

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

People's Democratic Republic of Algeria

Ministry of Higher Education

And Scientific Research

جامعة محمد الصديق بن يحيى جيجل

Faculty of Life and
Nature Sciences
Departement of
Environmental Sciences
and Agronomic Sciences



كلية علوم الطبيعة والحياة
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End of Study Memory

In Order to Obtain the Degree of Academic Master in Environmental
Sciences and Agronomic Sciences

Option: Applied Phytopharmacy

Title :

**Contribution to the Study of Diseases Caused by
*Phytophthora spp . in Citrus***

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School Year 2020-2021

Session : September 2021

Reference Number (Library):

Acknowledgement

I am very grateful to my supervisor, Professor Bouziane.Z., for her guidance, encouragement, patience, advice, ideas, constructive criticism, support and help throughout my thesis. I would like to thank the jury members Mr kisserli.O., and Mrs Benabdalkader.M., to have agreed to chair this jury.

I am indebted to the staff and students in the Departments of Environmental Sciences and Agronomic Sciences.

Special thanks should be made of my wonderful and amazing friends and my parents whose continuous patience, encouragement and support have made this thesis a certainty.

I would like to thank my family especially my old sister for her hope in me to do something in my life despite my all disappointments, relatives and friends and my mother and my father for their encouragement and prayers.

Dedication

I dedicate this modest work to my dear parents and grandparents who have always been there for me, who supported me and encouraged me all along my course.

To my dear friends and comrades: Lamia, Houda, Masouda , Amira, Sara, Khadidja, Somia, Yasmin, Salwa, Samira, Iman, and Rahma.

To all promotion M2 Phytopharmacie , for all the unforgettable moments that we shared during my cours.

To all those who helped me closely or far

To all those I have forgotten.

Table of contents

Table of contents

Acknowledgement	i
Dedication	ii
List of Tables	iii
List of Figures	iv
List of Abbreviation	vi
General Introduction	1

CHAPTER I : CITRUS CROPS

I.1. The History and Origin of Cultivated Citrus	3
I.2. Citrus Rootstocks and their Characteristics Historically	4
I.3. Genetic Classification of Citrus	5
I.4. The Botanical Character of The Genus Citrus	6
I.5. Citrus Fruits	6
I.5.1. Growth and Development of Citrus trees	7
I.5.2. Growth and Development of Citrus Fruits	8
I.5.3. Factors Influenced Citrus Growth and Citrus Quality	8
I.5.3.1. Temperature	8
I.5.3.2. Soil Moisture and Soil Solubility.....	8
I.5.3.3. Cultural Practices	8
I.5.3.4. Nutriments Influencing Fruit Growth and Fruit Quality.....	9
I.5.3.5. Other Properties of Soil Which Affect Citrus Fruits Growth	9
I.5.5. Nutrient Composition of Citrus Fruit	10
I.5.6. Some Benefits of Citrus Fruits	10
I.5.7. International Production of Citrus	11
I.5.8. Major Cultivar	12
I.5.9. Production of Citrus Fruits in Algeria	13

CHAPTER II: *PHYTOPHTHORA SPP.*

II.1. The History and Origin	15
II.2. The Genus <i>Phytophthora spp.</i>	15
II.3. Evolutionary Placement.....	16
II.4. Variability of <i>Phytophthora spp.</i>	16
II.4.1. Life Cycle	17
II.4.2. Host Range	18
II.4.3. Mating System.....	18
II.5. Identification of <i>Phytophthora spp.</i>	19
II.5.1. Taxonomic Key	19
II.5.2. Cultures	20
II.5.3. Morphological Character	21
II.5.4. Sporangia	22
II.5.5. Chlamydospores and Hyphal Swellings	22
II.5.6. Sexual Structure	23
II.6. Pathogenicity of <i>Phytophthora spp.</i>	23

CHAPTER III : *PHYTOPHTHORA SPP. DISEASE ON CITRUS*

III.1. Pathogen Contributing to Replant Disease on Citrus Trees	25
III.2. Diseases by <i>Phytophthora spp.</i>	25
III.3. Causal Agents of <i>Phytophthora spp.</i>	25
III.3.1. <i>Phytophthora nicotianae</i>	26
III.3.2. <i>Phytophthora citrophthora</i>	26
III.4. The Disease Cycle and Epidemiology	26
III.5. Symptomatic Diagnosis	28
III.5.1. Dumping off of Seedlings in Citrus Tree	28
III.5.2. Foot Rot Gummosis	29
III.5.3. Fibrous Root rot	30

III.5.4. Brown Fruit rot	31
III.5.5. Dieback of Twigs and Leaves	33
III.6. Means of Spread of <i>Phytophthora</i>	33
III.7. Soil and Environmental Factors that's Help <i>Phytophthora</i> to Evaluate and Survive ..	34
III.7.1. Soil Moisture	35
III.7.2. Soil Aeration	35
III.7.3. Soil Temperature	35
III.8. Economic Importance	36
Conclusion.....	37
Bibliographic references	39
Summary	

List of tables

LIST OF TABLES

Table.1. Genetic Classification of Citrus.....	5
Table.2. World Citrus Production and Utilisatioin 2000 /01(thousands of tons).	12
Table.3. Production of Citrus Fruits in Algeria from 2008 to 2019.	14
Table.4. Classification of the Oomycetes.	16
Table.5. Classifcation of <i>Phytophthora</i> into six Groups by Waterhouse (1963).	20

List of figures

LIST OF FIGURES

Figure 1. Different types of citrus fruits around the world.	4
Figure 2. Citrus fruits anatomy of different type.....	7
Figure 3. Life cycle of <i>Phytophthora spp.</i>	18
Figure 4. Antheridial attachment ,(A) paragynous, (B) amphigynous. Illustration from	20
Figure 5. Sporangium shape.	21
Figure 6. Sporangium papillation,(A) non-papillate, (B) semi-papillate and (C) papillate sporangia. Photographs from.....	21
Figure 7. Sporangiphore morphology Simple sympodium (left) Compound sympodia (middle) Umbellate sympodium (right).	22
Figure 8. Chlamydo spores and hyphal swellings produced by <i>Phytophthora spp.</i> prodigiosa. (A-C-D). Globose, small, sessile chlamydo spores on V8A, attached laterally to the hypha and with a thin wall. (B-E) Hyphal swellings of <i>Phytophthora spp.</i>	23
Figure 9. Disease cycle of <i>Phytophthora spp.</i> on citrus.	27
Figure 10. Coorg mandarin plant showing Coorg mandarin plant showing (A) -foot rot, (B) gummosis, (C) collar rot and (D) trunk slit.	28
Figure 11. Damping off caused by <i>phytophthora spp.</i> ((A) before the injury, (B) the beginning of injury, (C) after injury and damage and damping off).	29
Figure 12. Gummosis on citrus trees. (A) Gummosis on the main branch of sweet orange (citrus sinensis) tree and (B) tree dieback along the gummosis symptom on the main branch at Hurso 2011(Photo courtesy of Moges Mekonen).	30
Figure 13. Typical discoloration under the lemon's tree bark of the trunk of an active lesion caused by <i>P.nicotianae</i>	30
Figure 14. Typical gummy lesions caused by <i>Phytophthora nicotianae</i> in citrus bark.	30
Figure 15. Fibrous roots caused by <i>Phytophthora spp.</i> ,(A) decay of fibrous roots,(B) damaged roots of citrus tree,(C) roots are damaged and cause the tree stopped growing.	31
Figure 16. Brown Root Caused by <i>Phytophthora Citrophthora</i>	32
Figure 17. Sweet orange fruit with symptoms of brown rot caused by <i>Phytophthora citrophthora</i>	32
Figure 18. Brown Root Caused by <i>Phytophthora Citrophthora</i> on lemon's fruit.	32
Figure 19. Brown rot of orange fruit caused by <i>Phytophthora</i> species. Early symptoms are shown in the upper images. Late symptoms are shown for brown, leathery decay with a	

distinct pungent odor (bottom, left) and brown rot decayed fruit at high humidity with white mycelium on the fruit surface (bottom, right). 32

Figure 20. Brown rot of lemon fruit caused by *Phytophthora* species. Early symptoms are shown in the upper images. Late symptoms are shown for brown, leathery decay with a distinct pungent odor (bottom, left) and brown rot decayed fruit at high humidity with white mycelium on the fruit surface (bottom, right). 32

Figure 21. Dieback of twigs and leaves in citrus, (A) trees branches and leaves corrupt of citrus tree, (B) damaged branche,(C) decay of leaves and fully damaged. 33

List of abbreviation

LIST OF ABBREVIATION

TSS: Total soluble solids.

BPF: Bergamot Polyphenol Fraction which is a natural mixture of citrus flavonoids extracted from processed bergamot fruits.

CaCO₃: Calcium carbonate.

N: Nitrogen

General introduction

Introduction

The genus *Citrus*, one of the most important groups of fruit crops worldwide, belongs to the family Rutaceae comprising 140 genera and 1300 species distributed throughout the world. It is a long-lived perennial crop and is grown in more than 100 countries across the world (Swingle & Reece, 1967). Citrus is the third most important fruit crop in the world after apple and banana and accounts for the production of about 100 million tons with massive area of cultivation (Choudhari & *al.*, 2018).

Citrus is largest evergreen fruit crop in world trade. Its internal structure and long shelf life have facilitated its large-scale export as fresh fruit. Processed juice products have also become increasingly important worldwide, (Robbins, 1990) mentioned that citrus, best known for its vitamin C content, is generally perceived as a kind of health food. Thus, citrus fruits play an important role in human health. Slices of lemon are served as a garnish on fish or meat or with iced or hot tea. Lemon juice is primarily used for flavoring cakes, cookies, cake icings, puddings, sherbet, preserves and pharmaceutical products. The rind of the fruit and leaves are used for the production of oil, which is used in cosmetics industries. It is also the source of pectin and citric acid (Alam & *al.*, 2003).

Citrus orchards in Algeria cover an area of approximately 66 000 ha and are located mainly along the coastal zones (MADRP, 2016). According to Aouane & Ghezli (2001), citrus culture occupies a primary place and is one of the major concerns of decision-makers at the level of the Algerian Ministry of Agriculture. The INRAA in 2006 reported that Algeria holds a varietal collection composed of 277 citrus varieties.

Unfortunately, citriculture has been seriously damaged by many fungal diseases like *Phytophthora spp.* which it is perhaps the most important fungal diseases (MADRP, 2016). At least ten species of *Phytophthora* have been reported to attack citrus in the world, but the commonest species in commercial citrus orchards in Algeria are *P. citrophthora* and *P. nicotianae*.

Various factors are responsible for lowering the yield of citrus. Among the factors, plant diseases play an important role in lowering yield as well as citrus decline. Among these diseases, we find fungal diseases of citrus include root rot, collar rot, foot rot and gummosis. Fungal pathogens such as *Rhizoctonia solani* Kuhn and *Pythium spp.* occasionally cause some damage but *Phytophthora spp.* can kill citrus trees (Klotz, 1950). *Phytophthora* infections of citrus, caused by a number of *Phytophthora spp.* are probably the most widespread and serious diseases of citrus crops at all stages of development. There are at least

six *Phytophthora* spp. capable of infecting citrus plants, but the greatest reduction in fruit yield may be attributed to the following two spp:

Phytophthora parasitica Dast. (mostly in tropical and subtropical regions) and *Phytophthora citrophthora* . Leonian (mostly in temperate climates) (Timmer & Menge, 1988).

Phytophthora has a long history as a major problem in citrus production regions throughout the world. Outbreaks of the disease occurred in groves in Australia in 1860 and later in Florida in 1952 (Broadbent, 1977). In South Africa considerable loses to the citrus industry are caused by root pathogens including *Phytophthora* spp. (Kotze, 1984). On Elabered Estate Farm, Eritrea, the pathogen is a threat, although no formal assessment has been done (Menghisteab, 2001). During field visits to farms in KwaZulu-Natal, *Phytophthora* species was observed to be a threat to citrus tree health.

The objective of this study is to show the important of citrus in our life and to determine the different diseases caused by *Phytophthora* and to describe their symptoms on the citrus. On other hand, to set up the macroscopic and microscopic characters and the infectious mode of *Phytophthora*, in order to suggest a promising solution in the near future to minimize the phytosanitary problems associated with this pathogen.

This brief is divided into three chapters, except for the introduction and conclusion. The first chapter gives general considerations on citrus; the second and third relate to the description of the pathogen (*Phytophthora* spp.) and their infectious process followed by the various diseases associated with this host.

Chapter I : Citrus crops

I.1. The History and Origin of Cultivated Citrus

Citrus are some of the oldest cultivated tree crops (Ray & Walheim, 1980). Castle (1987) stated that earliest mention of citrus was in Chinese literature. which shows that citrus trees have been cultivated for over 4000 years. The center of origin of citrus is believed to be Southern Asia from eastern Arabia, east to the Philippines, and from the Himalayas, south to Indonesia. Within this large region, northern India and northern Burma were believed to be the primary center of origin, but recent evidence suggests that Yunnan province in South-central China may be as important due to the diversity of species found and the systems of rivers that could have provided dispersal to the South (Spiegel-Roy & Goldschmidt, 1996). However, the exact origin of citrus, and its ancestral types and systematics, are still unknown.

Extensive movement of the various types of citrus probably occurred within the general area of origin before recorded history. Many types of citrus are believed to have moved west to Arab areas, such as Oman, Persia, Iran, and even Palestine before Christ (Spiegel-Roy & Goldschmidt, 1996). Ray & Walheim (1980) characterized the movement of citrus to the west in one statement saying, "citrus followed the Cross". The report by Spiegel-Roy & Goldschmidt (1996) supported this idea and stated that the establishment of mission stations by the Roman Catholic Church aided the movement of citrus to North America. These missions established plantings of various fruit, including citrus, (particularly limes and oranges), which were introduced to South America by the Spanish and Portuguese settlers, and missionaries accompanying them. Major types of citrus include (Davies & Albrigo, 1994) (see figure 1):







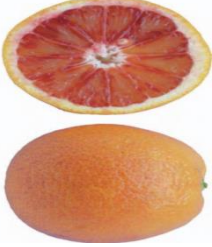


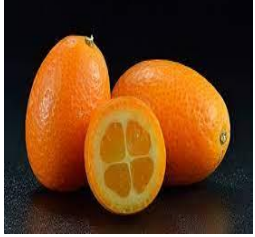
				
<i>Lime (Citrus aurantifolia Swingle)</i> (OCDE, 2010)	<i>Shaddock or Pummelo (Citrus grandis [L.] Osbeck)</i> (OCDE, 2010)	<i>Citron (Citrus medica L.)</i> OCDE 2010	<i>Lemon (Citrus limon Burmann)</i> (Zech-Matterne & Fiorentino, 2017)	<i>orange (Citrus aurantium L.)</i> (Zech-Matterne & Fiorentino, 2017)
				
<i>Sweet-orange (Citrus sinensis [L.] Osbeck)</i> (Zech-Matterne & Fiorentino, 2017)	<i>Grapefruit (Citrus paradisi Macf)</i> (OCDE, 2010)	<i>Trifoliate orange (Poncirus trifoliata [L.] Raf.)</i> (OCDE, 2010)	<i>Mandarin (Citrus reticulata Blanco)</i> (OCDE, 2010)	<i>Kumquat (Fortunella margarita [lour.] Swingle)</i> (OCDE, 2010)

Figure 1. Different types of citrus fruits around the world (Davies & Albrigo, 1994)

Citrus is largest evergreen fruit crop in world trade. Its internal structure and long shelf life have facilitated its large-scale export as fresh fruit. Processed juice products have also become increasingly important worldwide (Spiegel-Roy & Goldschmidt, 1996). Ray & Walheim (1980) mentioned that citrus, best known for its Vitamin C content, is generally perceived as a kind of health food.

I.2. Citrus Rootstocks and Their Characteristics Historically

Citrus trees have been propagated from seedlings, due to the ease with which seeds can be propagated, and their convenience for transport as citriculture has expanded worldwide (Ray & Walheim, 1980; Castle, 1987; Davies & Albrigo, 1994; Spiegel-Roy & Goldschmidt, 1996). These authors identify the following disadvantages of propagation by seedlings:

1. Seedlings are thorny and have a relatively long juvenile period, which delays the first commercial harvest.

2. Once they enter their bearing phase, cropping can be erratic. Therefore, it takes longer to obtain a positive cash flow.

Despite these disadvantages, citrus seedlings were commonly used in much of the world until the mid-1800s (Castle, 1987). Root rot or foot rot (*Phytophthora spp.*) was recognized as a major disease of trees in the Azores in 1842, an event which initiated the practice of budding trees onto tolerant rootstocks. As *Phytophthora* spread to all major producing countries, so too did the need to use budded trees as a means of combating the disease (Castle, 1987). *Phytophthora* foot rot spawned a search for resistant rootstocks, and initially sour orange became dominant. However, difficulty was encountered with this rootstock in South Africa and Australia. Trees declined within a few years after planting because of a disease identified as Citrus *Tristeza* Virus (CTV). As a result rough lemon rootstock became popular in both countries and was adopted with considerable success (Castle, 1987). Rootstocks are now widely recognized for their favorable effects on tree health and horticultural traits. The subject has been reviewed by some authors: Batchelor & Rounds (1948), Castle (1987). Davies & Albrigo (1994) noted that rootstock selection is a major consideration in citrus growing operations, given that more than 20 horticultural and pathological characteristics are influenced by it. They also pointed out that there is no perfect rootstock. The choice of rootstock should be based on the most important limiting factor(s) to production in a particular region, local climate and soil conditions, cultivars and intended use (fresh or processed) of the crop and disease situation(s). The detailed description of the major citrus rootstocks related to their nursery characteristics, influence on tree growth, production and fruit quality, and response to various disease and soil factors.

I.3. Genetic Classification of Citrus

In our next table we have the genetic classification of citrus:

Table.1. Genetic Classification of Citrus (Swingle, 1948)

Family	Rutaceae
Subfamily	Aurantioideae
Tribe	Citreae
Subtribe	Citrinae
Genus	<i>Citrus</i>
Species	<i>Progenitors - Swingle - Hodgson- Tanaka</i>
Family	Rutaceae
Subfamily	Aurantioideae
Tribe	Citreae
Subtribe	Citrinae
Genus	<i>Citrus</i>
Species	<i>Progenitors - Swingle - Hodgson- Tanaka</i>

I.4. The Botanical Character of The Genus *Citrus*

Swingle 1948, describes the general distinctive character of the genus *Citrus*. These are small trees whose young twigs are cylindrical and thorny but their elderly branches are frequently in termed thin and non-tailed with the limb, except in the case of the species *C. media*.

The flowers are lonely or in small conymbiform cluster, perfect or male by abortion more or less pistil, calice at 4 or 5 sepals, the number of the stamens is usually four times higher than that of the petals and sometimes 6 to 10 times more. The ovary is more globular and distinct from thin, or truncatd, fusiform or sub cylindrical style progressively passing to a thick style close to that of the upper part of the ovary. The style ends abruptly in a subglobulous stigma or flattened sphere.

The fruit is formed of segments containing seeds placed in the inner corner. The segments are surrendered by a white endocarp outside which there is a bark with many gasoline glands, becoming yellow or orange mature (Meziane, 2013).

I.5. Citrus Fruits

I.5.1. Citrus Fruits Anatomy

Anatomically, citrus fruits are superior ovaries composed of 6 to 20 united carpels which form locules. The pericarp exterior to the locules is subdivided into the exocarp (flavedo or exterior peel), mesocarp (albedo or interior peel) and endocarp (locule or segment membrane). The juice vesicles, which are the edible portion of citrus fruit and therefore of economic value, arise from epidermal or subepidermal primordia on the surface of the endocarp and grow to fill the locular cavity (Roth, 1977) (see figure 2).

<p>Anatomy of citrus fruits (lemon) (Rafiq & <i>al.</i>, 2018).</p>	<p>Orang fruit anatomy (Iglesias, 2007).</p>	<p>Diagrammatic equatorial cross-section through a citrus fruit. (From (Koch & <i>al.</i>, 1986; McCready, 1977).</p>

Figure 2. The anatomy of different Citrus types

I.5.2. Growth and Development of Citrus Trees

Flowering in citrus lasts for a month or so under subtropical climate. There are several flushes under tropical climates and citrus trees flower year-round. Out of several thousand flowers that open, very few (2.5–5.4 percent) set fruit, as observed in Valencia oranges (Bain, 1958). ‘Nagpur’ mandarins and many other commercially grown citrus fruits are similar. Setting also depends on climatic factors, as high to very high temperatures (about 38–40°C) and dry spells during and after set may lead to drop of fruitlet. The duration of growth and maturation varies with variety. The growth and development of a citrus flower’s ovary into a fruit ready to harvest takes 6–18 months or more depending upon the type of fruit, particular cultivar, and climate. For early varieties such as Hamlin and Navels, harvesting commonly starts 6–7 months after bloom, whereas for the late Valencia variety, harvesting starts about 12 months after bloom. Harvesting also continues during a ‘tree storage’ period that lasts several months after the maturity of fruit (Bain, 1958). The late oranges and grapefruits have two crops on the tree at the same time: both the fruitlets and the bloom of the new crop and the mature fruits of old crop can be seen (Bain, 1958). The growth of citrus can be shown as a single sigmoid curve: two stages of slow growth with a period of rapid growth in between. Usually early growth is marked by cell division followed by cell expansion. Washington Navel fruit completes growth within 245 days from anthesis to maturity, while Valencia

oranges take 413 days. The growth rate (g weight/day) was recorded 1.22 and 0.36 in Washington Navel and Valencia respectively (Bain, 1958).

I.5.3. Growth and Development of Citrus Fruits

Citrus fruit mature on the tree for six to twelve months (longer in the cooler subtropics). Growth and development of citrus fruit occurs in three stages. From four to nine weeks after fruit set, fruit size and weight increase due to growth of the peel by cell division and enlargement. Then, in the cell enlargement period, the fruit increases in size by cell enlargement alone as the albedo tissue differentiates and expands. Finally, in the maturation period, juice sac acids decrease, while the peel turns from green to yellow or orange and increases slightly in thickness (Bain, 1958).

I.5.4. Factors Influenced Citrus Growth and Citrus Quality

I.5.4.1. Temperature

In subtropical climates, the temperature usually falls below 70°F for several months during winter. This period of cool temperatures causes growth to cease and citrus trees to become dormant for about three months. The cool temperatures during this dormant period promote floral induction. When warm spring temperatures, among other things, stimulate the resumption of vegetative growth, induced buds grow and produce flowers. In tropical climates, there is no period of cold temperature to induce dormancy (Zekri, 2011).

I.5.4.2. Soil Moisture and Soil Solubility

However, with periods of less than ample soil moisture (drought stress), flushes of bloom and vegetative growth normally follow these drought periods (Yaalon, 1957). It is well documented that vegetative and reproductive fruit growth competes for available resources, such as carbohydrates (sugars) and mineral nutrients. Flushes of heavy vegetative growth will reduce the resources available to developing fruit, resulting in fruit with lower total soluble solids (TSS) (Yaalon, 1957). A period of dormancy, during which there is little or no vegetative growth, reduces this competition for resources and results in fruit with increased TSS. The competition for resources between vegetative and reproductive growth is one of the reasons that citrus fruit grown in tropical climates tend to have lower TSS than that grown in subtropical climates (Yaalon, 1957).

I.5.4.3. Cultural Practices

Although citrus trees develop largely in response to their genetic endowment and the climate, good production practices can have favorable influences on fruit production and quality. Cultural practices that attempt to cope with climatic or weather problems include irrigation and nutrition. Irrigation is of particular importance during the spring, which

coincides with the critical stages of leaf expansion, bloom, fruit set and fruit enlargement. Proper irrigation increases fruit size and weight, juice content and soluble solids: acid ratio. Soluble solids per acre may increase due to yield increase (Srivastava & Ram, 2000). However, soluble solids per box and acid contents are reduced. Through its tendency to stimulate vegetative growth, irrigation in the dry fall and winter may reduce soluble solids in the fruit. Decline in total acid levels can also be aggravated by excessive irrigation. Citrus trees require a good water management system and a balanced nutrition program formulated to provide specific needs for maintenance and for expected yield and fruit quality performance. Adequately watered and nourished trees grow stronger, have better tolerance to pests and stresses, yield more consistently and produce good quality fruit (Srivastava & Ram, 2000).

I.5.4.4. Nutriment Influencing Fruit Growth and Fruit Quality

The most important nutrients influencing fruit quality are nitrogen, phosphorus and potassium. However, when any other nutrient is deficient or in excess, fruit yield and quality are negatively altered. Nitrogen (N) increases juice content, TSS per box and per acre, and acid content. However, excessive N can induce excess vigor and promote a vegetative rather than a flowering tree, and can result in lower yields with lower TSS per acre. In contrast, low N levels promote extensive flowering, but fruit set and yields are poor. Phosphorus reduces acid content, which increases soluble solids: acid ratio. Potassium (K) increases fruit production, fruit size, green fruit and peel thickness. Foliar spray of potassium nitrate or monopotassium phosphate in the spring often increases fruit size of tangerine and grapefruit, and fruit size and total pound solids of Valencia orange. Foliar application (six to eight weeks before bloom) of urea can increase flowering and fruit set.

Even though citrus trees can tolerate shade and still flower and fruit, maximum flowering occurs when trees are grown in full sun and light penetration through the canopy is maximized (Zekri, 2011).

I.5.4.5. Other Properties of Soil which Affect Citrus Fruits Growth

Also we have soil salinity Citrus trees are quite sensitive to excess salts (Srivastava & Ram, 2000), and tolerance to soil salinity is correlated with its ability to restrict the entry of toxic ions [sodium (Na), chlorine (Cl), and boron (B)] into roots and onward transport to shoots. And soil PH which it one of the properties that dictates the nutrient availability. Soil pH range of 5.5–6.0 it's usually appropriate, we have also soil acidity ironically, soil acidity is the major production constraint but the world's top citrus production is obtained from those soils (Srivastava & Singh, 2001a). Soil acidity is defined as a soil system having proton-

yielding capacity during its transition from a given state to a reference state. The soil acidity is partitioned into pH-dependent acidity and exchange acidity collectively known as total acidity. And we have Calcareousness is characterized by the presence of calcium carbonate (CaCO₃) which has relatively high solubility, reactivity, and alkalinity (Yaalon, 1957).

I.5.5. Nutrient Composition of Citrus fruit

The composition of citrus fruit is affected by factors such as growing conditions, maturity, rootstock, variety and climate (Kale & Adsule, 1995). Citrus fruits contain N (1–2 g/kg on a wet basis), lipids (oleic, linoleic, linolenic, palmitic, stearic acids, glycerol, and a phytosterol), sugars (glucose, fructose, sucrose), acids (primarily citric and malic, but also tartaric, benzoic, oxalic, and succinic), insoluble carbohydrates (cellulose, pectin), enzymes (pectinesterase, phosphatase, peroxidase), flavonoids (hesperidin, naringin), bitter principles (limonin, isolimonin), peel oil (d-limonene), volatile constituents (alcohols, aldehydes, ketones, esters, hydrocarbons, acids), pigments (carotenes, xanthophylls), vitamins (ascorbic acid, Vitamin B complex, carotenoids), and minerals (primarily calcium and potassium) (Ammerman & Henry, 1991). The nutrient content of citrus BPF is influenced by factors that include the source of the fruit and type of processing (Ammerman & Henry, 1991).

I.5.6. Some Benefits of Citrus Fruits

Citrus is full of nutritional benefits, it is source of vitamin-C which help in absorption of iron, zinc and other foods. As compared to other fruits, citrus has higher contents of antioxidants which catalysed our immune system and also protects us from heart diseases and cancer (Baghurst, 2003). Citrus fruit contains good quantity of dietary fibre which improves food digestion and protect from constipation. Low level of sodium and high content of potassium are good to maintain normal blood pressure. Rich source of phytochemicals which protect human body from cancer, blood clotting and heart diseases as well as have anti-inflammatory properties (Baghurst, 2003). Flavonoids which are good against allergies, viral and fungal infection, inflammatory and heart diseases are also component of citrus fruit. Folate is a component of citrus fruit which helps in genetic stability and protect children from neural tube problem. Citrus fruit is good source of polyphenols which are good against viral infection and have anti-carcinogenic, anti-proliferative and anti-inflammatory properties (Baghurst, 2003).

I.5.7. International Production of Citrus

Citrus fruits rank first in the world with respect to production among fruits. They are grown commercially in more than 50 countries around the world. In addition to oranges, mandarins, limes, lemons, pummelos, and grapefruits, other citrus fruits such as kumquats, Calamondins, citrons, Natsudaidais, Hassakus, and many other hybrids are also commercially important. The contribution of the citrus industry to the world economy is enormous (estimated at more than 10 billion US\$ annually) and it provides jobs to millions of people around the world in harvesting, handling, transportation, storage, and marketing operations. Citrus fruit production recorded a handsome increase during the 1990s, and recently reached more than 100 million tons. Considering the therapeutic value of these fruits and the general health awareness among the public, citrus fruit are gaining importance worldwide, and fresh fruit consumption is likely to increase. Postharvest biology and technology has evolved into a branch of science that combines biology and engineering. It has evolved rapidly over the past four or five decades, although scattered research efforts in various aspects of this field have been made previously all over the world (see table.2). Increased citrus production combined with concern about growing population accelerated research and stimulated the development of new technologies in basic and applied areas (Ladanyia & Ladaniya, 2010).

Table.2. World Citrus Production and Utilisation 2000/01 (thousands of tons) (FAO, 2004)

	Totale citrus	Exports (fresh fruit)	% Totale Export (fresh fruit)	Processed	%Totale processed
World	89071	9423	11.0	27439	31.0
Northern Hemisphere	63569	7705	9.0	15754	18.0
USA	14049	1084	1.0	10969	12.0
Mediterranean Region	17779	5243	6.0	3025	3.0
Greece	1229	423	0.5	307	0.3
Italy	3011	232	0.3	1362	2.0
Spain	5401	2859	3.0	704	1.0
Israel	630	194	0.2	314	0.5
Mrocco	965	393	0.5	40	0.04
Egypt	2508	226	0.3	115	0.1
Cyprus	193	98	0.1	40	0.05
Turkey	1902	449	0.5	102	0.1
Mexico	6143	277	0.3	728	1.0
Cuba	884	72	0.04	671	1.0
China	8783	164	0.2	202	0.2
Japan	1504	5	0.005	119	0.1
Southern Hemisphere	25502	1718	2.0	11686	13.0
Argentina	2808	413	0.5	1136	1.0
Brazil	16498	154	0.2	9846	11.0
Uruguay	339	115	1.0	54	0.06
Australia	563	181	0.01	190	0.2
South Africa	1527	757	1.0	457	05

I.5.8. Major Cultivars

Oranges constitute approximately 65% of the world's citrus production followed by mandarins at 19%, lemons and limes at 11% and grapefruit at 5%. The most popular orange varieties are the Navel and 'Valencia'. Other important orange varieties include 'Hamlin', 'Pera' and 'Salustiana' oranges. Clementine mandarins are highly favored by consumers for their seedlessness, portability and ease of peeling. Pigmented grapefruit is gaining popularity over white varieties, with the 'Ruby Red', 'Rio Red', 'Ray Ruby' and 'Star Ruby' cultivars most common (Ismail & Zhang, 2004).

I.5.9. Production of Citrus Fruits in Algeria

For centuries, citriculture has been considered as the most important fruit crop sector in Algeria and part of its traditional agriculture. Before the French colonisation (1830), more than 22 000 citrus trees, mainly orange trees, were already grown in the Mitidja area. Until the end of the 2nd world war, the Algerian citriculture was considered as one of the most important in the Mediterranean basin and showed fluctuations in production, with a positive peak in 1950 (Rebours, 1950).

Algeria, a major producer and exporter of citrus fruits from the countries of the Mediterranean basin in the past, is experiencing a considerable decline in production due to aging orchards, lack of maintenance and investment (Ibrahim & Bayaa, 1989).

Citrus orchards in Algeria cover an area of approximately 66 000 ha and are located mainly along the coastal zones (MADRP, 2016). The citrus orchards are concentrated in five main areas or wilaya, namely Blida (in the Valley of Mitidja), Mascara, Chlef, Mostaganem and Annaba, which together represent about 80 percent of the total surface of citrus. Furthermore, citrus production represents an important agricultural and economic activity in the country. Oranges and mandarins are traditionally produced for local consumption and also for export

A big part of the Algerian citrus orchards concentrate in the plain of the Mitidja (Algeria), known by its agricultural vocation. The cultivated species are variable (orange, lemon, mandarin, grapefruit,...)(Karima & *al.*, 2018).

In 2019, citrus fruit production for Algeria was 1.58 million tonnes. Citrus fruit production of Algeria increased from 508,168 tonnes in 1970 to 1.58 million tonnes in 2019 (see table.3) growing at an average annual rate of 2.90%. And Algeria considered to be the 8 worldwide in the production of the oranges fruits and limes and lemons (Mascianà, 2015).

Table.3. Production of Citrus Fruits in Algeria from 2008 to 2019 (Mariam, 2021)

DATE	VALUE	CHANGE, %
2019	1,584,098	7.18 %
2018	1,478,022	9.90 %
2017	1,344,861	11.72 %
2016	1,203,775	-10.34 %
2015	1,342,559	5.58 %
2014	1,271,556	5.49 %
2013	1,205,401	10.75 %
2012	1,088,382	-1.71 %
2011	1,107,299	40.41 %
2010	788,607	-6.67 %
2009	844,939	21.09 %
2008	697,768	-

Chapter II: Phytophthora spp.

II.1. The History and Origin

Association of *Phytophthora* in destruction of citrus plants was recorded in 1836 when highly flourishing citrus plants of 200 to 300 years age started disappearing in Azore Island, much before the famous potato famine of Ireland in 1845 and after 31 years of famine, in 1876, Anton de Bary described the fungus as *Phytophthora* means “Plant destroyer”. He described potato late blight fungus, *Phytophthora infestans* (Montag) de Bary as the type species for the new genus. Bonavia (1888) probably reported the first *Phytophthora* epidemic of citrus in Azoreisland during 1832- 1836. Later *Phytophthora* epidemics of citrus were reported in 1841 from France, 1845 in Portugal, 1855-1889 killed all lemon trees in Italy, 1869-1880 killed all lemon and citron trees in Greece, 1860 – 1879 in Australia, 1871 in Spain, 1875 in California, 1876 in Florida, 1906 in Cuba, 1911 in Paraguay, 1917 in Brazil and 1920 in Mexico and in 1935 in Trinidad (Fawcett, 1936). An extensive damage was done by the fungus to the citrus plantation in Florida on rough lemon during 1920 – 1940 and in Australia where citrus plantation was replaced with *Phytophthora* resistant rootstocks like trifoliolate orange and Benton citrange (Broadbent, 1977). Latest evidences and occurrence of certain citrus species in wild forms suggest the origin of citrus in Asia which extends from the Himalayan foothills of North-eastern India to North Central China, Myanmar, Thailand, Indonesia in South-east. Before the invention of the terrarium in 1827 by Dr Nathaniel Ward, probably the citrus is disseminated from its natural home to other parts of the world through seeds. Thus the seedling trees were virtually free from viruses and *Phytophthora*, having highly productive life up to 200 to 300 years or more. The first appearance and notice of citrus gummosis in Azore Islands was probably because Azores islands were the mid-ocean stop for providing vital supplies for ships returning from Africa, Asia, and the Americas (Naqvi, 1999a).

II.2. The Genus of *Phytophthora spp.*

Phytophthora of Bary 1887 is a cosmopolitan genus of Oomycete obligate plant pathogens containing approximately 60 described species (Erwin & Ribeiro, 1996). *Phytophthora* species attack a wide range of plants, and are responsible for some of the world’s most destructive plant diseases - examples include the European potato famine of the 19th century caused by *P. infestans* (Bourke, 1964). *Phytophthora* diseases have been well studied in the temperate regions of the world. However, *Phytophthora* diseases are very common throughout the wet tropical regions of the world and cause significant diseases losses in many tropical fruit crops in the form of root rots, collar rots, stem cankers, leaf blights and fruit rot. For example, *P. palmivora* alone causes a myriad of

severe diseases on many different crops including: black pod of cocoa; root, stem and fruit rot of pawpaw; root rot and blight of citrus; bud rot in palms; and root rot, trunk canker (Bonavia, 1888).

II.3. Evolutionary placement

Phytophthora is a member of the order Peronosporales within the Class Oomycetes in the Kingdom Chromista (Hawksworth & al., 1995) (see table.4). The Oomycetes includes four orders, two of which, the Saprolegniales and the Peronosporales, contain important plant pathogens. The other two orders contain small groups of mainly aquatic fungal-like organisms. Within the Peronosporales, the family Pythiaceae contains a number of genera, the best-known of which are *Phytophthora* and its sister group, *Pythium*, a genus of approximately 120 species (Van der Plaats-Niterink, 1981).

Table.4. Classification of the Oomycetes (Hawksworth & al., 1995).

KINGDOM	CLASS	ORDER	FAMILY	GENUS
Chromista	Oomycetes	Lagenidiales		
		Leptomitales		
		Saprolegniales	Saprolegniaceae	<i>Achlya</i> <i>Saprolegnia</i>
		Peronosporales	Pythiaceae	<i>Pythium</i> <i>Phytophthora</i>
			Peronosporaceae	<i>Bremia</i> <i>Peronospora</i>
			Albuginaceae	<i>Albugo</i>

II.4. Variability of *Phytophthora* spp.

Waterhouse (1963) reviewed the species described up to 1960 and helped sort out synonyms or invalidly described species and brought order to the genus by developing a key based on the morphological features of the sporangia and antheridia . Erwin (1983), further increasing the difficulty in accurately describing species. The vegetative mycelial stage was interpreted to be haploid, diploid and even polyploid (Romero & Erwin, 1969; Elliott & Macintyre, 1973). Ribeiro (1978) collated much of the available information on taxonomy, genetics, ultrastructure, morphological characteristics, methods to induce asexual and sexual production...). During the decade 1970–1980 the physiology of *Phytophthora* spp. was

elucidated by various researchers, and chemically defined media were developed that allowed a precise study of the nutritional requirements for growth and reproduction (Ribeiro, 1983). Concurrently, the requirements for sexual reproduction were being elucidated by (Brasier, 1972). During this period the department of plant pathology at the University of California, Riverside, rose to the forefront of research on all aspects of *Phytophthora spp.* covering its biology, epidemiology, physiology, genetics and pathogenicity. In 1981 a seminal international conference was held at the University of California, Riverside, to recognize the increasing worldwide importance of *Phytophthora spp.* and to honour the numerous contributions made by Professor George Zentmyer towards our understanding of this genus (Lamour, 2013).

II.4.1. Life Cycle

Phytophthora spp. commonly overwinters as oospores, thick walled sexual spores, or as chlamydospores, thick cells that act as asexual spores. Both can be found in plant debris. Chlamydospores germinate and form hyphae that must be near a host plant for infection to occur. Oospores also germinate to do the same thing in drier conditions, but when free water is available, produce a reproductive structure called a zoosporangium from which the motile zoospores are produced. The zoospores swim and are splashed by water from the soil and leaf litter on to the plant leaves, where they encyst, then infect and invade a susceptible host (see figure 3). The disease cycle is repeated as oospores are produced from sexual combination and once again the *Phytophthora* overwinters in fallen leaves and plant debris (Martin & al. 2012).

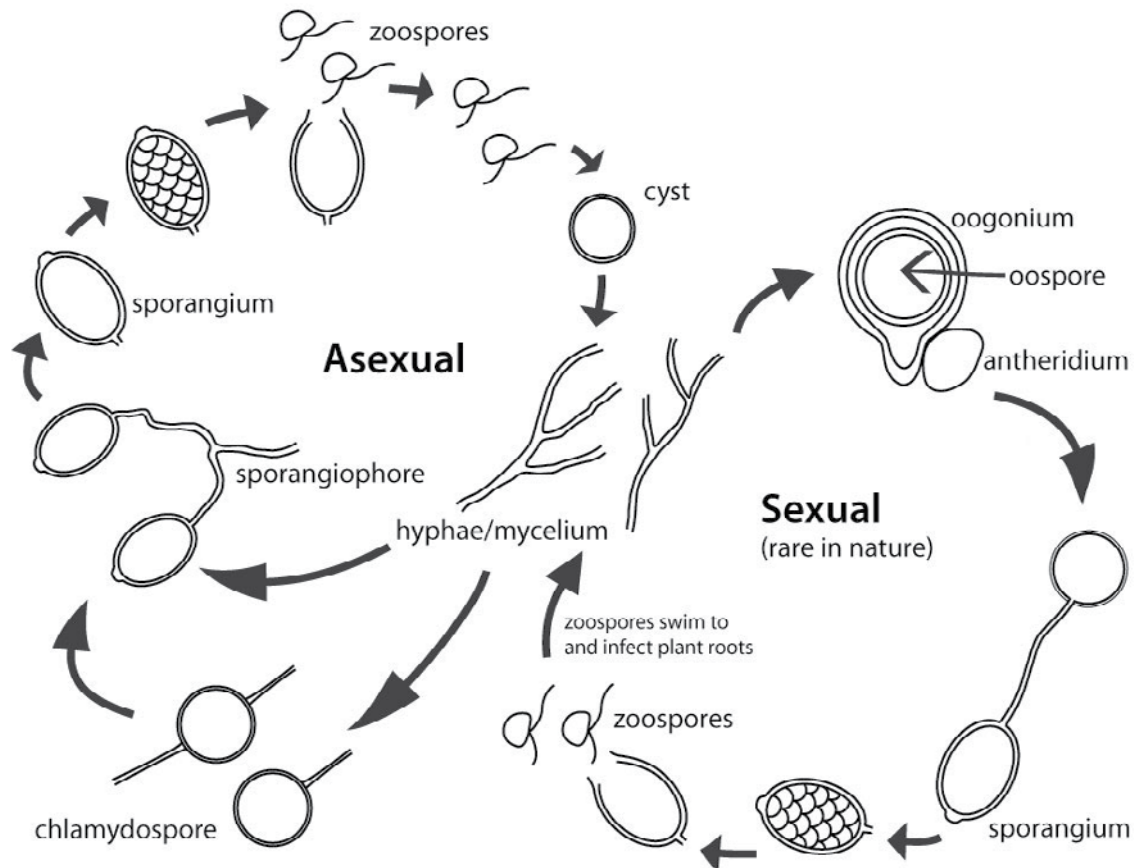


Figure 3. Life cycle of *Phytophthora* spp. (Martin & al., 2012)

II.4.2. Host Range

Species of *Phytophthora* vary greatly in their degree of host specificity. *P. fragariae* var. *rubi* infects a single host species (Kennedy & Duncan, 1995), while *P. cinnamomi* is able to attack over 1000 different host plant species (Erwin & Ribeiro, 1996), and other species occupy a continuum between these two extremes. Broad host range *Phytophthora* species tend to attack their hosts using enzymes which affect relatively unspecialised host chemical and mechanical resistance mechanisms (Brasier, 1983), whereas some host specific species are known to possess virulence genes which interact specifically, in a gene-for-gene system, with host resistance genes (Thompson & Burdon, 1992).

II.4.3. Mating System

All isolates of *Phytophthora* are potentially bisexual, that is, they are able to produce both male and female sexual structures, oogametes (Galindo & Gallegly, 1960). However, only about half of the species of *Phytophthora* are homothallic, and able to produce oospores rapidly and abundantly in single culture. The remaining species are heterothallic, and produce gametes only in response to chemical stimulation from an isolate of the opposite mating

type. The system of heterothallism involving A1 and A2 mating types is universal throughout the genus. Isolates of opposite mating types from different species are often able to reciprocally stimulate gametangial formation (Ko, 1978). The mating system of a *Phytophthora* species determines its ability to outbreed: homothallism allows frequent selfing, whereas heterothallism encourages outbreeding. However, both homothallic and heterothallic species do have a range of reproductive options. Homothallic species have recently been shown to undergo low levels of outbreeding in vitro, while heterothallic species have been shown to inbreed at low levels (Brasier, 1992).

II.5. Identification of *Phytophthora* spp.

Many species of *Phytophthora* can be easily identified. However, the morphological differences among some species are few and variable, making it difficult to classify the species accurately. Identification of *Phytophthora* is based on the taxonomic keys of Waterhouse (1963) and (Stamps & al., 1990). Characters which are used to classify species of *Phytophthora* include: sporangium morphology; morphology of sexual structures such as antheridia, oogonia and oospores; presence or absence of chlamydospores, and morphology of hyphae.

II.5.1. Taxonomic keys

The genus *Phytophthora* has been widely acknowledged as taxonomically ‘difficult’ (Brasier, 1983), as many of the characters used for species identification are plastic, highly influenced by environment, show overlap between species, and have an unknown genetic basis. Nonetheless, since a major review of the genus was performed by Waterhouse (1963), morphological characters have remained the basis for species identification and taxonomy (Newhook, 1978; Stamps & al., 1990). Waterhouse classified species based primarily on papillation and caducity (easy detachment) of sporangia, type of antheridial attachment (figure 4), and mating system. Based on this analysis, the genus was divided into six major groups (table.5), which were intended solely as an aid to species identification, and were not meant to imply a natural classification (Waterhouse, 1963).

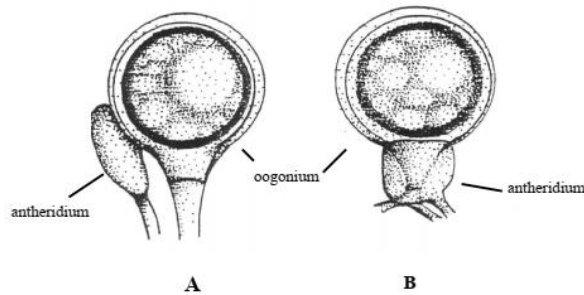


Figure 4. Antheridial attachment

(A) paragynous, (B) amphigynous. Illustration from

Table.5. Classification of *Phytophthora* into six Groups by Waterhouse (1963).

Group	Sporangia	Antheridial attachment	Examples
I	Papillate	Paragynous	<i>P. cactorum</i> , <i>P. clandestina</i>
II	Papillate	amphigynous	<i>P. capsici</i> , <i>P. palmivora</i>
III	semi-papillate	Paragynous	<i>P. inflata</i> , <i>P. multivesiculata</i>
IV	semi-papillate	amphigynous	<i>P. infestans</i> , <i>P. ilicis</i>
V	non-papillat	Paragynous	<i>P. megasperma</i> , <i>P. soja</i>
VI	non-papillate	amphigynous	<i>P. cinnamomi</i> , <i>P. drechsleri</i>

II.5.2. Cultures

Ideally, the culture used for species identification should be obtained from a hyphal tip, or a single germinated zoospore cyst, sporangium or oospore. It is important to remember that on selective media most *Phytophthora* species will not sporulate and form characteristic propagules for identification. Therefore, cultures should be incubated at the optimum temperature for the suspected species, on a natural medium such as V8 juice, carrot agar or Lima bean agar.

In order to identify an isolate of *Phytophthora* to species level, it is necessary to induce the production of asexual and sexual structures that will aid in species identification. In addition, characteristics of the mycelium, and whether the culture produces chlamydospores will also assist in identification (figure 5) (Lamour, 2013).

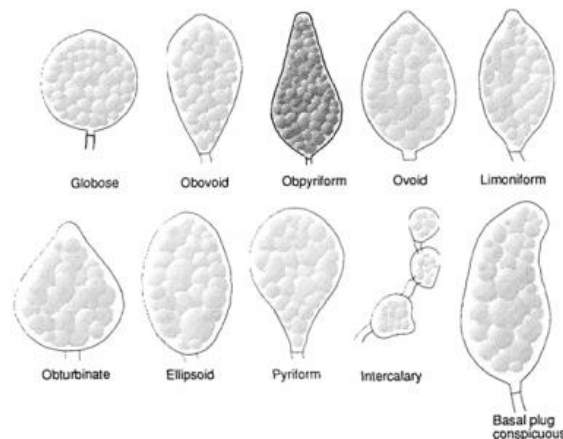


Figure 5. Sporangium shape (Lamour, 2013)

II.5.3. Morphological Characters

There are a number of morphological characters upon which identification of *Phytophthora* species is based. These include: sporangium shape, papillation, and caducity; sporangiophore morphology (figure 6); presence of chlamydozoospores and hyphal swellings; antheridial attachment, and whether sexual reproduction is heterothallic or homothallic (Erwin & Ribeiro, 1996).

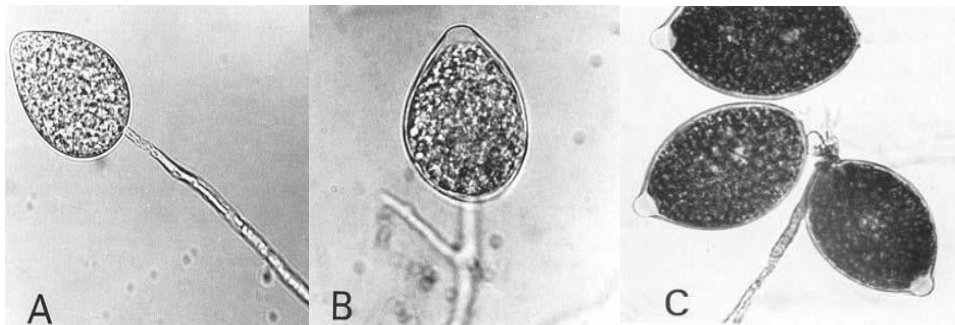


Figure 6. Sporangium papillation

(A) Non-papillate, (B) semi-papillate and (C) papillate sporangia. Photographs from (Erwin & Ribeiro, 1996).

II.5.4. Sporangia

Sporulation in *Phytophthora* cultures provides important clues for species identification. Important characters to observe are:

- sporangium morphology (shape, size, length:width ratio) (see figure 5 above).
- papillation of the sporangium (see figure 6 above).
- caducity (shedding of the sporangium at maturity).
- length of the pedicel on the sporangium.
- proliferation of sporangium (production of new sporangium within a sporangium that has germinated directly).
- branching of the sporangiophores on which the sporangia are borne (see figure 7).

(Erwin & Ribeiro, 1996).

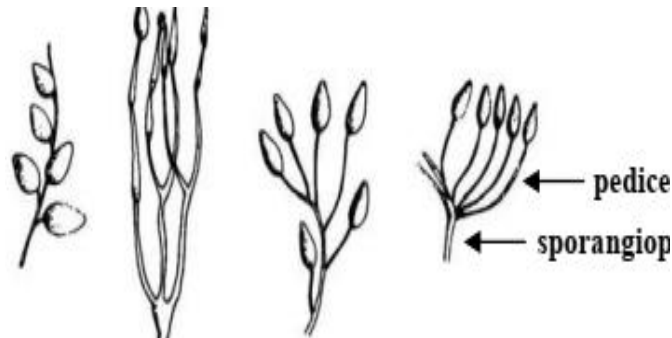


Figure 7. Sporangiophore morphology **Simple sympodium** (left) **Compound sympodia** (middle) **Umbellate sympodium** (right) (Erwin & Ribeiro, 1996)

II.5.5. Chlamydospores and hyphal swellings

Chlamydospores are thick-walled spores that function as a resting spore. Chlamydospores can be intercalary (formed between hyphae) or terminal (on the ends of hyphae). They differ from hyphal swellings by having thick walls and are delimited from the mycelium by septa. The morphology of chlamydospores does not differ greatly between species and therefore these spores are of limited use in species identification. However, the presence (for example, *P. palmivora*) or absence (for example, *P. heveae*) of chlamydospores can aid species identification (figure 8). Chlamydospores are generally produced readily in agar or water culture (Lamour, 2013).

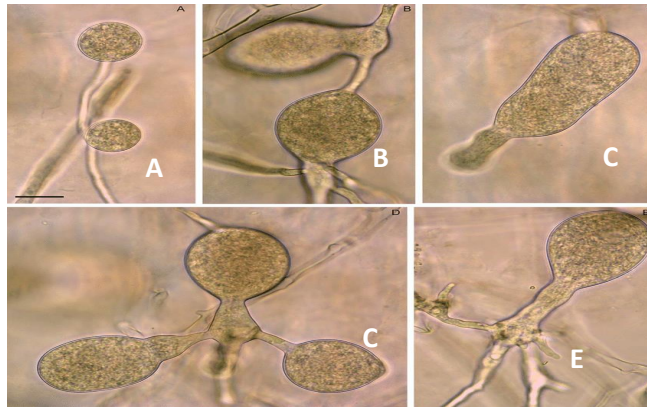


Figure 8. Chlamydospores and hyphal swellings produced by *Phytophthora spp.*

prodigiosa. (A-C-D). Globose, small, sessile chlamydospores on V8A, attached laterally to the hypha and with a thin wall. (B-E) Hyphal swellings of *Phytophthora spp.* (Babadoost, 2006)

II.5.6. Sexual Structures

Production of oogonia, antheridia and oospores and mating type tests for heterothallic species. Approximately half of the species of *Phytophthora* are homothallic. They will therefore produce oogonia, antheridia, and oospores in single culture. The remainder are heterothallic, with two mating types, A1 and A2. Heterothallic species produce gametangia (oogonia and antheridia) only in the presence of an isolate of the opposite mating type on the same plate. For species identification, it is important to determine if a culture is homothallic or heterothallic, and whether the antheridium is amphigynous (around the oogonial stalk) or paragynous (next to the oogonial stalk). It is important that mating type tester isolates are obtained from a reliable culture collection (Ko, 1978).

II.6. Pathogenicity of *Phytophthora Spp.*

Almost all species within the genus *Phytophthora* are formidable plant pathogens. Hence, we have to ask the question what makes these organisms such effective plant pathogens. The following factors are involved (Lamour, 2013):

- The ability to produce different types of spores such as sporangia and zoospores for short-term survival and spread, and chlamydospores and oospores for more longterm survival.
 - Rapid sporulation on host tissue such as leaves typically within 3-5 days after infection.
- These results in a rapid build up of inoculum in a multicyclic fashion, leading to epidemics under favorable environmental conditions.

- Ability of zoospores of *Phytophthora* to be attracted to root tips through a chemical stimulus (positive chemotaxis) coupled with the mobility of zoospores to actually swim to the actively growing root tips, encyst, and infect young, susceptible root tissue.
- Ability to survive in or outside the host tissue as oospores or chlamydospores for long periods of time. Oospores are also known to survive passage of the digestive systems of animals (for example, snails).
- Production of sporangia, which can be airborne and may travel reasonable distances on wind currents and infect neighbouring fields. These sporangia can directly infect host tissue. These same sporangiospores also have the ability to differentiate into 4- 32 zoospores under humid and cool conditions and cause multiple infections from the one sporangium. However, zoospores can travel only short distances as they are susceptible to desiccation.
- *Phytophthora* pathogens belong to the Kingdom Chromista and as such have different biochemical pathways to the true fungi. Therefore many fungicides are not very effective against *Phytophthora* pathogens.
- *Phytophthora* pathogens thrive under humid and wet conditions, which make them difficult to control, as protectant fungicides are difficult to apply and least effective under such conditions. *Phytophthora* pathogens can cause many different diseases and disease symptoms on a wide range of plant species. In the next section, the disease symptoms most often encountered are discussed.

***Chapter III : Phytophthora
spp. Disease on citrus***

III.1. Pathogens Contributing to Replant Disease on Citrus Trees

The major soilborne pathogens of citrus include the citrus nematode *Tylenchulus semipenetrans*, *Phytophthora nicotianae* and *Phytophthora citrophthora* (Le Roux & al., 1998). *Fusarium solani* is also a known soilborne pathogen of citrus, but its role as pathogen and symptoms caused have been somewhat unknown as it is also known as being involved with dry root rot of citrus. The severity of disease is influenced by the virulence of the organism or infectious agent involved the susceptibility and growth stage of the host and environmental conditions such as moisture and temperature (Dreistadt, 2012). Soilborne pathogens that infect the root system and trunk of citrus trees are few in number, but cause a variety of symptoms, resulting in yellowing of foliage, dieback of terminal shoots and branches, poor growth, leaf drop, gradual decline, girdling and death of the entire tree (Graham & Menge, 1999). Fibrous roots, which take up nutrients and water from the soil are primarily affected, followed by infection of major roots and trunk tissue. The roots and trunk tissue deliver water, nutrients and photosynthetic products between roots and shoot systems. Soilborne pathogens play a direct or indirect role in reducing fruit size, total yield, postharvest quality and tree survival under favorable pathogen conditions (Graham & Menge, 1999). Among the soil borne pathogens, the diseases caused by *Phytophthora* species are wide spread in all the citrus growing belts of the world and cause incalculable losses. These pathogens cause trouble because of their persistence and spread in infested soil and water, and their ability to attack a wide variety of plants. Some species cause root rot, whereas others cause foliar blight and shoot dieback. Several species can infect plant parts both above and below the ground (Graham & Menge, 1999).

III.2. Diseases by *Phytophthora* spp.

Citrus trees may be susceptible at any growth stage to *Phytophthora* spp. It causes seed rot or pre-emergence rot. Damping-off caused by *Phytophthora* spp. is similar to that caused by *Rhizoctonia* and *Pythium* spp. . The pathogen also causes decay of fibrous roots. The cortex turns soft, becomes somewhat discolored, and appears water soaked. The fibrous roots slough their cortex, leaving only the white thread-like stele, which gives the root system a stringy-like appearance (Graham & Menge, 1999).

III.3. Causal Agents of *Phytophthora* spp.

The most widespread and important *Phytophthora* spp. are *P. nicotianae* Breda de Haan (synonymous with *P. parasitica* Dast.) and *P. citrophthora* (Sm. & Sm.) Leonian (Feichtenberger, 1992; Graham & Menge, 1999).

III.3.1. *Phytophthora nicotianae*

Is the most common species occurring in subtropical areas of the world, and causes foot rot and root rot but usually does not infect far above the ground, *Phytophthora nicotianae* was first isolated from tobacco at the end of the 19th century and is considered one of the most widespread oomycete plant pathogens, with a host range of more than 255 species. Brown rot epidemics are associated with prolonged wet periods and a temperature range of 23°C to 32°C (Graham & Timmer, 1992). Foot rot occurs when *Phytophthora* spp. infect the scion area near the ground level, which results in lesions that extend upward to the bud union on resistant rootstocks, or up the trunk into main branches (Graham & Feichtenberger, 2015).

III.3.2. *Phytophthora citrophthora*

Causes both gummosis and root rot; it also attacks aerial parts of the trunk and major limbs. It is the most common cause of brown rot in Brazil (Feichtenberger, 1992) and in Mediterranean climates, though brown rot is also caused by *P. hibernalis* in the latter. The reported symptoms include trunk and branch cankers, gum exudates on branches and similar root rot symptoms associated with *P. nicotianae* induced root rot (Alvarez & al., 2008). Lesions can spread around the trunk, slowly girdling the tree, leading to the appearance of pale green leaves with yellow veins (Schutte & Botha, 2010). A study by Schutte and Botha (2010) showed that *P. citrophthora* is more active at moderate temperatures below 30°C, and *P. nicotianae* at higher temperature >30°C. This confirmed the high activity and isolation of *P. citrophthora* in Mediterranean climatic areas (Schutte & Botha, 2010). *Phytophthora citrophthora* also infects the root cortex leading to the decay of fibrous roots.

III.4. The Disease Cycle and Epidemiology

Phytophthora spp. is endemic in the soil of citrus orchards from most areas. Infection usually occurs by means of zoospores, which are released from sporangia produced in infected roots. Zoospores are attracted to wounds or to the zone of elongation of root tips, which are extremely susceptible to pathogen infection. Zoospores are probably attracted to the zone of elongation of new roots by nutrients, which are naturally exuded from this root zone (Graham & Menge, 1999). Chemotaxis of zoospores to the root can be an important factor in pathogenesis. Upon contact with a root, zoospores encyst, germinate and then infect along the zone of elongation. Once the fungus has entered the root tip, the infection may advance in the cortex, resulting rot of the entire rootlet. Severe outbreaks are associated with prolonged periods of wet weather. The cycle can repeat itself as long as conditions are favorable and susceptible tissue is available (Graham & Menge, 1999). Penetration of young leaves and fruits can occur without wounding. Fruit and leaf lesions on citrus are confined to the lower

50 cm of trees unless secondary inoculum is splashed from infected low-hanging lesions to the higher tree parts. Brown rot epidemics are usually restricted to areas where rainfall coincides with the early stages of fruit maturity (Graham & Menge, 1999). The pathogen requires a wound or natural growth crack for infection of suberized tree trunks (Timmer & Menge, 1988) and foot rot or gummosis occurs when zoospores or other propagules are splashed onto the trunk above the bud union. Infection occurs through wounds or natural cracks in the bark when moisture is present on or around the base of the trunk. This condition is favored by high soil moisture, heaping of soil against trunks, deep planting, low budding and cultivation injury (see figure 10). *Phytophthora spp.* usually survives unfavorable periods as chlamydozoospores or oospores in soil, or as hyphae or sporangia in decayed roots or other organic matter (Timmer & Menge, 1988).

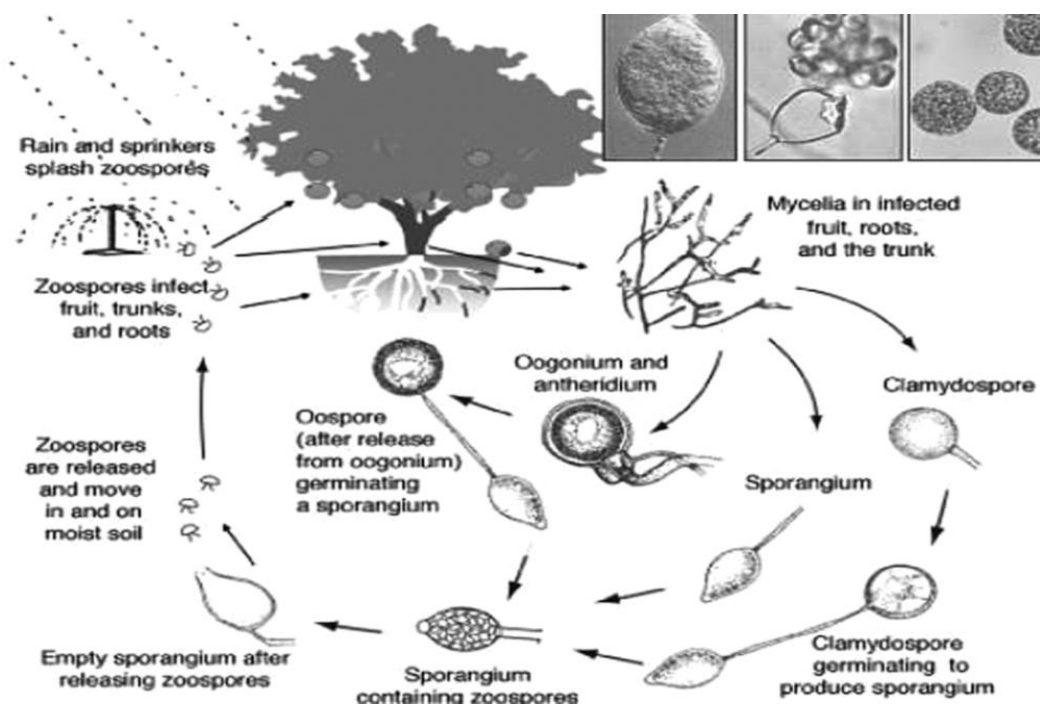


Figure 9. Disease cycle of *Phytophthora spp.* on citrus (Aglave, 2018)

III.5. Symptomatic Diagnosis

Different species of *Phytophthora* cause serious and economically important soilborne diseases of citrus throughout the World. Tree and crop production losses occur from damping-off of seedlings in the seedbed, root and crown rot in nurseries, foot rot and fibrous root rot, and brown rot of fruit in groves (see figure 11) (Iqbal & *al.*, 2020).

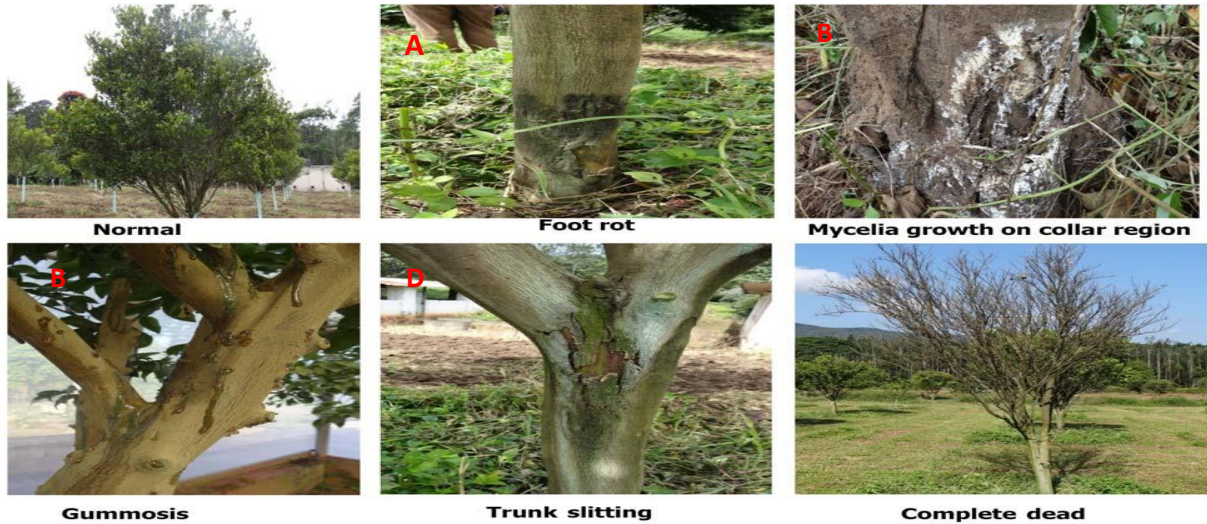


Figure 10. Coorg mandarin plant showing (A) foot rot, (B) gummosis, (C) collar rot and (D) trunk slit (Sonavane & Venkataravanappa, 2017)

III.5.1. Damping-off of seedlings in citrus Tree

Damping-off of seedlings in nursery bed is a widespread problem of citrus industry and frequently occurs in citrus orchards where phytosanitary conditions are difficult to maintain. Damping-off of citrus seedlings is caused by *Phytophthora parasitica*, *P. citrophthora* and *P. palmivora*. Typical symptoms of damping-off result when the soil or seed-borne fungus penetrates the stem just above the soil line and causes the seedling to topple. *Phytophthora* spp. also causes seed rot or pre-emergence rot. Infected seedlings are killed rapidly when moisture is abundant and temperatures are favorable for fungal growth (Klotz, 1969). Plants usually become resistant to damping-off once true leaves have emerged and the stem tissue at the soil line has matured. Damping-off *Phytophthora* spp. can also infect the root cortex and cause decay of fibrous roots. Root rot also occurs on susceptible rootstocks in fruit-bearing groves where damage rarely kills the tree, but the tree declines in vigor and fruit production (see figure 12). Water and mineral nutrient uptake are impaired, and carbohydrate reserves in the roots are depleted by the repeated attacks. According to

researches carried out in the United States, the damage in terms of yield losses caused by root rot is on average about 5% (Menge & Nemeč, 1997).

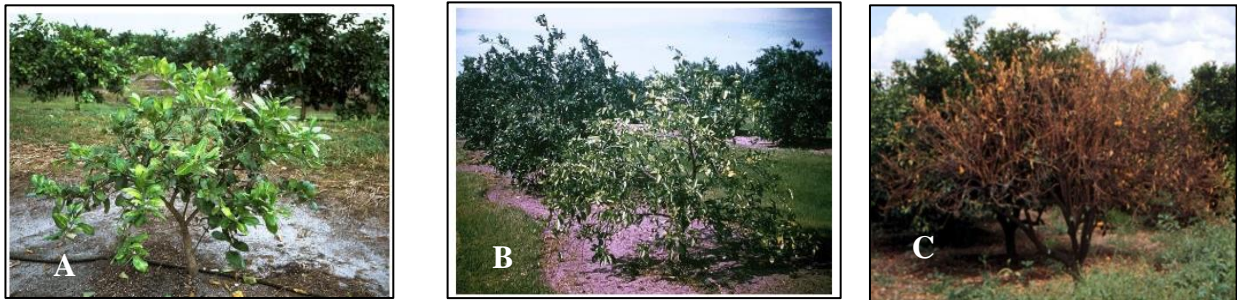


Figure 11. Damping off caused by *Phytophthora spp.*

((A) before the injury, (B) the beginning of injury,(C) after injury and damage and damping off) (Sonavane & Venkataravanappa, 2017)

III.5.2. Foot rot or Gummosis

The most serious disease caused by *Phytophthora spp.* is foot rot, also known as gummosis, where in drier climates the water-soluble gum is not washed from the trunk by rainfall (Graham & Menge, 1999). Infection of the scion occurs near the ground level, the specific symptoms of this facies of the disease are the cankers and gummosis at the base of the trunk (Fawcett, 1936). Gum oozes proceed from longitudinal cracks of the bark around necrotic areas, which have a distinct water-soaked discoloration. The dead bark turns soft and sloughs off the central cylinder below which a callous is formed around the edges of the lesion. If the canker affects more than 50% of the trunk circumference, the plant shows symptoms of decline in the canopy, chlorosis of the veins and also midrib of the leaf, phyllotaxis, thinning of the canopy and dieback of branches (see figures 13 and 14 and 15). Gum exudation can be seen on trees especially between the end of spring and beginning of summer. The gum is water-soluble, but even if it is washed away by the rain, the discoloration on the cortex is still visible. Since the cankers are often formed below ground, it may be necessary to scrape away the soil around the collar to see them and evaluate the severity of infection. According to researches carried out in the United States, the damage caused by gummosis was estimated to be on average about 1% (Menge & Nemeč, 1997).



Figure 12. Gummosis on citrus trees

(A) Gummosis on the main branch of sweet orange (*Citrus sinensis*) tree and (B) tree dieback along the gummosis symptom on the main branch at Hurso 2011 (Photo courtesy of Moges Mekonen) (Sonavane & Venkataravanappa, 2017)



Figure 13. Typical discoloration under the bark of the trunk of an active lesion caused by *P. nicotianae* (Olsen, 2000)

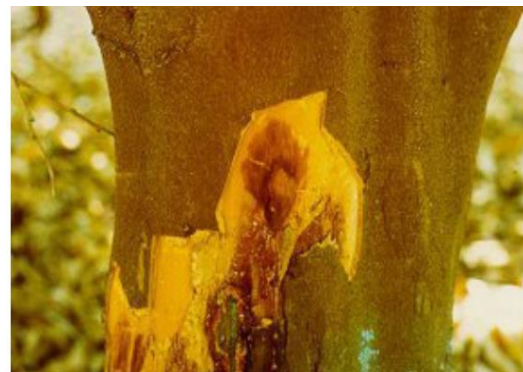


Figure 14. Typical gummy lesions caused by *Phytophthora nicotianae* in a lemon's tree bark (Olsen, 2000)

III.1.5.3. Fibrous Root rot

This is the most difficult facies to diagnose by visual inspection, since similar symptoms can be caused by different factors, like poor soil aeration and excessive salt content in irrigation water (Klotz, 1950). The root cortex sloughs off easily, leaving the stele bare, with the tip of the rootlet appearing thread-like. The plant reacts to the infection by forming new rootlets (see figure 16). Adult plants may show no symptoms even when there is a very high percentage of infected rootlet. The symptoms of decay on the canopy appear when

the plant is no longer capable of producing new rootlets to substitute the rotten ones (Klotz, 1950).



Figure 15. Fibrous roots caused by *Phytophthora* spp.

(A) decay of fibrous roots, (B) damaged roots of citrus tree, (C) roots are damaged and cause the tree stopped growing (Olsen, 2000)

III.5.4. Brown Fruit rot

When fruits are infected by *Phytophthora*, it produces a decay in which the affected area is light brown, compared to the adjacent rind. Under humid conditions, the white mycelium grows on the rind surface. In the orchard, fruits near the ground become infected when splashed with soil containing the fungus. The disease spreads to fruits throughout the canopy, if favorable conditions of optimum temperature (75-82°F) and long periods of wetting (18 plus hours) continue, the pathogen produces sporangia on the fruit surface. For *Phytophthora* spp. with caducous sporangia, such as *P. palmivora* (Butler) Butler and *P. hibernalis* Carne, sporangia are spread by splash or wind-blown rain to fruit throughout the canopy (Graham & Feichtenberger, 2015). Most of the infected fruits soon abscise, but harvested fruits may not show symptoms until they have been kept in storage for a few days. If infected fruit is packed, brown rot may spread to adjacent fruits in the container (see figures 17 and 18 and 19). In storage, infected fruit has a characteristic pungent, rancid odor (see figures 20 and 21). Brown rot epidemics are usually restricted to areas where rainfall coincides with the early stages of fruit maturity (Graham & Feichtenberger, 2015). In continuously wet weather conditions about 24 hrs or more, *Phytophthora* splashed along with rain drops to low hanging fruits and caused a typical brown rot of fruits and leaf fall (Agtap & al., 2012). All citrus cultivars are affected, especially early season oranges and lemons (Agtap & al., 2012).

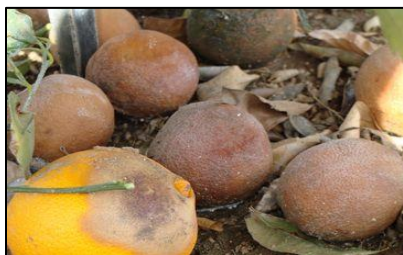


Figure 16. Brown root caused by *Phytophthora citrophthora* on orange (Eckert, 2012).



Figure 17. Sweet orange fruit with symptoms of brown rot caused by *Phytophthora citrophthora* (Khanchouch & al., 2017).



Figure 18. Brown Root Caused by *Phytophthora Citrophthora* on lemon's fruit (Eckert, 2012).



Figure 19. Brown rot of orange fruit caused by *Phytophthora* species. Early symptoms are shown in the upper images. Late symptoms are shown for brown, leathery decay with a distinct pungent odor (bottom, left) and brown rot decayed fruit at high humidity with white mycelium on the fruit surface (bottom, right) (Khanchouch & al., 2017)



Figure 20. Brown rot of lemon fruit caused by *Phytophthora* species. Early symptoms are shown in the upper images. Late symptoms are shown for brown, leathery decay with a distinct pungent odor (bottom, left) and brown rot decayed fruit at high humidity with white mycelium on the fruit surface (bottom, right) (Khanchouch & al., 2017)

III.5. Dieback of Twigs and Leaves

Dull chlorotic foliage is the first symptoms of such affected plants where mid rib, main lateral veins and bands of leaf tissue bordering them became yellow-leaved rest of the leaf normal in color (see figure 22) . The diseased plants thus had comparatively fewer fibrous roots than healthy plants. In severe cases, where regeneration of feeder root did not cope with the rate of destruction, the affected plant shown, starvation, less canopy volume with naked branches, dieback and slow decline symptoms (Agtap & *al.*, 2012).



Figure 21. Dieback of twigs and leaves in citrus trees : (A) branches and leaves corrupt of citrus tree,(B) damaged branche,(C) decay of leaves and fully damaged (Khanchouch & *al.*, 2017)

III.6. Means of Spread of *Phytophthora spp.*

The primary means by which *Phytophthora spp.* are spread through citrus orchards is by use of infested nursery stock, (Graham & Timmer, 1992). Mechanisms of dispersal of *Phytophthora* species were reported, (Ristaino & Gimpertz, 2000). Dispersal from root to root in soil involves either root growth to inoculums, inoculum movement to roots or root to root contacts. Inoculum dispersal in surface water. Splash dispersal from soil to aerial parts of the plant. Aerial dispersal from sporulating lesions on leaves, stem, fruits or other aerial parts of the plant and dispersal by human or invertebrate activities including movement of soil, plants or propagules. The pathogen may be present in soil or infected roots even though disease symptoms are not readily apparent. The fungus is also carried in soil on equipment when vehicles move from infested to non-infested groves or nurseries. Propagule densities decline sharply when soil is air-dried, reducing the probability of spread. Irrigation water may also move the pathogen from area to area. Within groves, dispersal by irrigation water occurs especially where furrow or flood irrigation is used. Surface water following heavy rains may carry the fungus as it drains from the grove. More serious problems can arise in irrigated

citrus areas where run-off water carries the pathogen into canals or ponds. Use of water from these sources may then contaminate previously noninfested areas. Wind is not a major factor in dispersal of *Phytophthora spp.* However, wind-borne soil carries *Phytophthora spp.* and may recontaminate fumigated soils. Windblown rain can disseminate sporangia produced on the surface of aboveground plant parts. Environmental factors also play an important role in disease development, severity, dispersal and survival of *Phytophthora* in causing epidemics, (Cheema & al., 1954). Thus, *Phytophthora* remains active throughout the year in citrus nurseries and irrigated orchards, (Naqvi, 2002b).

III.7. Soil and Environmental Factors that's Help *Phytophthora* to Evaluate and Survive

Phytophthora spp. are parasites but are poor competitive saprophytes (an organism that lives off any dead or decaying organic matter) in soil. The fungus grows well on nutrients obtained from the living plant and, under favorable conditions, undergoes repeated cycles of mycelium to sporangia, zoospores, and more mycelium. *P. nicotianae* is most active during the warmer seasons of the year and forms chlamydospores and oospores for survival during unfavorable periods. *P. citrophthora* is active during cooler periods and is believed to survive as resistant sporangia and possibly chlamydospores and oospores. Because less is known about the life cycle of *P. citrophthora* discussion of the spore forms in relation to soil environmental factors will be limited to *P. nicotianae*.

Optimal germination of chlamydospores occurs in well-aerated, moist environments when temperatures are favorable for root growth (Naqvi, 2002b).

Chlamydospores require nutrients in the form of root exudates for germination. Oospores which occur in low numbers are thick walled and probably resistant to desiccation and cold temperature. They may mature more slowly than chlamydospores, but once matured they germinate in response to nutrients from roots. Oospores form when the opposite mating types are present, which may occur infrequently in some citrus soils. Thus, the importance of oospores in the disease cycle is not known, except that they provide new sources of variation in the population when sexual recombination occurs between the different mating types (Graham, & Timmer, 1992).

Under well-aerated, moist conditions, chlamydospores and oospores can germinate immediately to form a sporangium that liberates zoospores. Zoospore release for *Phytophthora spp.* is optimal in saturated soils. Diurnal soil temperature changes may serve to synchronize zoospore release. Zoospores are motile and can swim short distances by flagellar movement or can be carried long distances by soil water. Zoospores are attracted to roots and

swim to the root and encyst upon contact. Zoospore cysts then germinate and penetrate the root cortex (Graham, & Timmer, 1992).

III.7.1. Soil Moisture

Host susceptibility is affected when roots are stressed or damaged in saturated or in dry soil. Root exudates released by living, but damaged or stressed roots attract zoospores. Soils with drainage restricted by hardpans or clay layers or those with shallow water tables that temporarily rise into the root zone provide ideal conditions for fibrous root infection and rapid buildup of *Phytophthora* propagules. Also, the frequency and duration of irrigations can influence fungal activity and predispose roots to rot. If soils become saturated during an irrigation zoospores are released which can then infect roots to form more sporangia. When soils do not dry sufficiently between irrigations sporangia can survive until the next irrigation and zoospores will be released again. Citrus roots do not grow in saturated soil due to low oxygen availability. Thus, new roots will not develop if the soil is not allowed to adequately dry between irrigations. Disease development is usually more severe on soils with restricted drainage and soil pH between 6.0 and 6.5. Stress from either excess moisture or low moisture can also increase susceptibility of some hosts to infection (Erwin & Ribeiro, 1996).

III.7.2. Soil Aeration

Oxygen availability in the soil is closely related to soil moisture because the amount of pore space occupied by water directly determines the remaining pore space occupied by air. When roots are subjected to low oxygen (anoxia), they are damaged by reduced forms of minerals and by toxic metabolites produced by microorganisms on the roots themselves. Root regeneration is restricted and root exudation increases under flooded conditions. Prolonged oxygen deprivation makes roots more attractive to zoospores and increases infection. Moreover, chronic anoxia due to flooding of the root system will in itself damage roots even in the absence of *Phytophthora*.

Little is known about the host specificity of *P. nicotianae* and *P. citrophthora* and the relative aggressiveness of different isolates within each species. *P. nicotianae* has a broad host range. The virulence of *P. nicotianae* isolates from tomato and other noncitrus hosts toward citrus is low, but all isolates of *P. nicotianae* are pathogenic on tomato. Aggressiveness of *P. nicotianae* on sweet orange seedlings varies widely, but that does not appear to be the case for *P. citrophthora* (Graham & Timmer, 1992).

III.7.3. Soil Temperature

Populations of *P. nicotianae* do not drop significantly in soil temperatures rarely fall below 59°F. The increase in *P. nicotianae* populations coincides with a spring flush of roots

that occurs when soil temperatures rise above 68-73°F. This population response is probably not temperature dependent, but due to the greater abundance of roots available for infection *Phytophthora* grows actively at temperatures between 10°C and 35°C within an optimum of 26°C (Erwin & Ribeiro, 1996). Similarly, citrus root growth ceases at soil temperatures of below 13°C or above 36°C (Davies & Albrigo, 1994). Thus, both the pathogen and citrus root development require similar soil temperatures. The pathogen causes the most damage during summer months when optimal growth conditions occur and there is an abundance of feeder roots and elevated soil moisture (Davies & Albrigo, 1994).

III.8. Economic Importance

Phytophthora species are responsible for significant economic losses on many important food, fiber, and ornamental crops (Erwin & Ribiero, 1996) and one of the major pathogens of many horticultural crops causing incalculable losses. Thousands of fruit trees succumb to *Phytophthora* diseases every year in India. Besides gummosis and brown rot, the root rot caused by *Phytophthora* alone reduces 46 % yield of Citrus plants in California and Citrus industry loses about \$ 12.9 million annually due to this pathogen whereas to *P. citrophthora* \$ 5 million annually. Recognizing the importance of this devastating pathogen, Indian Council of Agricultural Research has launched a National Network Project on *Phytophthora* diseases of Horticultural Crops (PHYTONET) in 1997 to combat the problem. *Phytophthora* is the major problems of Citrus industry world over. *Phytophthora* species cause most serious diseases of citrus and infect almost every part of citrus plants right from damping off of seedlings in nursery beds, decay of fibrous roots, crown rot, foot rot, gummosis, and brown rot of fruits in groves and as post-harvest decay during storage and transport. Recently, *Phytophthora* diseases of citrus and management strategies have been reviewed (Naqvi, 2003).

Conclusion

Conclusion:

Citrus are one of the most important deciduous fruit crops being produced in the world, contributing to the revenue produced from exporting fruit, mainly to Europe and the Middle East. Losses due to soil borne diseases are of economical concern for citrus growers in many countries among them Algeria, where citrus considered one of the most important products. Based on the literature study there are still many gaps in literature based on the management of citrus replant disease in the world. The pathogens involved have not yet been characterised using modern molecular studies combined with phylogeny and within the list of the server fungal diseases attack citrus, the most common disease founds in citrus seedbeds is dumping-off which is caused by a fungus such as *Rhizoctonia*, *Sclerotinia* or *Phytophthora*. A thracnose (*Colletotrichum sp*) and scab (*Sphaceloma sp*) are sometimes also serious problems. Several insects as (aphids and beetles...) and mites also common pests of citrus seedbeds. *Phytophthora spp.* is the most serious, dangerous and the most important which caused many damages, and it is a pathogen that has begun to be recognised as harmful to citrus plants. This pathogen attacks seedlings, stem, roots and fruit. Stem rot is very difficult to control because pathogens can generally survive as mycelium and chlamydo spores (thick-walled resistant spores) on infected plant material such as roots, stem or in the soil for long periods of time. For this purpose, we should use a healthy seeds, tolerant rootstock, soil solarisation, water management, garden and plant sanitation; biological control with biological agents and fungicides if necessary. In Algeria, *Phytophthora* and foot rot are present in most Algerian groves, and trees are budded about 2 feet above ground level. No rootstock trials are evident (Graham & Menge, 1999).

Here some other sanitary recommendations in citrus nurseries can be summarised as follows:

In the first year of growth in the garden, water the tree at intervals of 4 - 7 days, depending on the weather. Regular watering is essential during hot, dry, windy periods. When the tree is well established, watering may be limited to a good soaking with a hose and sprinkler every week.

The root system of citrus trees is usually concentrated in the top 30 cm of soil, many of the important fibre roots will be found within 10 cm of the surface. Take care when digging around the tree not to damage larger roots, as an injury may result in invasion by wood-rotting *Phytophthora* fungi.

It is important to reduce the sources. Remove fallen fruit from the ground as soon as it drops as well as remove damage fruit from the tree. To increase air circulation, prune out dead limbs and prune trees.

The best solution for endemic *Phytophthora spp.* has been and will continue to be resistant rootstocks for minimizing risk of *Phytophthora* disease-related losses. Most rootstocks are at least moderately resistant to bark infection, but vary widely in their susceptibility to root rot depending on the predominant *Phytophthora spp.* (Naqvi, 2003).

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Contribution to the study of diseases caused by *Phytophthora spp.* in Citrus

Summary:

Citrus represent one of the main crop in the world belongs to the family Rutaceae. Citrus cultivation is one of the major contributors to the Algerian wealth and it's a part of the traditional agriculture of the country. Citrus facing a lot of fungal diseases and the most important one are caused by *Phytophthora spp.*, which is considered one of the most important diseases carried by the soil. This disease causes the death of newly planted plants and slow plant development, as well as causing yield losses in productive plants, and it causes: foot rot, root rot, and brown rot. Citrus do not need high moisture, especially during the flowering season and excess water is bad for their growth, as it encourages fungal pathogens such as *Phytophthora spp.*

In Algeria, citrus fungal diseases are numerous and diverse, and the most dangerous and serious are caused by *Phytophthora spp.*

In this study, we discussed the different benefits of citrus and the knowledgment of *Phytophthora spp.*, their symptoms and damage that causes different losses in citrus orchards, and to have an idea to ignore this problem and find appropriate management strategies to control this fungal disease.

Keywords: *Phytophthora spp.*, citrus, foot rot, brown root, root rot, fungal diseases

Résumé:

Les agrumes représentent l'une des principales cultures de la famille Rutaceae. La culture des agrumes est l'un des principaux contributeurs à la richesse algérienne et fait partie de l'agriculture traditionnelle du pays. Les agrumes confrontés à beaucoup de maladies fongiques et les plus importantes sont causés par *Phytophthora spp.*, qui est considérée comme l'une des maladies les plus importantes véhiculées par le sol. Cette maladie entraîne la mort de plantes nouvellement plantées et le développement lent des plantes, ainsi que des pertes de rendement dans les plantes productives, et il cause : la pourriture du pied, la pourriture des racines et la pourriture brune. Les agrumes n'ont pas besoin d'humidité élevée, surtout pendant la saison de floraison et l'excès d'eau est mauvais pour leur croissance, car il encourage les pathogènes fongiques tels que *Phytophthora spp.*

En Algérie, les maladies fongiques des agrumes sont nombreuses et diverses, et les plus dangereuses et graves sont causées par *Phytophthora spp.*

Dans cette étude, nous avons discuté des différents avantages des agrumes et de la connaissance de *Phytophthora spp.*, leurs symptômes et les dommages qui causent des pertes différentes dans les vergers d'agrumes, et d'avoir une idée sur la manière d'ignorer ce problème et de trouver des stratégies de gestion appropriées pour contrôler cette maladie fongique.

Les mots clés : *Phytophthora spp.*, les agrumes, pourriture des pieds, pourriture de la racine et pourriture cultivée, les maladies fongiques

ملخص :

تمثل الحمضيات أحد المحاصيل الرئيسية في العالم حيث تنتمي لعائلة Rutaceae ، تعتبر الحمضيات إحدى العوامل المساهمة في الثروة الجزائرية وهي جزء من الزراعة التقليدية في البلاد . تواجه الحمضيات العديد من الأمراض الفطرية أهمها مرض *Phytophthora spp.* ، والتي تعتبر من أهم الأمراض الفطرية الموجودة في التربة . يتسبب هذا المرض الفطري في موت النباتات الياقعة ويبيط من نموها، كما يتسبب في خسائر في المنتج النباتي ويسبب كل من : تعفن ساق الشجرة وتعفن جذورها وتعفن ثمارها كذلك. إن أشجار الحمضيات لا تحتاج إلى رطوبة عالية خصوصا في موسم الإزهار ولا تحتاج إلى كميات كبيرة من الماء ، لأن ذلك سيء لنموها و يجذب الفطريات لها كـ *Phytophthora spp.*

توجد في الجزائر العديد من الأمراض الفطرية المختلفة أهمها وأخطرها هي *Phytophthora spp.* ، في هذه الدراسة تطرقنا إلى التحدث عن الحمضيات ومختلف فوائدها و عن مختلف المعلومات المتعلقة بـ *Phytophthora spp.* وعما تسببه من أضرار وخسائر في مزارع الحمضيات ، و لمعرفة كيفية تجنب هذا المشكل وإيجاد حلول وإستراتيجيات للسيطرة على هذا المرض الفطري.

الكلمات المفتاحية:

Phytophthora spp. ، الحمضيات، تعفن الساق، تعفن الجذور، تعفن الثمار الأمراض الفطرية