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Grapevine Twigs Tip Die-Back Disease in Egypt

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Several fungi cause grapevine twigs tip die-back disease in Egypt. *Botryodiplodia theobromae* Pat.; *Phomopsis viticola* Sacc. and *Fusarium solani* (Mart.) Sacc. were the most frequently isolated fungi from diseased twigs. These fungi caused the disease symptoms either in the current growing season or in the next. Among the isolated fungi *B. theobromae* was the most pathogenic one in the current growing season. It showed twigs tip die-back and long necrotic parts in pathogenicity test, while, *P. viticola* and *F. solani* were the lowest, in this respect. The fungi tested were able to cause twigs tip die-back and buds mortality in the following season. *B. theobromae* isolates were categorized into 3 biotypes, biotype 1 included El-Sharkia and El-Giza isolates, while, biotype 2 included North Sinai isolates and the third one included El-Ismaelia isolates.

Key words: *Botryodiplodia theobromae, Fusarium solani,* grapevine, twigs tip die-back and *Phomopsis viticola.*

Grapevine (*Vitis vinifera* L.) is the most widely distributed orchard crop in the world, covering an area of approximately 10 million hectares. It grows from temperate to tropical regions but most vineyards are planted in area with temperate climate. The grape is produced for multi uses.

Diseases can result in substantial losses in grape production. Die-back disease attacks twigs of grapevine and causes great losses in the yield (Hewitt, 1988; Ferreira *et al.*, 1989 and Machowicz-Stefaniak *et al.*, 1991). Through the few past years, the disease was found to be spread out in several governorates in Egypt. Under local conditions few reports have been published about the disease incidence (El-Goorani and El-Mcleigi, 1972 and Farag, 1998).

Thus, this work was aimed to isolate and identify the fungi associated with samples of grapevine twigs tip die-back disease collected from different governorates in Egypt. The pathogenic capabilities of the isolated fungi were also studied. Pycnidia, conidiophores length and width of *B. theobromae* isolates, using light and scanning electron microscopes, were used to classify the pathogen into biotypes.

Materials and Methods

1. Survey of grapevine twigs tip die-back disease incidence in Egypt under natural infection :

Grapevine twigs tip die-back disease survey was carried out during 1996 and 1997 growing seasons on different grapevine cultivars (El-khalily, Flame seedless, Thompson seedless, King Robi, Romi Ahmer and Bez El-Anz) at North Sinai (Nakhl and El-Arish); El-Ismaelia (El-Cassasein and El-Salhiya); El-Sharkeia, (Menyah El-Kameh and Belbase); El-Gharbia (Met-Mimona) as well as El-Giza (Embaba and El-Saff) governorates. The survey was conducted by calculating the percentage of the disease incidence (disease infection) through counting the number of infected and non-infected grapevine twigs in each governorate orchard tested. The disease severity (DS) estimated according to Barakat *et al.* (1990) with some modification as follows: $D.S. = \frac{\sum n \times V}{5N}$

Where:

n = the number of diseased branches in each infected category (grade).

v= Numerical value of the grade as follows:

0 = No infection (healthy).

1 = 10% infection.

2 = 11-24% infection.

3 = 25-50% infection.

4 = 51-75% infection.

5 = Maximum disease severity grade.

N = Total number of inspected branches.

2. Isolation, purification and identification of fungi associated with grapevine twigs tip die-back disease:

Twigs from grape showing symptoms of grapevine die-back were collected and surface sterilized with 0.001 sodium hypochlorite for 3 min, then rinsed three times with sterile distilled water. Sections from the infected xylem were dissected with sterile scalpel and placed on potato dextrose agar (PDA) medium containing 1000 ppm streptomycin sulfate and incubated at 25°C for one week.

Observation was daily carried out and any emerged fungus was picked up and cultured on fresh PDA plates . All the isolated fungi were purified using either single spore or hyphal tip techniques (Dhingra and Sinclair,1985) and sub-cultured on PDA medium. Isolates obtained were identified at Pl. Pathol. Lab., Agric. Bot. Dept., Fac. Agric., Zagazig Univ. according to Ellis (1971) and Barnett and Hunter (1987).

Identification of isolated fungi were kindly confirmed by Division of Fungal Taxonomy, Pl. Pathol. Res. Inst., ARC, Giza. Stock cultures were maintained on PDA slants and kept in refrigerator at 5°C for further study. Stocks were routinely sub-cultured on fresh slant every three months. The frequency of the isolated fungi were calculated separately for each of the collected samples.

3. Pathogenicity tests for the isolated fungi:

3.1. Grapevine plants:

One year-old rooted vines produced from cuttings of the same surveyed cultivars were planted in pots (40 cm in diam) contained 3 kg mixture of peat-moss; sand and clay (1:1:1 w/w). The plants were irrigated with tap water when necessary.

3.2. Inoculation methods:

3.2.1. In twigs of the current growing season:

The purified fungi were grown individually on PDA medium for 7 days. Each isolate was inoculated in grape twigs of the same cultivar from which it was isolated. Small disk (3 mm in diam) of the desired fungal growth was inserted in a split (5 mm length and 2 mm depth) made in the base of new-shoots which measure about 20 cm length for their respective cutting as mentioned by Peros and Berger (1994). Control treatments were done by the same method using PDA medium without fungus.

Treated plants in greenhouse were covered for overnight with polyethylene bags. Number of plants exhibiting die-back symptoms and the average of necrotic area (in mm) around the infection wounds produced by fungi tested were estimated after 4, 21 and 28 days from inoculation.

3.2.2. In twigs of the next growing season:

Each isolate was inoculated individually as mentioned before but at the end of seasonal vegetative growth of shoots. The plants were pruned to 2 bud/plant in January and symptoms were observed during the second vegetative growth cycle by calculating the number of mortality buds and twigs with tip die-back.

4. Morphological differentiation between B. theobromae isolates:

Some experiments were conducted according to Krupinsky (1983) and Leavitt and Munnecke (1987) to differentiate morphologically between *B. theobromae* isolates collected from different governorates in Egypt.

The spores of *B. theobromae* (one and two-celled spores) obtained from individual pycnidia as well as grouped pycnidia were compared with each other using light and scanning electron microscope (SEM). Also, differences in length and width between spores and pycnidia of isolates were determined.

The ability of *B. theobromae* isolates tested to produce red pigment as well as linear growth ratio at 36°C was studied on PDA medium. The medium poured in sterile Petri-dishes (9 cm in diam).Three plates for each isolate were inoculated at the center with an equal disk (7 mm in diameter) taken from 7-day-old culture of each isolate. The plates were incubated at 36°C. The isolates tested were examined for the presence of visible red pigment. The linear growth was determined daily for each isolate by measuring the two dimensions of growth in each plate and the mean was estimated. This experiment was terminated whenever, the mycelial growth covered the plate surface in any treatment.

Results and Discussion

Grapevine (*Vitis vinifera* L.) is the leading fruit crop all-over the world including Egypt. Under the Egyptian conditions grapevine is subjected to invasion by many phytopathogenic fungi causing annually considerable losses in the yield. Die-back disease of grapevine has not been thoroughly studied since 1972 when it was recorded for the first time by El-Goorani and El-Meleigi, (1972) followed by Farag (1998). The observed symptoms during survey were the same as mentioned before by many research workers (El-Goorani and El-Meleigi, 1972; Leavitt and Munnecke, 1987 and Hewitt, 1988). Field survey carried out at different localities in five governorates (North Sinai; El-Ismaelia; El-Sharkia; El-Gharbia and El-Giza) showed that, grapevine twigs tip die-back disease existed in the five inspected governorates. The percentage of natural infection ranged from 9.0% to 32.15% at the growing season 1996 while it ranged from 8.93 % to 20.0 % at the growing season 1997 (Table 1). On the other hand, data in Table (1) reveal also that, the disease severity ranged from 11.16% to 29.26% at the growing season 1996 and from 4.23% to 23.94% at the growing season 1997. In the present study, thirteen fungi were frequently isolated from the diseased twigs (Table 2).

Table 1. Percentage of grapevine twigs tip die-back disease incidence and severity at different governorates during 1996 and 1997 growing seasons

Governorate	Grapevine twigs tip die-back disease incidence (%)		Mean	Disease severity (%)		Mean
	1996	1997		1996	1997	
North Sinai	32.15	20	26.07	29.26	23.94	26.6
El-Ismaelia	27.16	19	23.08	23.8	9.88	16
El-Sharkeia	9	11.5	10.25	11.16	6.37	8.76
El-Gharbia	14.8	8.93	11.86	13	5.69	9.34
El-Giza	16.26	16.94	16.6	19.26	4.23	11.74
Mean	19.87	15.57	17.44	19.29	10.02	14.66

The most frequently isolated fungi were *B. theobromae* followed by *Alternaria* sp. and *A. alternata* while, *Aspergillus niger* was the least isolated one. Differences of the disease occurrence as well as frequency of the isolated fungi among the investigated governorates might be due to varietal differences and environmental factors favorable for infection by the causal organisms. These results agree with those obtained by Farag (1998) who isolated *B. theobromae* and *Alternaria* sp., from grapevine twigs tip die-back; Kozar *et al.* (1992) isolated *Phomopsis viticola* and Machowicz- Stefaniak *et al.* (1991) isolated *B. theobromae*

Table 2. Percentage of isolated fungi from infected sample of grapevine which exhibited typical symptoms of twigs tip die-back disease during 1996 and 1997 growing seasons

Isolated fungi	North Sinai		El-Ismaelia		El-Sharkeia		El-Giza		El-Gharbia		Total	
	96	97	96	97	96	97	96	97	96	97	96	97
B. theobromae	5.48	6.51	28.33	21,8	0	3,26	12,66	8,56	0	0	46,47	40,1
F. semitectum	0	0.34	1,7	0,34	0	0	0,65	0,17	0	0	2,35	0,86
F. solani	0.78	0.34	0,26	1,03	0	0	0	0,51	0	0	1,04	1,89
Fusarium sp.	0.39	0	2,22	0	0,52	0	0	0,86	0,26	0	3,39	0,86
Alternaria sp.	2.87	4.29	7,05	9,95	3,26	4,17	6,53	7,55	1,17	0,51	20,9	26,4
A. alternata	1.44	1.38	4,44	3,43	2,09	3,09	6,79	8,92	0,91	0,67	15,7	17,3
P. viticola	0	0.34	1,04	1,89	0,13	0,34	0	0	0	0	1,17	2,57
Curvularia sp.	0.71	3.26	1,96	0	1,17	2,23	0,26	0	0	0	5,09	5,49
Cladosporium sp.	0	0	0	0	1,04	0,17	0	0,17	0	0	1,04	0,51
Helminthosporium sp.	0	0	0.13	0,34	0,26	1,72	0	0	0	0	0,39	2,06
Stegmenia viticola	0.13	0.34	0,65	0,34	0,52	0	0	0	0	0	1,3	0,69
Aspergillus niger	0	0	0,39	0,51	0	0	0	0	0	0	0,39	0,51
Nigrospora sp.	0	0	0,52	0,69	0,26	0	0	0	0	0	0,78	0,68
Total	12,8	16,8	48,7	40,3	9,26	15	26,9	26,8	2,35	1,2		

and *P. viticola* from grapevine twigs tip die-back. On the other hand, many phytopathologists isolated *B. theobromae* (*Diplodia natanlensis*); *P. viticola* and *F. solani* from other fruit trees that exhibited die-back symptoms (Adaskaveg *et al.*, 1999 and Turney, 1999).

The present study also revealed out that, there were more than one fungus causing die-back in Egypt: *B. theobromae*; *P. viticola* and *F. solani*. These fungi proved to be the most pathogenic causing die-back symptoms either in current growing season or in the next growing season. It was also found that, isolate No. 1 of *B. theobromae* (North Sinai isolate) was the most virulent one in the current growing season. This isolate gave the highest percentage of twigs tip die-back and longest necrotic parts.

No plants exhibited twigs tip die-back due to infection by *P. viticola* and *F. solani* (Table, 3). The symptoms of infection by those fungi only showed necrotic parts. Data in Table (3) also indicate that, *B. theobromae* isolates; *P. viticola* and *F. solani* were able to cause both twigs tip die-back and mortality in buds in the second growing season (February–March). The highest percentage of twigs tip die-back was due to infection by *B. theobromae* isolate No. 2 (El-Ismaelia isolate). On the other hand, the highest percentage of mortality in buds was due to infection by *B. theobromae* isolate No. 3 (El- Sharkia isolate). While, *F. solani* caused the lowest effect either in buds mortality or twigs tip die-back. It is worthy to mention that, *P. viticola* was intermediate in this concern. These results agree with those obtained by Latham *et al.* (1992); Sharma and Gupta (1999) and Kuo *et al.* (1999).

Table 3. Pathogenicity test of the isolated fungi from grapevine twigs tip with die-back disease symptoms

Isolate tested	In the current growth season			In the next growing season		
	No. of inoculated twigs	Twigs with tip die-back (%)	Necrosis around inoculated twigs wound (mm.)	No. of tested buds	Mortality in Buds (%)	Twigs with tip die-back (%)
B. theobromae-1	10	56.67	44.33	10	56.66	40.33
B. theobromae-2	10	20.00	41.66	10	20.00	60.33
B. theobromae-3	10	46.67	42.66	10	43.33	50.66
B. theobromae-4	10	43.33	39.33	10	53.33	50.00
P. viticola	10	0.0	38.66	10	26.66	50.00
F. solani	10	0.0	16.00	10	13.33	30.66
Control	10	0.0	0.0	10	0.0	0.0
The rest isolates did not cause any symptoms of die-back						

L.S.D value for:	at 5%	at 1%
Twig die-back (A)	14.80	20.54
Necrosis (B)	6.932	9.621
Mortality in buds(C)	10.81	15.00
Twigs tip die-back (D)	1.666	2.321

Differences in morphological characteristics (the measurement of pycnidia and spores) were observed in different isolates of *B. theobromae* isolated from grapevine twigs grown in different governorates (Table, 4). Results obtained indicate that, pycnidia of isolate No. 4 were bigger in length (226.54μ) and width (214.94) than other isolates tested followed by isolates No. 1 and 2 (225.18 and 200.22 μ as well as 185.79 and 173.3 μ, respectively). Isolate No. 3 was the smallest one in length and width (185.38 and 173.22 μ, respectively). Data in Table, (4) also indicate that, there was no correlation observed between pycnidial size and width of one or two celled spores.

Variation in mycelial linear growth at 36°C, mycelial color as well as red pigment production was detected between isolates tested. According to Leavitte and Munecke (1987), they classified *B. theobromae* isolates into two biotypes, biotype 1 grew rapidly at 36°C and produced red pigment. Biotype 2 grew slowly at 36°C without producing red pigment. Data in Table (5) reveal that, isolates of *B. theobromae* under Egyptian conditions were categorized according to their properties into 3 biotypes, biotype 1 includes isolate No. 3 (El-Sharkeia isolate) and isolate No.4 (El- Giza isolate) which produced red pigment; and biotype 2 includes isolate No. 1 (North Sinai isolate) and the third biotype group which includes isolate No. 2 (El-Ismaelia isolate). These results are in harmony with those reported by Douglas Barbe and Hewitt (1965) who found that, some isolates of *B. theobromae* produced red pigment in PDA medium and also in the mycelium structure. Red pigment production responds to high temperature. Photographs obtained from Light and Scanning Electron Microscope (SEM) cleared that there are two types of pycnidia, type 1 stromatic pycnidia included isolate No. 4 only, where pycnidia were embedded in stromatic tissues. The other isolates produced pycnidia

Table 4. Differences in pycnidium and spore length and width of *B. theobromae* isolates

Isolate	Pycnidia		Spores			
	Length (μ)	Width (μ)	One-cell spores		Two cells spores	
			Length (μ)	Width (μ)	Length (μ)	Width (μ)
B. theobromae-1	138.2-300	117.6-366.6	15.71-25.71	10-11.42	15.71-24.28	8.71-11.42
Average	225.18	200.22	20.23	10.05	19.57	10.29
B. theobromae-2	117.6-258.8	117.6-252.9	15.71-25.71	9-12.85	15.71-24.28	9-12.36
Average	185.38	173.3	21.47	10.43	20.85	10.38
B. theobromae-3	117.6-258.8	117.6-252.9	15.71-25.71	8.75-12-85	15.57-21.42	8.71-12.42
Average	185.38	173.22	21.62	10.1	18.63	10.05
B. theobromae-4	147-287.8	123.5-266.6	15.71-25-71	8.71-11.42	15.71-24.28	8.71-11.42
Average	226.54	214.94	20.23	9.34	19.99	9.91
L.S.D. at 5 %	10.61	11.57	0.683	0.524	0.674	0.529

Table 5. Effect of incubation at 36°C on linear growth (mm), mycelium colour and red pigment production of different *B. theobromae* isolates

Isolate	Linear growth (mm) intervals (days)							Mycelium colour	Red pigment production
	1	2	3	4	5	6	Mean		
B. theobromae (1)	0	18.8	34	42	57.3	80	38.7	White	-
B. theobromae (2)	0	25	40.3	51	70	90	46.0	Brown	-
B. theobromae (3)	0	19	37	49	72	90	44.5	Gray	+
B. theobromae (4)	0	14	25	39	50	63	31.8	black	+
Mean	0	19	34.1	42.3	62.3	80.8			

L.S.D. at 5 % for Isolates (I)= 3.74 Periods (P)= 4.82

with stromata (Fig. 1 A, B and C). On the other hand, the status; single or grouped, of pycnidia varied among isolates tested. Isolates No. 1, 2 and 4 produced both single and grouped pycnidia while isolate No. 3 produced grouped pycnidia only (Fig. 2 A, B and C). Such results are also in harmony with those obtained by Hassan, (1980) who found that, *B. theobromae* isolates obtained from different hosts varied in the formation of pycnidia, pycnidiospores and stromata. Accordingly, isolates obtained were categorized under two main groups divided into two subgroups, subgroup 1 included single and grouped pycnidia and subgroup 2 included pycnidia in groups. Further studies are needed to clarify the taxonomic relationships among biotypes of *B. theobromae* isolates.

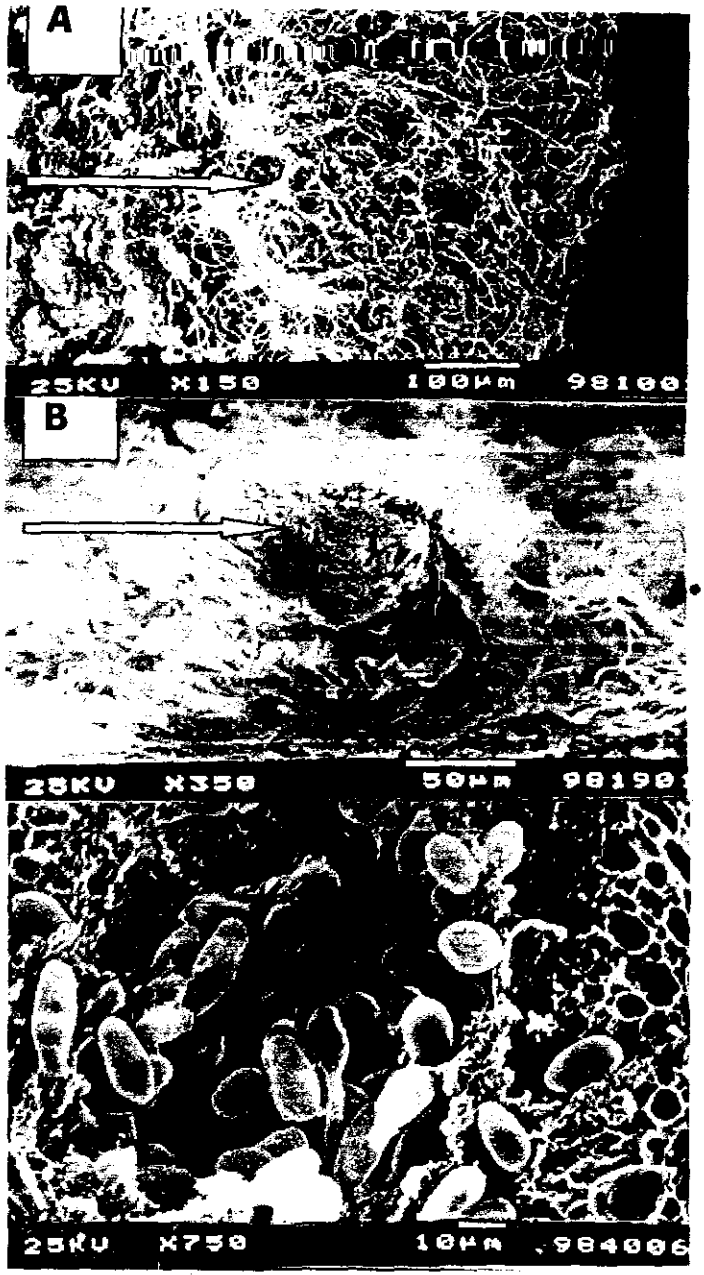

Fig. 1. Scanning electron microscope photograph of *Botryodiplodia theobromae* isolate No. 4. Arrows indicate the ostiole of immature pycnidium (A), arrow indicate the mature pycnidium embedded in stromata (B) and cross section in mature pycnidium (C).

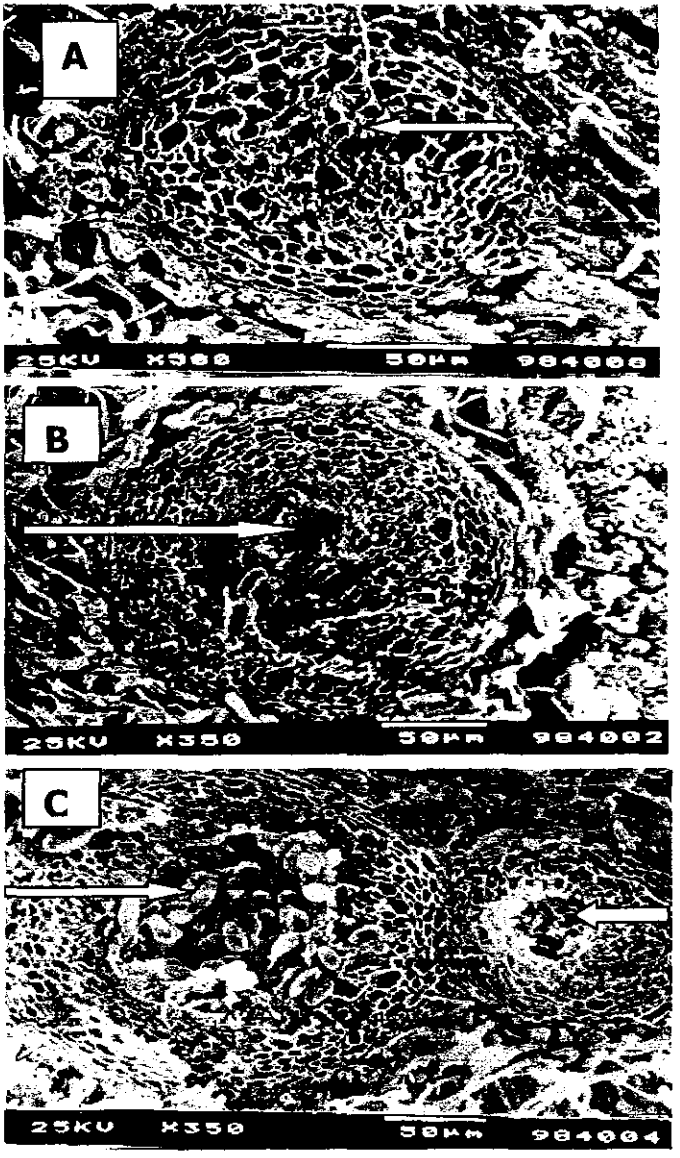

Fig. 2. Scanning electron microscope photograph indicating differences between pycnidia of *Botryodiplodia theobromae*: A, pycnidium of isolate No. 2 (notice undistinguished ostiole, by arrow). B, pycnidium of isolate No. 1 (notice ostiole, indicated by arrow). C, two pycnidia associated together in one group and spores found in pycnidial cavity of isolate No. 3 (arrows).

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مرض الموت الرجعي لأطراف أغصان العنب فى مصر

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تسبب العديد من الفطريات مرض الموت الرجعي لأطراف أغصان العنب في مصر، و قد تم تكرار عزل فطريات البتريوديلوديا ثيوبرومي و الفومبوبسس فيتيكولا و الفيوزاريم سولانى من أغصان العنب المصابة . أدت الفطريات المعزولة إلى حدوث أعراض المرض فى نموات نفس الموسم أو الموسم التالى. و قد كان الفطر بتريوديلوديا ثيوبرومي أكثر الفطريات قدرة على إحداث المرض على نموات نفس الموسم حيث أظهر اختبار العدوى ظهور أعراض مرض الموت الرجعي لأطراف أغصان العنب مع ظهور أجزاء طويلة متقرحة بينما كان الفومبوبسس فيتيكولا و الفيوزاريم سولانى اقلهم قدرة على إحداث المرض. كما كانت الفطريات المختبرة قادرة على إحداث مرض الموت الرجعى على أطراف الأغصان مع موت البراعم فى نموات الموسم التالى. و قد تضمنت عزلات البتريوديلوديا ثيوبرومي على ثلاث طرز وراثية الأول منها عزلات محافظة الشرقية و الجيزة بينما احتوى الطراز الثانى على عزلات شمال سيناء، أما الطراز الأخير فاقتصر على عزلات الإسماعيلية.