

# بسم الله الرحمن الرحيم





# شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





# جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



## يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





بالرسالة صفحات  
لم ترد بالأصل







# بعض الوثائق الأصلية تالفة



B 11544

**PATHOLOGICAL STUDIES ON CROWN  
CANKER AND ROOT-ROT OF APPLE  
INCITED BY *Phytophthora cactorum***

**BY**

**SAHAR SHARKAWY ABD-ALLA**

B.Sc. Agric. (Plant Pathology) - Cairo University 1988  
M.Sc. Agric. (Plant Pathology) – Cairo University 1994

**THESIS**

**Submitted in Partial Fulfilment of the  
Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**in**

**PLANT PATHOLOGY**

**Department of Plant Pathology  
Faculty of Agriculture  
Cairo University**

**2002**

## APPROVAL SHEET

**NAME :** SAHAR SHARKAWY ABD-ALLA

**TITLE :** PATHOLOGICAL STUDIES ON CROWN CANCER AND  
ROOT-ROT OF APPLE INCITED BY *Phytophthora cactorum*

**This Thesis for Ph.D. Degree has been approved by:**

**Prof. Dr. Abd El-Moniem I.I. El-Fiki**

Professor of Plant Pathology,

Faculty of Agriculture, Moshtohor, Zagazig University

A. I. I. El-Fiki

**Prof. Dr. Mohamed F.M. Attia**

Professor of Plant Pathology,

Faculty of Agriculture, Cairo University

M. F. Attia

**Prof. Dr. Moustafa M. Fahim**

Professor of Plant Pathology,

Faculty of Agriculture, Cairo University

M. Fahim

**Prof. Dr. Khairy A. Abada**

Professor of Plant Pathology,

Faculty of Agriculture, Cairo University

K. A. Abada

Date: 25/6/2002

Committee in charge

<b>Name of Candidate:</b>	Sahar Sharkawy Abd-Alla	<b>Degree:</b>	Doctor of Philosophy
<b>Title of Thesis:</b>	Pathological Studies on Crown Canker and Root-rot of Apple Incited by <i>Phytophthora cactorum</i>		
<b>Supervisors:</b>	Prof. Dr. M.M. Fahim, Prof. Dr. K.A. Abada, Prof. Dr. H.M. Fouly		
<b>Department:</b>	Plant Pathology		
<b>Branch:</b>		<b>Approval:</b>	

#### ABSTRACT

Natural infection with crown canker (Cc) and root rot (Rr) was observed on apple trees and seedlings grown in different locations. Isolation trials from collected diseased samples yielded many fungal isolates. *Phytophthora cactorum* (Pc) isolates were found to be the most dominant ones. Pathogenicity tests of eight Pc isolates on MM 106 and Malus rootstocks revealed that they were all pathogenic and both isolates No. 1 and No. 3 were the most virulent, therefore they were used in the following experiments. The tested two Pc isolates caused infection to both apple and pear seedlings. Gradual increase in the inoculum level of the two tested isolates caused also gradual increase in Cc and Rr severity. Severity of Cc and Rr gradually decreased by the increase of rootstock age. The highest Cc and Rr severities were recorded on MM 106 rootstocks grown in clay soil, while the lowest infection was observed on rootstocks grown in soil consisted of 4 sand and 1 clay. Regarding natural soil, the highest infection occurred on rootstocks grown in loam soil. Meanwhile, the lowest infection was recorded on rootstocks grown in clay loam soil. Severities of Cc and Rr were gradually decreased by increasing irrigation intervals. All the used eight Pc isolates were able to grow on the tested eight solid media. The best media were corn meal agar and potato agar, while both carrot agar and tomato agar media were the least suitable. Maximum mycelial weight was obtained when corn meal medium was used followed by oat-meal, potato and carrot media. All Pc isolates failed to form any sporangium on oat meal agar medium and maximum production was achieved by isolates No. 1, No. 3 and No. 4 on onion agar medium. Moreover, all (Pc) isolates failed to form any oospore on both oat meal and corn meal agar media, with the exception of isolate No. 5 when grown on corn meal agar medium. All Pc isolates can grow at 10-30°C, while four of these were able to grow at 35°C on oat meal agar medium. Enzymatic activities of the tested Pc isolates revealed that PG enzyme activity was increased within 15 minutes. This increase was further recorded after 30 minutes with the exception of isolates No. 1 and No. 3, which recorded their maximum increase after 15 minutes. Moreover, great fluctuation was recorded for PME activity by the tested Pc isolates. Furthermore, in most cases Cx activity differed among the tested isolates, with the exception of isolate No. 8 which no Cx

*M. Fahim*  
*K. A. Abada*



activity. Root exudates of both MM 106 and Malus rootstocks caused significant reduction to the linear growth of the tested Pc isolates. Moreover, root-exudates of MM 106 rootstock were less efficient in this respect compared with those of Malus rootstocks. Root exudates of MM 106 rootstock contained more amounts of sugars and amino acids, while Malus rootstocks contained more amount of conjugated and total phenolic compounds. Both isolates (No. 1 and No. 3) of Pc were able to survive in different soil types for at least 24 months. Genetic relationships among different isolates were successfully utilize molecular (protein and DNA-based) markers. Isolate as well as trait-specific markers were also detected for some isolates and traits indicating the efficiency of using molecular tools in discriminating different isolates.

All isolates of genus *Trichoderma* were more antagonistic to Pc isolates than isolates of genus *Epicoccum*, while both isolates of actinomycetes were of low antagonistic action. Considerable reduction in Cc and Rr severities (isolates No. 1 and No. 3) was attained by the used antagonistic microorganisms. All the three used fungicides caused significant reduction to both the linear growth of all Pc isolates and the infection with isolates No. 1 and No. 3 of Pc.

**Key words:** Apple, crown canker, root rot, *P. cactorum*, fungi, chemical control and biological control.

M. Fahim

K-A-Abada

## ACKNOWLEDGEMENT

**First of all, ultimate thanks are due to Allah,  
who without his aid this work could not be done.**

*The author wishes to express her sincere gratitude and appreciation to Dr. M.M. Fahim, and K.A. Abada Professors of Plant Pathology, Plant Pathology Department, Faculty of Agriculture, Cairo University, for their fruitful, creative supervision, and orientation during this investigation.*

*The author greatly indebted to the late Prof.Dr. M.R. Mahdy, Late Professor of Plant Pathology, Plant Pathology Research Insititure, Agric. Res. Center for suggesting the problem of this thesis and continous encourage during his supervision. May God bless his soul.*

*Thnaks to Dr. H.M. Fouly for his sincere supervision during the course of this investigation.*

*The author wishes also to express her deep thanks to Prof. Dr. M.S. Khalil, Director of Plant Pathology Research Institute, Prof. Dr. M.A. Sherif, Water Requirements and Onfarm Irrigation Dept., Soil and Water Research Insititute, Prof. Dr. A. Bahieldin, Dept. of Genetics, Faculty of Agriculture, Ain Shams University and Dr. S.M. Mahmoud, Senior Researcher, Manager of Central Lab. Plant Pathology Research Insititute, Agric. Res. Center for their indispensable help, sincere advising and encouragement during this research work.*

*Finally, thanks to all the staff members of Plant Pathology Research Insititute, Agric. Res. Center, Giza, Egypt.*

# CONTENTS

	Page
<b>INTRODUCTION.....</b>	<b>1</b>
<b>REVIEW OF LITERATURE.....</b>	<b>3</b>
1. Disease history and its world-wide distribution: .....	3
2. The causal pathogens:.....	5
3. Symptoms .....	5
4. Isolation .....	6
5. Pathogenicity tests:.....	8
6. Rootstock reaction.....	10
7. Host range.....	11
8. Some factors affecting disease severity: .....	11
a. Inoculum level:.....	11
b. Soil type and soil moisture.....	11
c. Plant age.....	12
9. Physiological studies .....	13
a. Growth and spore formation.....	13
b. Enzyme activity.....	14
c. Root exudates studies.....	14
c.1. Effect of root exudates on fungal growth .....	14
c.2. Chemical composition of rootstocks .....	14
10. Survival .....	15
11.a. Electrophoretic analysis of protein of different isolates .....	15
11.b. Random Amplified Polymorphic DNA (RAPD).....	16
12. Biological control of the disease: .....	18
13. Chemical control.....	19
<b>III. MATERIALS AND METHODS.....</b>	<b>22</b>
Survey of the disease at different locations .....	22
Isolation, purification and identification of the causal pathogen, <i>Phytophthora cactorum</i> .....	22
A. Capture method .....	22
B. Isolation from the naturally infected tissues .....	23
Pathogenicity tests and detection of the virulence of <i>P. cactorum</i> .....	23
4. Rootstock reaction.....	25
5. Host range.....	26
6. Some factors affecting disease severity .....	26
6.1. Inoculum level.....	26
6.2. Effect of plant age.....	27
6.3. Effect of soil texture and type.....	28
6.3.1. Effect of soil texture.....	28
6.3.2. Effect of natural soil type.....	28
6.4. Effect of irrigation intervals .....	30
7. Physiological studies .....	31
7.a. Effect of different media on fungal growth and spore production .....	31
7.b. Effect of different temperature degrees.....	32
7.b.1. Cellulase assay.....	32
7.b.2. Polygalacturonase assay .....	33
7.b.3. Pectin-methyl-esterase-assay method.....	33
7.c. Root exudates .....	33
7.c.1. Collection of exudates from roots .....	33
7.c.2. Effect of root exudates on fungal growth.....	34
7.c.3. Determination of total amino acids in root exudates .....	34
7.c.4. Determination of sugar content.....	35
a. Picrate-picric solution prepared as follows.....	35
b. Sodium carbonate solution .....	36
7.c.5. Determination of phenolic components.....	36



1. Free phenols.....	36
8. Survival of the causal fungal isolates .....	37
Electrophoretic detection of protein patterns by SDS-PAGE .....	38
SDS-protein electrophoresis.....	38
Molecular markers.....	41
DNA isolation.....	41
Polymerase chain reaction (PCR) conditions .....	43
Marker nomenclature .....	45
Data analysis.....	45
Biological control.....	46
A. Antagonistic effect of different bioagents against <i>P. cactorum</i> in vitro .....	46
10.B. <i>In vivo</i> .....	46
11. Chemical control .....	48
11.a. <i>In vitro</i> experiment.....	48
11.b. <i>In vivo</i> experiment.....	50
12. Statistical analysis .....	50
<b>IV. RESULTS .....</b>	<b>51</b>
1. Severity of natural infection with crown canker and root rot in different orchards.....	51
2. Occurrence and frequency of fungi isolated from roots of apple trees .....	56
3. Pathogenicity tests.....	58
3.a. Pathogenicity test of <i>P. cactorum</i> isolates on apple rootstocks (MM106) under controlled temperature (greenhouse experiment) .....	58
3.b. Pathogenicity test of eight <i>P. cactorum</i> isolates on two apple rootstocks (MM 106 and Malus) under natural temperature (Rootstock reaction):.....	60
4. Host range.....	61
4.1. Severity of crown canker and root rot disease on MM 106 apple rootstock and Communis pear rootstock .....	61
5. Some factors affecting disease severity incited by two isolates of <i>P. cactorum</i> .....	64
5.1. Effect of irrigation intervals .....	64
5.2. Effect of different inoculum levels on the severity of crown canker and root rot.....	66
5.3. Effect of apple rootstock ages on the severity of crown canker and root rot.....	68
5.4. Effect of soil texture and soil type on disease severity.....	68
5.4.A. Effect of soil texture on disease severity .....	68
5.4.B. Effect of different natural soil types .....	71
6. Physiological studies.....	72
6.1. Effect of different natural media.....	72
6.1.a. On the linear growth.....	72
6.1.b. Effect of different liquid media on the mycelial dry weight.....	74
6.1.c. Effect of some natural solid media on sporangial and oospore formation.....	76
A. Effect on sporangial formation .....	76
B. Effect on oospore formation .....	79
6.2. Effect of different degrees of temperature on linear growth.....	81
6.3. Enzymes activity of the tested isolates in vitro.....	81
6.3.A. Activity of polygalacturonase (PG) and pectin methyl esterase (PME) .....	81
6.3.B. Activity of cellulase (cx).....	84
6.4. Root-exudates studies .....	86
6.4.1. Effect of different concentrations of root exudates of two rootstocks on the linear growth of the eight <i>P. cactorum</i> isolates.....	86
6.4.2. Sugars, phenolic compounds and amino acids content of root-exudates.....	86
7. Survival of two <i>P. cactorum</i> isolates in soil.....	89
8. Electrophoretic studies .....	91
8.1. Protein markers.....	91
8.2. Random amplified polymorphic DNA (RAPD) markers .....	91
8.3. Isolate-specific markers.....	91
8.4. Phytogenic relationships among isolates of <i>P. cactorum</i> .....	92
8.5. Trait specific markers.....	98
9. Biological control.....	101

9. Biological control.....	101
9.1. <i>In vitro</i> .....	101
9.2. <i>In vivo</i> experiments.....	104
10. Chemical control.....	107
A. <i>In vitro</i> .....	107
B. <i>In vivo</i> experiment.....	109
<b>DISCUSSION .....</b>	<b>113</b>
<b>V. SUMMARY.....</b>	<b>137</b>
<b>VI. REFERENCES.....</b>	<b>146</b>
<b>ARABIC SUMMARY.....</b>	

## INTRODUCTION

Apple (*Pyrus malus* L.) is considered an economical dicotyledonous fruit trees grown in many Egyptian Governorates. The total area of apple trees cultivated in Egypt reached about 70,474 feddan during 1997. This area, however, was reduced to 64,000 feddan during 1999 (**Ministry of Agriculture, Statistic Dept., ARC 1997 and 1999**).

The most important apple rootstocks grown in Egypt are MM 106 and Malus. Also, there are other rootstocks such as MM 111 and Balady which are less important or rarely used.

*Phytophthora cactorum* (Lebert and Cohn) Schroter, and less frequently *P. syringae* (Klebahn) were the only fungal species reported as causals of crown, collar and root rots of apple (Harris, 1991). However, *P. cactorum* is world wide distributed (**Anon, 1984**). In addition, there are some other species belonging to the genus *Phytophthora*, i.e. *P. megasperma*, *P. camivora* and *P. drechsleri* have been reported to be associated with the root rot of apple in various regions of the world (**Harris, 1991**).

The disease prevails in several apple orchards, especially, in the Northern region of Egypt. The main causal fungus was successfully isolated from different diseased apple trees, purified and identified as *Phytophthora cactorum*.

Based on the available literature, this is the first report on the occurrence of apple crown canker and root rot in Egypt caused by *P. cactorum*.



The main objective of the present study was to determine some pathological aspects of the crown canker and root rot disease of apple and their casual organisms. Further investigations were carried out in order to study some factors affecting disease incidence in the rootstock stage. Extensive physiological studies were carried out regarding hyphal growth and sporangial and oospore formation. The present study was also extended to determine enzyme activities of the fungal isolates, effect of root exudates on growth of the fungal isolates and chemical constituents of root exudates. Electrophoretic detection of protein patterns by SDS-PAGE and RAPD analysis was employed to differentiate between the different isolates of *Phytophthora cactorum* as well as to detect markers linked to some pathogenic and physiological traits of the fungus. Also, the potential of controlling the disease by biological means (antagonistic fungi and actinomycetes) and chemical compounds was investigated.