PHYSIOLOGICAL STUDIES AHD HOST RANGE OF PHYTOPHTHORA CACTORUM (LEB. & COHF.) SCHROET, THE CAUSAL ORGANISM OF LEATHER ROT OF FRUITS AND COLLAR ROT OF STRAWBERRY IN EGYPT

BY

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ABSRACT

Phytophthora cactorum (Leb. & Cohn.) Schroet. Botrytis cinerea Pers. ex. Fr. Rhizoctonia (Corticium) solani (Prill. and Delacr) Bourd. & Galz. and Rhizopus nigricans Ehrenb. ex. Fr. were isolated from diseased fruits and plants of four strawberry cultivars namely; Fresno, Tioga, Aliso and Balady. Phytophthora cactorum was the most virulent pathogen causing most of the damage to rotted fruits. Strawberry cultivars differed as regards their resistance to infection and could be arranged as descendingly as Aliso, Freano, Tioga and Balady. Phytophthora cactorum had a wide host range in Egypt, ARE. i.e., Tomato, Potato tuber, Navel orange, Lemon and Apple. The optimum temperature, of mycelial growth ranged between 22-25°C and the best medium was Carrot Dextrose Agar (CDA) and V-8 Juice for sporulation. Fruit contact with the soil and soil moisture content were mainly responsible for fruit rot.

INTRODUCTION

Strawberry (Fragaria sp.) is among the most favourable and delicious fruits on which the demand increased in ARE during the last ten years. It is produced in some regions of the country such as El-Deir, Khanka regions in Kalubia, Tahrir province near Alexandria, Ismaelia and Gharbia Governorates. Fruits are not only consumed fresh, but may be canned or processed in different forms as well as fresh strawberry fruit are exported in considerable quantities.

The cultivated area increases gradually during the last few years and repelled about 1045 feddans in 1979 producing approximately 2102 tons with average of 2.01 tons per feddan.

(Annual Report of Agric. Statistical Dept. 1980 min. of Agric. ARE).

Strawberry fruit rots cause much losses to fruits both in quantity and quality. in field and during marketing. Numerous pathogenic fungi cause rots of the fruits such as, Leather Rot caused by Phytophthora cactorum. Grey mold caused by Botrytis cinerea. Rhizopus leak caused by Rhizopus nigricans and Hard Brown Rot caused by Rhizoctonia (Corticium) solani. One or more of the previous fungi was recording as the causal organism for strawberry fruit: diseases by different investigators i.e. Bauer (1914), Jorsted (1923), Rose (1924), Wode (1950), Hennebert and Gills (1959), Jarvis (1966), Goverova (1970) and Anbery (197a).

Phytophthora cactorum was isolated from diseased fruits for the first time in ARE by the authors in 1979 and could be considered one of the important causal of rot of strawberry fruits in Egypt, ARE.

This investigation is mainly concerned with some physiological studies and host range of Phytophthora cactorum.

MATERIALS AND METHODS

These studies were carried out in vitro and greenhouse at the Faculty of Agricultural Science at Moshtohar, Zagszig University, Kalubia and Plant Pathology Institute, Agricultural, Res. Center, Gisa; ARE, during 1979 and 1980 seasons.

Isolation of causal organisms:

Diseased fruits of different strawberry cultivars were collected from different localities i.e. Kalubia (El-Deer and El-Khanka), Ismaelia and Gharbia Governorates (Tahrir, Provines) showing various types of rots. Diseased fruits of cultivars (Balady, Tioga, Fresno and Aliso) were rinsed several times in sterilized distilled water. Fruits were surface sterilized.

Specimens of diseased fruit parts with adjacent healthy tissues were placed on plates containing different media, i.e. Potato Dextrose Agar (PDA), Corn Meal Agar Pimarcin Vancomycin

(CMAPV) (Ocana & Taso, 1965) V-8-Juice Agar (Miller 1955), Lima Bean Agar and Carrot Dextrose Agar (CDA) to isolate different causal fungi, Plates were inoculated at 25°C for 3 to 7 days. Hyphal tips were transferred to other plates and isolated fungi were purified, by using single spore or hyphal tip technique and identified microscopically (Hildebrand, 1938). These identifications were verified by the Identification and Taxonomy of Fungi Department, Plant Pathology Institute Agricultural Res. Center, Giza, ARE.

Pathogenicity tests:

Healthy fruits of four cultivars namely i.e., Balady, Tioga, Fresno, Aliso were used in these studies. Fruits were surface sterilized, Forty fruits from each cultivar were inoculated by each of the isolated fungi after wounding each fruit at the base stem and by a sterilized needle glass arid other forty fruits, were inoculated without wounding. All were incubated at 25°C for 3-7 days and the percentage of rotted fruits were recorded.

Reisolation of causal fungi from artificially inoculated fruits was undertaken and the resultant fungi were cohered with the original cultures.

Phytophthora cactorum the new recorded fungus was used in physiological studies and host range as follows:

Disease: assessment:

Disease readings ware determined for each fruit according to disease severity rating which was made to include the average diameter of the infected areas. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

0 = No rot.

1= Scattered small rots.

2= Rots coalescing and including about ¼ - ½ fruit area.

3=More than ½ of the fruit area was infected.

Readings were converted to disease index according to the equation suggested by Townsed and Heuberger (1943) as follows:

$$\text{Disease index} = \frac{\sum (n - r)}{3N} \times 100$$

where, n is the number of fruits in which numerical rate, r and N is the total number of inoculated fruits multiplied by the maximum numerical rate 3.

Host range

Healthy mature fruits of Tomato, Apple, Lemon and Navel orange together with Potato tubers were surface sterilized using ethyl alcohol 95% wounded using a sterilized scalpel. Ten fruits or tubers of each host were inoculated, each with equal disc (Ø5mm) of Ph. cactorum growths grown on CDA (7 days old and incubated at 25°C). Healthy fruits were used as controls. The severity of infection was recorded after incubation period of 7 days.

Media:

Four different media i.e. CDA, V-8-Juice, PDA and CMA were used to study their effect on the fungal growth and spoliation of Phytophthora cactorum. Equal discs (Ø5mm) of the fungal growth (grown on plain agar-medium,7 days old) were placed in Petri-dishes containing the aforementioned media and then incubated at 25°C. The linear growth was measured, daily, and the experiment was terminated when one of the dishes was filled with mycelial growth.

Temperature:

Seven different, degrees of temperature i.e.5,10,15,20, 25, 30, 35°C were used to determine their effect on the fungal growth, equal discs of fungal, growths (grown CDA 7 days old) were plated on CDA. Four plates were used, for degree of temperature. All were incubated at the desired degrees of temperature. Results of the linear growth were recorded as before.

Soil moisture:

To study the effect of soil moisture on fruit, rot three levels i.e. 25, 50 and 100% of soil moisture were investigated. The experiment was carried out in plastic pots (No. 15) each filled with 690 gram formalin sterilized soil. Soil infested with spore

suspension (300.000 Zoospores/ml) prepared, by harvesting the sporangia using sterilized camel hair brush from a 7 day fungal growth on CDA medium in sterilized distilled water (10 ml/dish), then kept at 10 - 12°C for 1- 2 hours to enhance zoospores formation. The inoculum was added to pots at the rate of 25 ml per pot. Healthy offsets (runner plants) of strawberry were transplanted ;one per pot. Five replicates were used for each treatment.

Water holding capacity was determined in a sample of the soil according to Piper (1950). The weights of different quantities of water were calculated and added to reach either 100 or 50 or 25% of WHC of the soil and the weights of planted pots were recorded.

The previous moisture contents of the soil ware' leapt at the desired levels until harvest by adding, the lost quantities of water due to evaporation after weighing the pots every other days. Four replicates for each treatment were used.

At harvest, fruits yield of each replicate were collected. The fruits that were in touch with soil were collected separately for each, treatment, in order to calculate the percentage of leather rot.

RESULTS AND DISCUSSION

Table (1) shows the different isolated fungi from rotted strawberry fruits collected from different regions of ARE.

Table (1): Survey of strawberry fruit rots in different provinces of ARE, with percentage of isolation frequencies of different causals in season 1979-1980.

	The percentage of infection in provinces			
	Tahreer	Gharbia	Kalubia	Ismalia
Botrytis cinerea	25	15	25	20
Phytophthora cactorum	20	10	20	15
Rhizoctonia solani	10	5	5	10
Rhizopus nigricans	5	10	15	5

The following fungi were isolated and purified by single spore or

hyphal tip technique and identified as: Phytophthora cactorum (Leb. & Cohn) Schroet, Botrytis cinerea Pers. ex Fr., Rhizoctonia (Corticium) solani (Prill & Delacr) Bourd & Galz., and Rhizopus nigricans (Ehrn. ex. Fr.) Lind. Phytophthora cactorum and Botrytis cinerea were obtained-more than the other fungi. However, Phytophthora cactorum was isolated easily on Carrot Dextrose Agar medium "CDAM". These results are in agreement with different investigators and one or more of the previous fungi was recorded as the causal organism for strawberry fruit diseases by the following investigators i.e. Bauer (1914), Jorsted (1923), Rose (1924) Wode (1950), Hennebert and Gills (1959), Powelson (1960), Jarvis (1966), Jarvis and Borecka (1969), Govorova (1970), D'Ercol and Canova (1974) and Anbery (1976).

Pathogenicity tests:

Wounded and unwounded strawberry fruits of four cultivars of strawberry namely; Balady, Tioga, Fresno and Aliso were used in this study. Forty fruits of each cultivar were sterilized and inoculated with each of the aforementioned fungi and incubated at 25°C. Percentages of infection and disease severity are recorded in Table (2).

Data in Table (2) show that Phytophthora cactorum was the most virulent fungus responsible for causing the highest damage to the fruits of different strawberry cultivars. Fruits of Balady cultivar were most susceptible to fruit rot disease, whereas Aliso was the least susceptible one. These results are in agreement with those obtained or recorded by many investigators such as Bauer (1914), Rose (1924), Wright et al. (1966), Govorova (1970), Molot and Nourrisseau (1970), D'Ercol and Conova (1974) Anbery (1976) and Kacharmazov & Mirkova (1976).

Results indicated that severe infection was caused by Phytophthora cactorum and are in conformance with isolation results. Thus it was used in further studies.

Table (2): Effect of different isolated fungi on the percentage of infection and disease severity on fruits of four strawberry cultivars.

Different isolated fungi	Different cultivars															
	Balady				Tioga				Fersno				Aliso			
	Unwounded Fruits		Wounded Fruits		Unwounded Fruits		Wounded Fruits		Unwounded Fruits		Wounded Fruits		Unwounded Fruits		Wounded Fruits	
	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity	Mean %of infection	Mean disease severity
Phytophthora cactorum	75	45.7	100	90.0	55	21.7	80	70.0	30	11.7	60	40.0	10	5.0	30	25.0
Botrytis cinerea	70	41.7	90	80.0	60	28.3	70	60.0	25	21.7	50	30.0	15	11.7	20	15.0
Rhizoctonia solani	15	11.7	50	40.0	10	8.3	60	50.0	10	5.0	40	20.0	5	5.0	10	10.0
Rhizopus nigricans	50	26.7	100	80.0	40	16.7	70	70.0	20	11.8	50	30.0	5	5.0	20	20.0

L.S.D. at 0.05	Unwounded Fruits		Wounded Fruits	
	% of infection	Disease severity	% of infection	Disease severity
Fungi (F):	10.4	8.4	5.9	4.6
Cultivars (C):	11.9	10.9	5.8	5.5
F x C:	33.9	21.9	11.7	15.4

Identification of Phytophthora cactorum:-

This was carried out since it was isolated for the first time from strawberries in Egypt. The non-septate mycelium, is more branched especially in the beginning of the growth (Fig., 1). The papiliatea sporangia are recognized easily and are (35-65µ) × (22-35 µ) with average of (36×28 µ) (Fig., 2). Sporangia germinate indirectly giving zoospores (Fig., 2) of directly forming hyphae (Fig. 3) or bearing another sporangium. The antheridium is paragynes. The zoospores are (22 - 30 µ) with average 27µ. Oospores are formed in the synthetic media (Fig., 4) and could be recognized easily (Fig., 5). These results are in agreement with Molot and Nourrissean (1970).

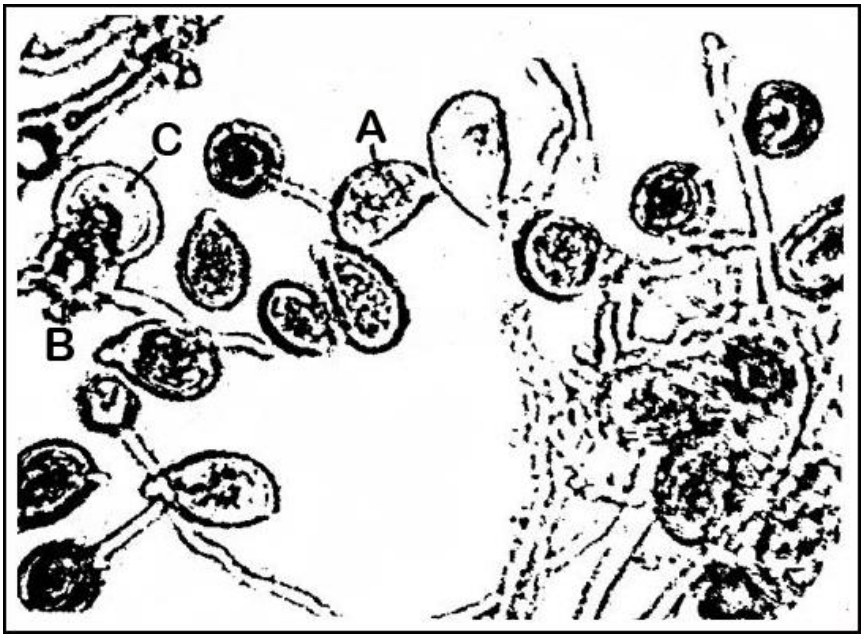

Fig. (1): General view of Phytophthora cactorum (Approx. X190).

A:Sporangium B: Antheridium C: Oogonium

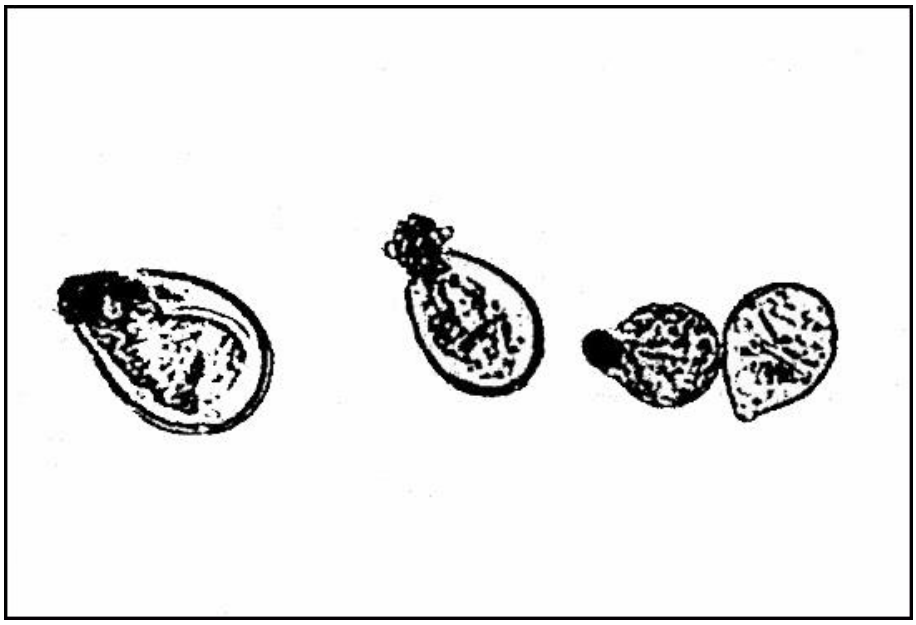

Fig. (2): Zoospores released from sporangium or direct germination of them (Asexual reproduction) (Approx. X 825).

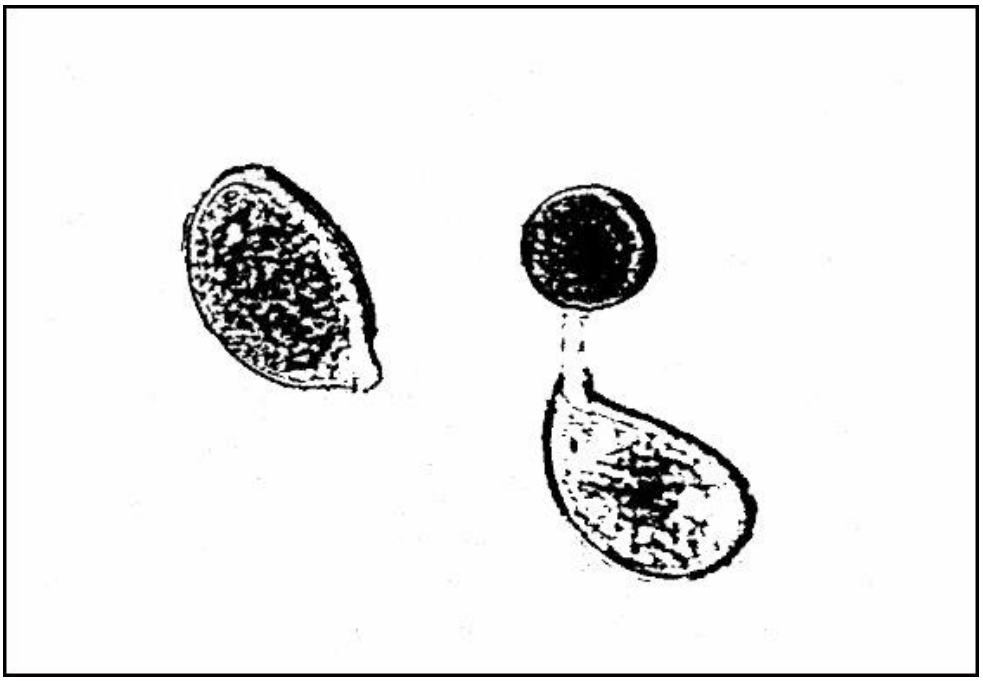

Fig. (3): Indirect germination of sporangium (Like conidia) giving hypha bearing sporangium (Approx. X305).

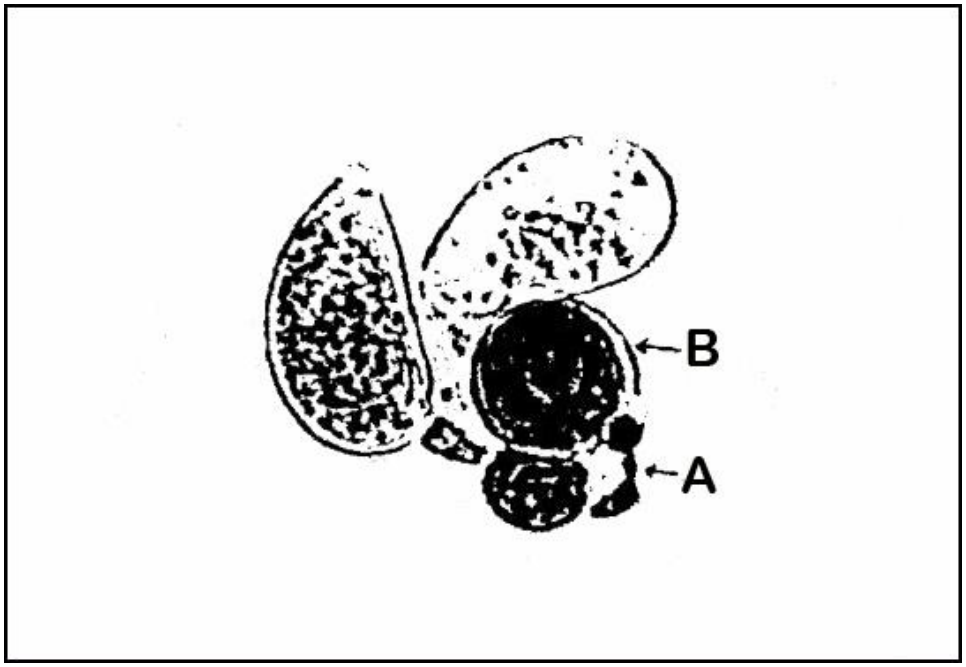

Fig. (4): Closed antheridium beside oogonium in middle stage of sexual spore production. (Approx. X 370).

A: Antheridium B: Oogonium

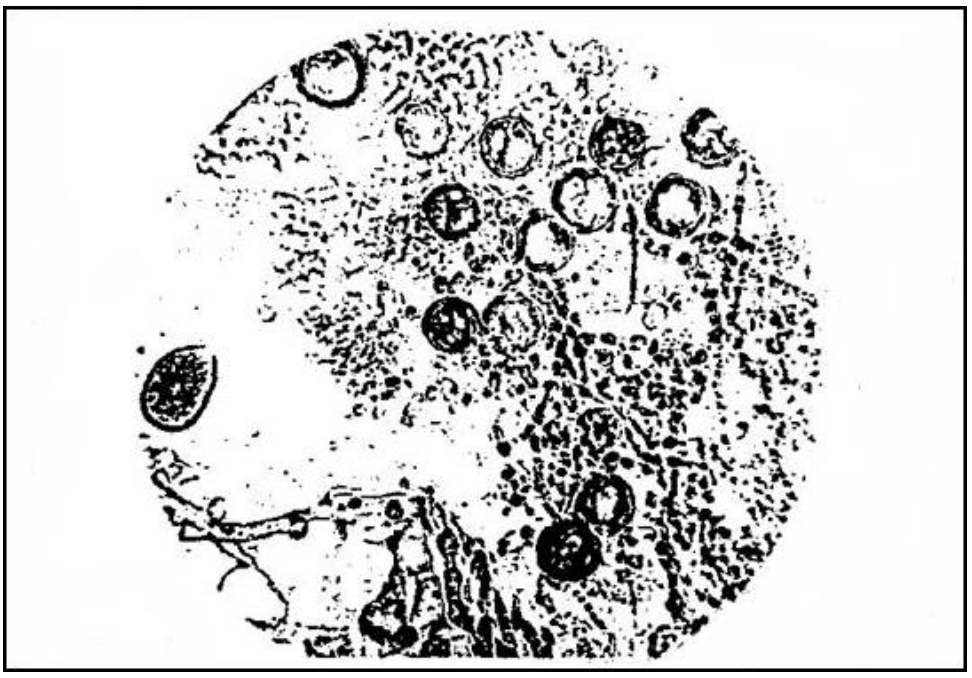

Fig.(5): Last stages of production of Oospores. (Approx. X 95).

Symptoms of disease caused by Ph. cactorum:

On fruits (Leathery Rot):

On green or immature fruits light brown rot with, purple edges. On mature fruits berries become brown or dark brown. Fully ripened fruits become slightly darkened and the tissue surrounding the affected part be corns rotted arid have a bitter taste. In later stages, the infected fruits become leathery.

b. On Plant

Symptoms appear at flowering and fruiting time as a sudden wilting followed by death of the plant. It is essentially a collar rot.

The above results hold fairly good with results recorded by different investigators i.e. Bauer (1914), Boss (1924), Wright et al. (1966) and Molot and Hourrisseau (1970).

Host range

Fruits of different plants together with potato tubers were surface sterilized and artificially inoculated with Ph. cactorum. Reaults are recorded and symptoms of infection are described (Fig. 5) as follows:

1) All fruits were, infected with differences, in symptoms and severity.

2) Symptoms are as follows:

A. Tomato : The infection zone was coloured with the white mycelial growth. Symptoms of infection were characterized with oozing like bacterial diseases.

B. Navel orange : light symptoms accompanied with a white mycelial growth on the inoculation zone. The depth of rotted tissue was about 2 cm.

C. Lemon fruits : Surface symptoms similar to those on Navel orange. However the depth of infection was about 1 cm.

D Apple fruits The Infection zone was coloured with an irregular dark brown area. The depth, of rotted tissues was about 3 cm.

E Potato tubers The infection zone was covered with a white of the mycelium. The depth of rotted tissues was about 1.5 cm.

Severity differed as regards the host and was 70, 50, 20 15, 10% on fruits of Tomato, Apple, Navel orange, Potato tubers and Lemon, respectively.

These results agree with the findings of Wright et al. (1966). They mentioned that wounded fruits of watermelon, tomato, Lemon, nectarine, apple and potato tubers were susceptible to infection, with Ph. cactorum.

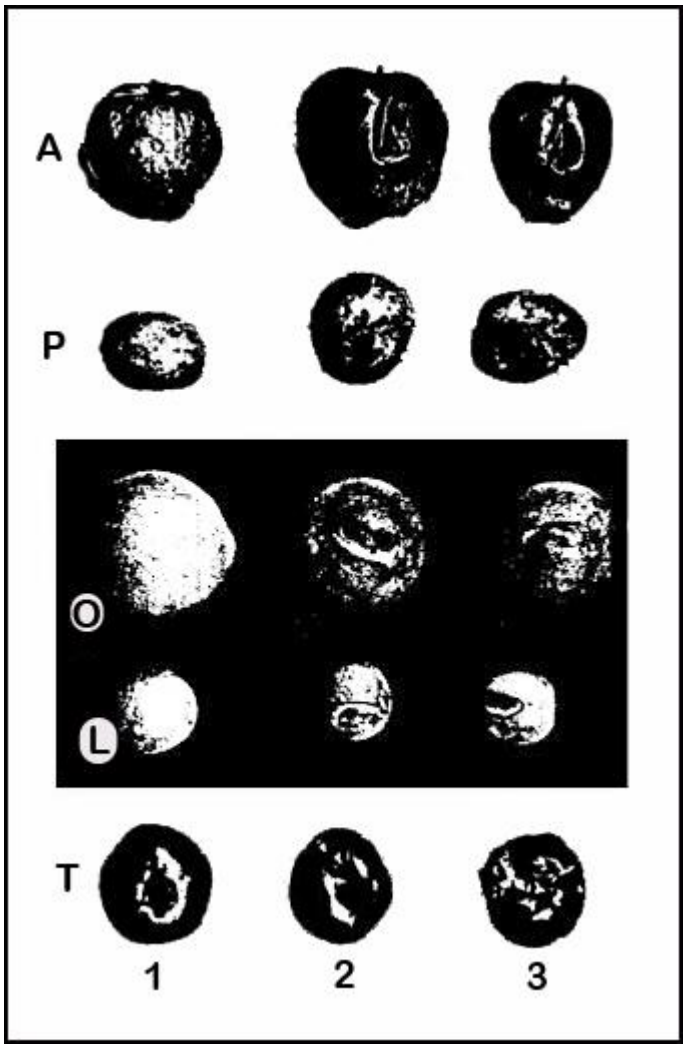

Fig.(6): Different symptoms of infection by Phytophthora cactorum on different host range.

A= Apple
P= Potato
O= Navel orange
L= Lemon
T= Tomato

1= Control unwounded
2 & 3= Wounded

Media:

The effect of media on mycelial growth and sporulation was studied. Four media were used i.e. CDA, V-8-Juice, PDA and CMA. Five plates were used for each medium. Results are recorded in Table (3) average diameters of linear growth. No. of sporangia, antheridia, oogonia and oospores formation.

Table (3): Effect of different media on the linear growth (in mm), no of sporangia and oospores formation of Ph. cactorum.

Physiological characters	Different media			
	CDA	V-8-	CMA	PDA
Mean diameter of linear growth	60	42.5	15	25.0
*Mean No. of Sporangia.	70	8.0	35	10.0
*Mean No. of Antheridia	30	5.0	10	15.0
*Mean No. of Oogonia	30	10.0	10	15.0
*Mean No. of Oospores	30	100.0	5	15.0

*** In a microscopic field L.S.D. 0.05 for media : 17.0**

Data of the above Table (3) show clearly that GDA medium was the best one for mycelial growth and No. of sporangia, antheridia and oogonia formed, whereas V-8-Juice medium was the best for oospore production.

The characteristics of fungal growths on different media were as follows:

1- **On CMA medium: Sporangia abundant Oogonia less frequently produced, antheridia were more than on V-8 Juice medium and oospores less produced and heterogenous.**

2- **On Juice medium: Few sporangia were produced, large number of oogonia antheridia were less than produced on CMA medium and oospores content more homogenous and the wall more pronounced.**

3- **On CPA medium: Sporangia abundant, few antheridia and oogonia were present, whereas little numbers of oospores were produced.**

4- **On PDA medium: Sporangia were produced in less number, whereas antheridia and oogonia were produced in a large number. Oospores were abundant. These results are in agreement with Wright et al. (1966) and Molot and Nourrisseau (1970).**

Temperature :

Data in Table (4) showed that the fungus- grew on a wide range of temperature and the minimum temperature was at 5°C, whereas the maximum was at 35°C. The optimum temperature 25°C The fungal growth, decreased significantly by increasing the temperature above 25°C, and under 5°C. Sexual reproduction as favoured by low temperature ranges from 10 - 20°C, where antheridia and oogonia were formed a large scale and consequently oospores. However at the higher temperatures 25 - 30°C. the fungus tended to form sporangia. Similar results were obtained by Wright et al. (1964 and 1966).

Table (4): Effect of different degrees of temperature on the linear growth (in mm), No. of sporangia, antheridia, oogonia and oospores formation of Ph. cactorum.

Physiological characters	Different temperature (°C)						
	5	10	15	20	25	30	35
Mean diameter of linear growth	5.75	14.0	26.0	30.0	46.3	12.5	6.0
*Mean No. of Sporangia.	--	--	--	15.0	35.0	40.0	--
*Mean No. of Antheridia	--	--	15.0	40.0	50.0	20.0	--
*Mean No. of Oogonia	--	10.0	20.0	50.0	45.0	25.0	--
*Mean No. of Oospores	--	--	20.0	100.0	30.0	5.0	--

* In a microscopic field
L.S.D. 0.05 for media : 17.0

Soil moisture:

WHC of the soil was determined as usual. Blastic (No.15) pots were used. Different. V-8 Juice levels were obtained by adding different quantities of water, then were weighed The lost amounts of water were added every other days. Results of rotted fruits., are recorded in Table (5).

Table (5): Effect-of soil moisture on the percentage of infection with Ph. cactorum on Tioga cultivar at different WHC-levels.

WHC-levels (%)	Mean of total fruits/plant	Mean. of total No. of infected	Mean of percentage of infection
100	3.5	2.8	81.87
50	6.9	2.1	31.19
25	2.0	0.4	11.94

L.S.D, 0.05 for % infection : 18.13

Data in Table (5) show generally that all fruits that touched

the soil were found to be infected with the fungus, while, others were free of infection in all treatments. The increase in fruit infection with, the in WHC as noticed in the above table is mainly due to the increase in crop and consequently the increase in the No. of fruits touching the soil, parallel to the increase in the harvest with increased WHC level, This agrees with the results of Bauer (1914), Wright et al. (1964), Gisi (1975) and Meyer and Schonbeck (1975).

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دراسات فسيولوجية والمدى العوائلي للفطر فيتوفثورا كاكتورام المسبب لمرض العفن الجلدي لثمار الشليك في مصر

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تم عزل الفطريات فيتوفثورا كاكتورم لأول مرة في جمهورية مصر العربية ورايزوكتونيا سولاني ،بوطرايتس سينيريا ، ريزوباس نجريكانس من ثمار الشليك المختلفة والمنزرعة في مناطق مختلفة من الجمهورية مثل الدير والخانكة والاسماعيلية وجنوب التحرير.

وجد أن الفطر فيتوفثورا كاكتورم يسبب خسارة شديدة على محصول الشليك كل عام تحت ظروف الحقل. كما وجد للفطر عوائل مختلفة يصيبها مسبب لها اعراض مختلفة مثل درنات البطاطس وثمار الطماطم والتفاح والبرتقال والليمون.

امكن تنمية الفطر على بيئات مختلفة مثل بيئة آجار الجزر والبطاطس والذرة والجوس واعطت بيئة آجار الجزر احسن نمو للفطر في حين كانت أحسن بيئة لإنتاج وتكوين الجراثيم البيضية هي بيئة جوس.

كما وجد أن درجة الحرارة المثلى لنمو الفطر تقع ما بين ٢٢-٢٥°م والدرجة الصغرى هي ٥°م والدرجة الكبرى ٣٥ °م.

وجد انه لتلامس ثمار الشليك للتربة والمحتوى الرطوبي لها هما المسئولان الاساسيان لحدوث مرض العفن الجلدي للثمار المتسبب عن الفطر فيتوفثورا كاكتورم.