. Plant materials

The seeds of tomato were collected from cultivars Flora of tomato crop that was growing under plastic tunnel of Tropical and Subtropical Institute of the Czech University of Live Science at Prague – Suchdol in October 2006. At the end of growing period, the most of the tomato plants are infested by *Phytophthora infestans*, the leaves and fruit carry out severe symptoms of late blight disease (Photo 3).

For planned experiments we are sampled fruits and leaves with characteristic symptoms of late blight disease during two weeks, when the fruit mature, and we transit these fruits to the laboratory of the Department of Plant Protection of Faculty of Agrobiology, Food and Natural Resources in October 2006.

At the laboratory the seeds of tomato from the cv. Flora were separated from fruits, which carry out severe symptoms of the late blight disease caused by *P. infestans*. According microscopy control of the washing water in which were washed extracted seeds, we are seen typical size and form of sporangia, and mycelium without septum of causal agent *P. infestans* (Microphoto 4).

3.1.1. Washing process of tomato seed was divided in to three steps

1. Samples of tomato fruits are cleaned, crushed and seed extracted by hand from tomato fruit matrix.
2. Extracted seed was washed by coarse sieve, and the mature seed stay on this sieve; and the most of the fruit tissues (the pulp, skins, undeveloped seed and other tomato material) float off throughout the coarse sieve.
3. Last step continue over night, as a short fermentation at the laboratory temperature (20 ± 2° C), is required to separate the seed from surrounding gelatinous matrix.

3.1.2. Drying tomato seed that are separated from diseased tomato fruits by *P. infest*ans

The cleaned seeds were dried at the laboratory **temperature** (20 ± 2° C) on trays with filter paper for several days. One parts of the dry seeds we are immediately used for the seedborne test and the second parts of dry seeds we stored several months at the same stored temperature for seedborne test after stored time period of 4 months in 2007.

3.1.3. Laboratory test for the detection of Phytophthora infestans in seed of tomato

For this test we use 19 sterile plastic plates with 6 x 4 wells fill up with 1 ml of sterile water and part of tomato leaflet disc cv. Flora with diameter 10mm and 1 dried seed of tomato. Each plate was covered with top and wrap up by transparent plastic envelope that

maintained adequate humidity for germinating of the seed, sporangiospores and growth of mycelium of *Phytophthora infestans*. Sterile plastic plates were kept in thermostat at 16 ± 1 ° C during testing period (3 weeks).

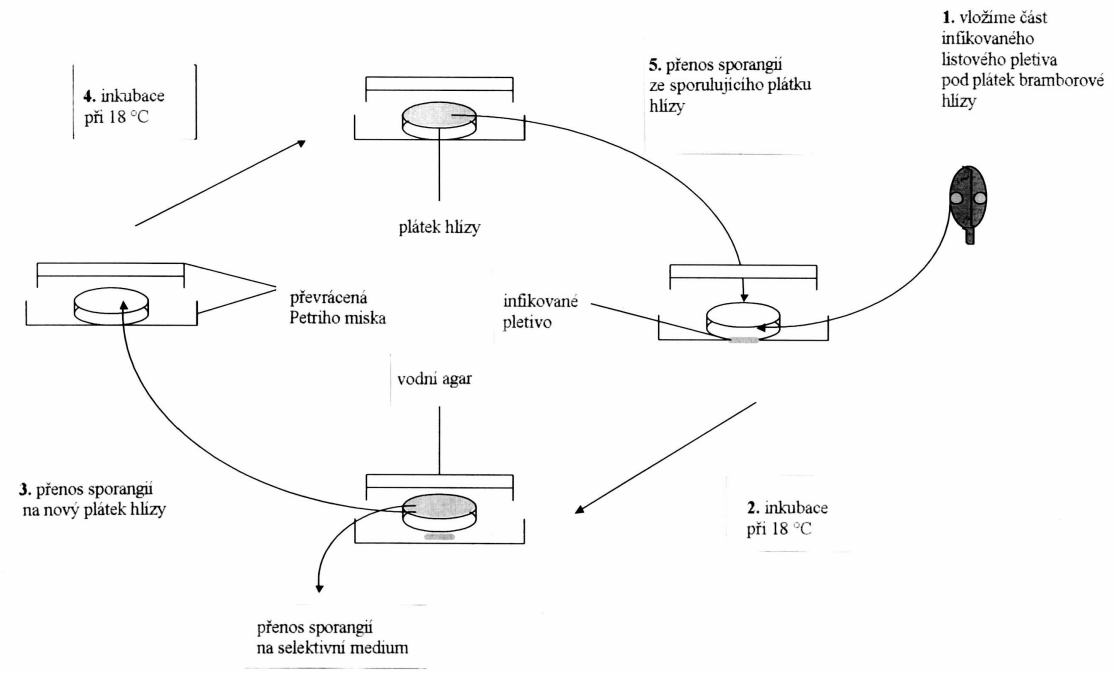
Germinating of the seed and growth of contaminants were evaluates three times by eyesight through covered plastic top of plates and again wrap up with transparent plastic envelope. After the third week was open each plastic plate and by microscopy control were determined contaminants according type and size of presented spores and mycelium. After each eyesight control plastic plate is wrapping up with transparent plastic envelope again. The final evaluation of germinated seeds was after one week later by microscopy. (See Microphoto documentation). For the microscopy evaluation we are used laboratory light microscopy NIKON and inverted microscopy NIKON. For laboratory test we are used plastic plates and each plate contents 24 wells. Dry seeds of cv. Flora prepared according washing process and stored only 1 week after drying. The temperature during the laboratory test in thermostat was 16 ± 1 ° C.

3.1.4. Isolation the causal agent *P. infestans* from leaflets with symptoms of late blight potato/tomato disease in pure culture

For isolation pathogen *P. infestans* utilize international method "CIP-Quito Isolation *Phytophthora infestans* method in laboratory of the Department of Plant Protection of Faculty of Agrobiology, Food and Natural Resources.

3.1.5. Isolation and maintenance of *P. infestans* in pure culture

Leaves and fruits of tomato with lesions were placed into a moist chamber (an inverted Petri dish with water agar). After the visible intensive sporulation mycelium with sporangia was transferred on a small piece of a surface-sterilized potato tuber of cv. Ditta. These pieces of the tuber were put on B rye agar (CATEN, JINKS, 1968) with antibiotics and fungicides. Petri dishes were incubated in the dark at 15 –18° C and re-inoculated every 2-3 weeks to maintain pure uncontaminated pure cultures of *P. infestans*. (See in CIP Quito http://www.cipotato.org).



Příloha č. 4: Postup izolace *Phytophthory infestans*.
(Převzato z http://www.cipotato.org)

Figure. 1 Isolation of *P*. infestans from the leaf to water agar and other steps of of purification of this isolate into pure culture semi artificial substrate in P. dish. according Mazáková 2000 Dipl. Thesis.

3.1.6. Biological test for determination of mating types A1, A2 of patogen *P. infestans*

The mating types were determined by pairing of tested isolates with standard isolate of the known mating type A2 on B rye agar or V8 juice agar. On one side of the Petri dish a tested isolate was placed and on the other side A2 standard isolate was placed. The Petri dishes were incubated at 15–18° C in the dark for 10 days approximately or until the oospores were formed. On the basis of observation of oospores tested isolates were classified as the mating type A1 or A2 (TOOLEY *et al*., 1989). The isolate that produced oospores with A2 standard isolate was characterized as A1 mating type isolate. Isolate that did not create oospores was determined as A2 mating type isolate (Fig. 1,). From group of A1 mating type isolates, one isolate was chosen as A1 standard isolate and all isolates were paired with this isolate to confirm mating type classification. Every isolates were again examined with standard A1 and A2 that was obtained from Scottish crop research institute in Dundee in 2004 by Miss. J. Mazáková from the Department of Plant Protection Czech University of Live Sciences in Prague.

3.1.7. Detection of oospores in the tissue of naturally infected tomato leaves

For this test we are used circle segments (15mm of diameter) tomato naturally infected leaves by *Phytophthora infestans* in glasshouse of ITS University of Live Science at Prague. These circle segments were boiled (5-10 minutes in 96% of ethanol bath water) to achieve clear microscopy field for determination of oospores under 40x magnified by Microscopy TMS Nikon (Cohen et al., 1997).

4. Results

4.1 Testing tomato seed if are infested by *P. infestans*

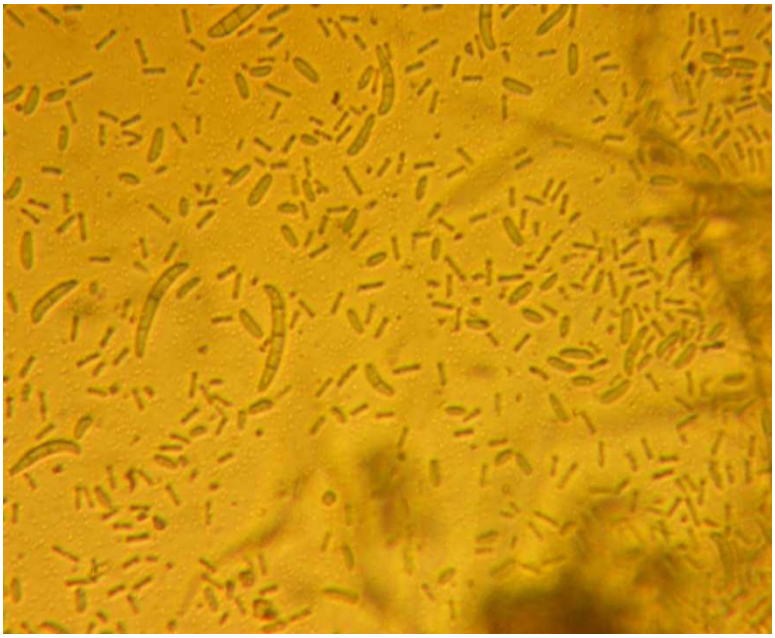
In the following table are results of the laboratory test for the detection of *Phytophthora infestans* in the seed of tomato harvesting from the tomato fruits infested by this pathogen. In this table you can see a situation about possibility seeds of tomato disseminate infection of *P. infestans.*

Table 2. The results of the laboratory experiment with pathogen *Phytophthora infestans* **presented after germinated in the seed of tomato on the floated disc from tomato leaves**

No. of Plate with 6 x 4 wells	No. of germination seeds	No. of not germination seeds	Brown Color of mycelium round seed	White Color of mycelium round seed	Presence of sporangium *P. infestans*
1	20	4	10	14	-
2	24	0	16	8	+ prim. inf.
3	12	12	14	10	+ prim. inf.
4	17	7	12	12	-
5	18	6	13	11	+? Contaminate
6	23	1	14	10	-
7	24	0	19	5	-
8	19	5	18	6	-
9	12	12	7	17	-
10	15	9	5	6	-
11	19	5	17	2	-
12	12	12	8	3	-
13	11	13	5	9	-
14	17	7	17	7	-
15	20	4	17	7	-
16	18	6	12	12	-
17	19	5	17	7	-
18	23	1	8	16	-
19	16	8	7	17	-
456wells	339	117	236	173	2
100 %	74,3 %	25,6 %	51,7 %	37,9 %	0,43 %

In the laboratory test we were used 456 seeds of tomato plants cv. Flora that are growing under plastic tunnel and were naturally infested with pathogen *P. infestans.* During second half of September and at the beginning of October the late blight disease of tomato was very intensive mainly on the fruits. From these fruits damage by late blight disease we have receive above mention seeds for laboratory test. According the results of laboratory test we can see,

that the seeds germinated on level 74,3 %, while seed not germinated was the rest, that is one four. Also contamination of the seeds was very high

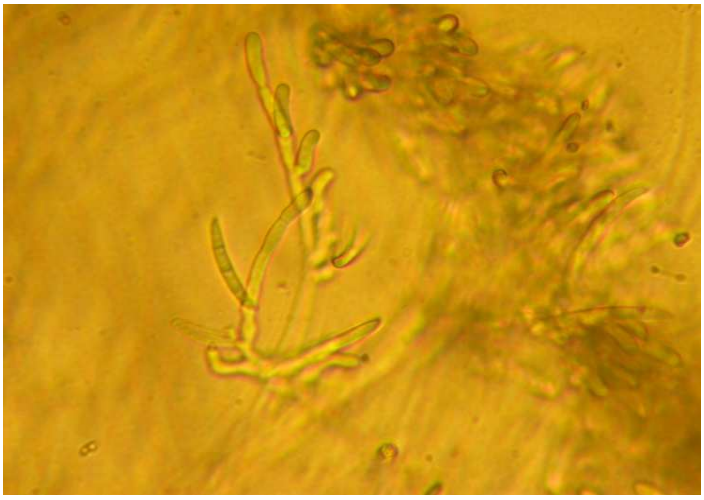


Microphoto 1 Contaminant of the tomato seeds (*Fusarium culmorum* and other different spore and mycelium

89.6 %, and affected both not germinated seeds and germinated seeds. Only, just less 10 % germinated seeds germinate without surroundings mycelium. Characteristic one cell sporangium-spores and without septum mycelium of *P. infestans* was present only in two cases that is only 0.43%. This result is corresponding with general agreement, that pathogen *P. infestans* is not seed-born with tomato seeds. A tomato seed is 3 to 5mm in size, silky in appearance, flat, and light cream to brown in color. Seed size is determined by both fruit type and degree of viability. Mature seed can remain viable for up to 4 years in hermetically sealed containers at seed moisture content 5.5%.

Prevail dark spores and mycelium was from *Alternaria* spp., *Botrytis cinerea Cladosporium* dark mycelium of *Rhizoctonia* spp., and prevail white spores and mycelium was *Fusarium* spp. with specific form of spores, white mycelium of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (mycelia sterilia).

Microphoto 2. *Alternaria* spp. Spores growth around tomato seed



Microphoto 3. Fusarium oxysporum spp. growth around tomato-seed

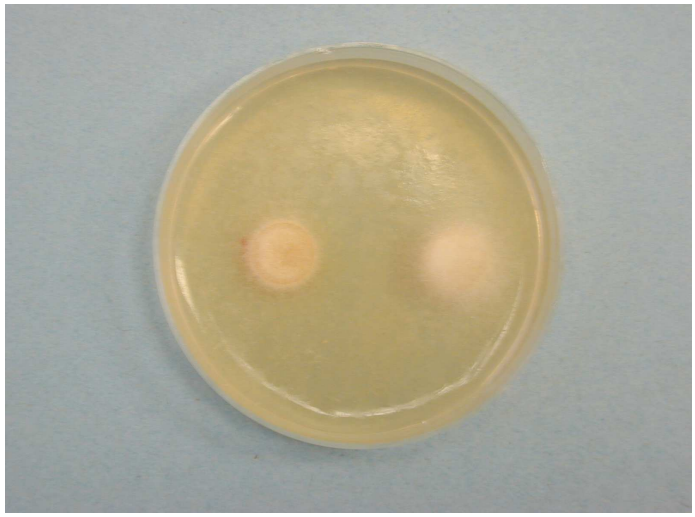
4.2. Isolation *Phytophthora infestans* in pure culture from tomato crop growing in plastic tunnel

By collection of sample leaves and fruit of tomato with the symptoms of late blight diseases, for isolation, serial geniculation and losses caused by bacterial and fungal contamination, we are received 4 isolates in pure culture; 2 isolates originated from infected tomato leaves and 2 isolate was from infected tomato fruit. On the Microphoto 1, we can see characteristic sporangia, mycelium without septum and sporangiospores.

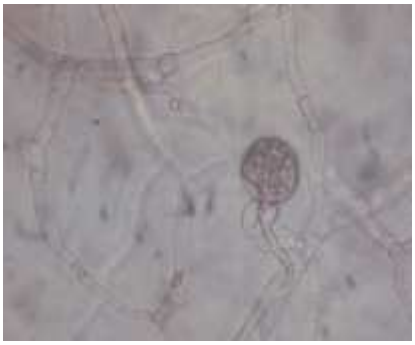
Microphoto 4. Sporangiospores of *P. infestans*

4.3. Biological test for determination of mating types A1, A2 of *P. infestans* isolate from tomato plant in plastic tunnel

All four isolates were used in biological test. A1 mating type was found in all cases. These findings corresponding with present knowledge of biological and diseases cycles of *P. infestans*, which is not seed-born and it is not mix mating types A1, A2 presented together in the location of local tomato crop. The same result received in the year 2006 and 2007 (Mazáková et al. 2007).



Microphoto 5. Pairing between mating types A1 + A2



Microphoto 6 and 7. After pairing of maiting types A1, A2 mycelium created oospores (Microphoto 6. detail of oospores)

5. Discussion

Causal agent of Late blight disease of potato and tomato *Phytophthora infestans* that is heterothallic organisms and requires two distinct mating types (A1,A2) to undergo sexual reproduction and produce the sexual oospores. Presence and importance of oospores appears to differ depending on where the crops (tomato and potato) are grown. In parts of different countries in Europe (The Netherlands, Poland, Russia) USA, Canada, Mexico, the oospores are more readily found and genetic evidence indicates grater diversity is present in populations, which presupposed sexual recombination. The causal agent is sometimes difficult to maintain in pure culture, though culture can be grown on V-8 juice, rye B agar and other media. Incubating the symptomatic tissue at law temperatures (bellow 20˚ C) and with high humidity will also encourage the causal organisms *P. infestans* to grow and produce sporangia after only one to a few days.

The recent increase in late blight severity coincides with worldwide changes in the populations genetic of *P. infestans*. Recent advance in molecular methods and DNA technology have enabled researchers to precisely characterized isolates and obtain some insight into reasons for this increased severity. Research reveals that *P. infestans* is a very complex organism. While all *P. infestans* isolates belong to one of two mating types, all individuals also simultaneously belong to one of series distinct clone lineages, which are asexual descendants from single genotypes. Prior to the 1980s, the various regions of the world were primarily populated by *P. infestans* isolates, which were of the A1 mating type and overwhelmingly belong to the US-12 clone lineage group that is sensitive to the widely used fungicide metalaxyl.

However, the recent serious outbreaks were often caused by novel lineages. In Nord America, four such lines caused significant damage to crops (potato and tomato): UD-6 MR (A1 mating type; resistant to metalaxyl; highly virulent to tomato), US-7 (A2 mating type; resistant to metalaxyl; highly virulent to tomato); US-8 (A2 mating type; resistant to metalaxyl; highly virulent to potato), and US-11 (A1 mating type; resistant to metalaxyl; highly virulent to potato and tomato) Goodwin et al. (1998). The occurrence and spread of these new pathogen lines is most likely due to the shipments of potato seed tubers, tomato fruit, and tomato transplants between areas and continents. The population genetics are in flux, because new clone lineages replace older ones a few seasons. Significant changes in *P. infestans* population structures have also been documented in Europe, South America, and Asia. These new aggressive isolates can reproduce more rapidly than older genotypes, thereby shortening the disease cycle to 5-7 days under favorable condition. Additional changes in

virulence and fungicide insensitive could occur more frequently in the future because sexual reproduction will likely be more common now, which both mating types are widely distributed. According Mazáková et al. (2006) the same situation is in Czech Republic.

One additionally parameter of *P. infestans* diversity is aggressiveness of isolates are highly virulent on potato. Therefore, *P. infestans* can be further divided up into tomato-aggressive and tomato non-aggressive isolates is present in a tomato field, than crop loss due to late blight is likely to be limited and fungicides may be needed less frequently. There is some evidence that A2 mating type occurs more frequently on tomato than on potato in open field condition Lergad et al. (1995).

Ours results confirm, that pathogen *P. infestans* are not significant seed-born. Only in two cases was presented seed-born of pathogen *P. infestans* by tomato seed on the level 0.43%. This tomato seed-born was obtain in the case by 2 tomato seeds, that we are tested immediately one week later after seed drying. As long as we were tested 336 tomato seeds after storage period time 4 months, we are not prove of pathogen *P. infestans* by tomato seed its provenance is from the tomato late blight fruits. On the contrary Porter and Jones (2004) published, that pathogen *P. infestans* survived in surface water.

6. Summary

The first of aim of this Diploma Thesis was isolation of causal agent of late blight of tomato/potato *Phytophthora infestans* and its isolation is very exacting for who star with its isolation into end of pure permanent culture. All the same is with maintain pure culture for long period time several months.

Also the second aim of my Diploma Thesis is possible do it only with pure culture of both standard mating type A1 and A2 of *P. infestans*, and so new isolates in pure culture too. According our results the pathogen *P. infestans* on tomato is mating type A1.

The third aim of my Diploma Thesis was very hard to do it tomato seed-born test for the patogen *P. infestans*. According our results we can say, that pathogen *P. infestans* is not significantly seedborne by tomato seeds. Sporadic seedborne on the level 0.43 % of pathogen *P. infestans* was determined only by the seed stored after washing two weeks but after storage seed for several months (4) over the winter time this pathogen was not by tomato seed born in any tested of 336 tomato seeds.

7. References

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8. ANNEX

Fruit for isolation tomato seed-born *P. infestans* test

Test of tomato seeds for seed-born *Phytophthora infestans* in plates with 24 well and wrap translucent plastic

Technique of laboratory seed-born test by *Phytophthora* infestans of tomato late blight

Pipet sterile water in each well of plastic

Tomato fruits disease by late blight from which were receive seeds for seed-born test of *P. infestans*

Lay the tomato seed from late blight disease fruit on segment of tomato leaf of each well

Wrap closed experimental plates in translucent envelope for maintain adequate humidity

Microscopy inspection of germination seeds for occurrence of sporangia *P. infestans*

Mycelium is hyaline and coenocytic, sporangiophores and sporangia of *P. infestans*

Tomato experimental plant growth in absence of *P. infestan*s for seedborne test