HISTOPATHOLOGY AND CONTROL OF *BOTRYODIPLODIA THEOBROMAE* ROT OF DATE PALM OFF-SHOOT (*PHOENIX DACTYLIFERA* L.) VARIETY ZAGHLOUL

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Abstract

The pathogenic capabilities of fifteen isolates of *Botryodiplodia theobromae* Pat. Isolated from nine hosts (Mango, Grapevine, Apple, Apricot, Plum, Peach, Pear, Persimmon and Palm) at various region of Egypt were studied on date palm off-shoot. All isolates were pathogenic on date palm off-shoot except the Mango isolates. Seven days after inoculating date palm off-shoots (Zaghloul cv.), with grapevine isolate the fungal hyphae were clearly noticed in parenchyma cells and spread towards phloem, xylem parenchyma and protoxylem causing necrosis. After 14 days from inoculation, the fungal hyphae completely colonized the meta-xylem. The linear growth of *B. theobromae* was completely inhibited at 10 ppm of Thiophanate-methyl (Hista 70% w.p) and Carbendazim (Actazime 50% w.p) followed by Pyrifenox (Dorado 200 E.c) at 40 ppm. and Propioconazol as Cornazol 10 Ec) at 50 ppm. Carbendazim and Thiophanat-methyl gave the best control followed by Propioconazol and Pyrifenox.

INTRODUCTION

Botryodiplodia theobromae Pat. is a wide spread plant pathogen in different parts of the world as well as in Egypt. It is a progressive and vigorous pathogen that causes several diseases to a large number of fruit trees such as Apple (Lathan and Dozier 1989), Avocado (Darvas and Kotze, 1987), Date Palm (Abdl-Megied and Gafar, 1966), Grapevine (Ammar, 1998 and Aly *et al.*, 2002), Guava (Baiuomy *et al.,* 2003), Mango (Al – Adawi *et al.*, 2003 and Aly *et al.,* 2004) and Peach and some pomes and stone fruit trees (Barakat *et al.,* 1990). Abdl-Megied and Gafar (1966) revealed that, *Diplodia phoenicum* is the causal fungus of Diplodia leaf stalk rot in date palm off-shoot whether still attached with mother tree or after they have been planted. This disease is characterized by yellowish brown lesion extending along the rachis. The

lesion becomes brownish and the terminal bud may become infected. Symptoms include wilt and eventual desiccation of the youngest leaves which become yellowish white in color. The new leaves can be easily detached by hand since their bases are soft rotted, dead and covered with the blackish mycelial growth of the fungus. Sometimes, mature leaves remain healthy for several weeks after the death of the bud. On the other hand, the fungus can invade the unwounded trunk and disintegrate its fibers causing charcoal soft rot. The infected area is usually characterized by constriction and deep cracks. Bhamsali (1989) described *B. theobromae* Pat. symptoms on date palm-off-shoot in India. Some of those symptoms were somewhat, similar to those described in Egypt especially on the leaves and the trunks. On the other hand, *B. theobromae* was found to be one of the causal pathogens of date palm diseases. Symptoms of infection appeared as necrotic tissues at the top of the palm, crown and terminal bud (Brun and Laville, 1995) and on young unopened crown leaves of off-shoots, however, yellowish brown streak might appear on leaves and might result in death to bud of the off-shoots which were experienced in Egypt.

The present investigation was carried out to study the pathogenic capabilities of *B. theobromae* isolates, originated from different hosts, on date palm off-shoots and showing a variety of disease symptoms. Also, the histpathological changes in the structure of date palm off-shoot tissues after artificial inoculation with *B. theobromae* was considered. Disease control using some fungicides was studied.

MATERIALS AND METHODS

1 – Sources of *Botryodiplodia theobromae* Pat. isolates

Fifteen isolates of *B. theobromae* obtained from nine different hosts showing different symptoms from various governorates of Egypt were used. These isolates were obtained from Research Department of Fruit and Woody Trees Diseases, Plant Pathology Research Institute, A. R. C. Details of the obtained isolates are tabulated as in Table (1):

Table 1. *Botryodiplodia theobromae* isolates used, their code number, hosts, symptoms and governorates

Code No. of isolates	Hosts	Symptoms	Governorate
4	"Mango" *Mangifera indica* L	Gummosis, die-back and vascular discoloration	Giza
B2	"Mango" *Mangifera indica* L.	Stem end rot (SER)	Ismaelia
900	"Mango" *Mangifera indica* L.	Stem end rot (SER)	Giza
777	"Mango" *Mangifera indica* L.	Stem end rot (SER)	Ismaelia
334	"Grapevine" *Vitis vinifera* L.	Die-back	Sharkia
410	"Apple" *Malus domestica* Borth	Root rot	Nobaria
T11	"Apple" *Malus domestica* Borth	Cankers and die-back	Monefia
811	"Apricot" *Prune armeniaca* L.	Root rot	Nobaria
555	"Plum" *Prune domestica* L.	Gummosis and die-back	Nobaria
880	"Peach" *Prune Persica* L.	Die-back	Monefia
901	"Peach" *Prune Persica* L.	Die-back	Gharbia
902	"Pear" *Pyrus communis* L.	Die-back	Monefia
590	"Persimmon" *Diospyros kaki* L.	Cankers	Gharbia
504	"Palm" *Phoenix dactylifera* L.	Basel rot and black spot	Nobaria
501	"Palm" *Phoenix dactylifera* L.	Basel rot and black spot	Monefia

2- Virulence evaluation of *Botryodiplodia theobromae* Pat. isolates on date palm off-shoots:

Plants:

Two years old date palm plants originating from seeds of the highly susceptible Zaghloul vr. (Sabet, *et al.,* 1995) were grown in plastic pots No. 40 filled with a mixtures of sand and clay soil (1:1 v:v). Through the period of growth, the plants were irrigated and fertilized when needed. Three replicate plants were used for each treatment

Fungal inoculum and inoculation.

B. Theobromae isolates were separately grown on PDA media in Petri-dishes for seven days at 25-30 °C. The inoculation was carried out by placing a PDA disk (5 mm in diameter) containing the mycelium and spores on wounds (5 mm, long x 2 mm deep) made in the basal parts of the leaves. The proper control sets were inoculated in the same way with fungal free PDA medium. Three replicates were used for each treatment. All treatments were incubated in a controlled greenhouse at 25 °C $\pm$2. Plants were covered with polyethylene bags to maintain high humidity level around the plants for 48 hrs. After 3, 7 and 10 days, the area of the rot lesions, appearing on the infected shoots was measured and expressed in mm^2.

Histopathological studies:

Tissue blocks from the base of healthy or previously artificially inoculated leaf stalk, by the most pathogenic grapevine isolate were cut into small portions (5- 10 mm. Long). The portions were washed in distilled water, dried between folds of sterilized paper towels, killed and fixed in formalin acetic acid alcohol solution (F.A.A) then dehydrated and embedded in wax. Sections were cut at 15- 20 µm thickness by the rotary microtome then stained with safranin and crystal violet (Johanson, 1940). The sections were mounted on slides in Canada - balsm, microscopically examined and photographed .

Evaluation of four different fungicides against *Botryodiplodia theobromae* isolates *in vitro*:

Four different fungicides, Propiconazole as Cornazol 10% EC, Pyrifenox as Dorado 200 Ec, Thiophanate-methyl as Hista 70 % wp. and Carbendazim as Actazime 50% wp were tested both *in vitro* and *in vivo*.

***In vitro* effect of fungicides on fungal linear growth:**

Different concentrations (0, 5, 10, 20, 30, 40, and 50 ppm.) of each fungicide tested were prepared according to their active ingredient and mixed with autoclaved PDA medium before solidification to study their effect on the linear growth of tested *B. theobromae* isolates. Five replicates of each concentration were inoculated with an equal disc (7 mm in diam.) of mycelial growth of each isolate obtained from 7 days old culture. The inoculated dishes were incubated at 25°C ± 2 until fungal growth completely covered the surface of the check treatment (fungicides free PDA medium). The two perpendicular diameters of the linear growth were measured and their mean was calculated.

***In vivo* effect of different fungicides:**

Rachis of young seedlings (originated from seeds of cv. Zaghloul) were cut into parts, 10 cm long, which were used to study the effect of fungicides showing an inhibitory effect on the linear growth of *B. theobromae* isolates on disease development. The excised plant parts were washed with ethanol, artificially wounded and inoculated as mentioned before. Each three parts were kept in sterilized Petri-dishes (9 mm diam.) containing a layer of sterilized cotton saturated with sterilized water. All treatments were incubated at 25°C ± 2. Lesion area was recorded after 10 days from incubation then, all treatments were sprayed with the recommended dose of the desired fungicides and incubated again at the same temperature. Control

treatments were sprayed with water only. Decrease in disease incidence (Pv) were calculated 7 days after fungicides treatment as follows : Pv = (Ic – Iv / Ic) 100 where: Pv = disease reduction %, Ic = lesion area (mm^2) on untreated rachis parts. (water treatment) and Iv = lesion area (mm^2) on treated rachis parts.

RESULTS AND DISCUSSION

Pathogenicity study:

Since the fungus B. theobromae is known to occur on a variety of host plants, it was important to uncover the pathogenic potential of fungal isolates obtained from different hosts to palm off-shoots.

Data in table (2) show that all mango isolates were non pathogenic to date palm off-shoot while isolates from other hosts were pathogenic.

Table 2. Virulence of *Botryodiplodia theobromae* isolates on date palm off-shoot cv. Zaghloul.

Code of *B. theobromae* Isolates	Average area of lesion in mm^2 , after (days)			Means
	3	7	10	
4	0.0	0.0	0.0	0.0
B2	0.0	0.0	0.0	0.0
900	0.0	0.0	0.0	0.0
777	0.0	0.0	0.0	0.0
334	23.2	44.0	53.0	40.0
410	10.0	24.0	35.2	23.0
T11	10.0	29.1	40.0	26.4
811	17.7	32.0	44.2	31.3
555	16.1	22.0	30.0	22.7
880	15.0	23.0	39.0	25.6
901	19.0	30.0	41.2	30.0
902	13.0	22.0	34.0	23.0
590	11.6	27.8	41.0	27.0
504	20.0	38.0	49.0	35.6
501	21.0	40.0	50.0	37.1

L.S.D at 0.05:

Days (D)	0.38
Isolates (I)	0.3
Lesions (L)	0.19
D x I	1.11
D x L	3.31
I x L	1.92
D x I x L	1.22

Grapevine isolate (isolate 334) was the most aggressive to date palm off-shoot (the infected area was 40 mm^2) followed by date palm isolates (isolate,501and 504) which recorded 37.1 and 35.6 mm^2 respectively. The least aggressive were apricot (555)and apple(410) isolates as the respective means of infected area were 22.7 and 23.00 mm^2 with no signficant difference. The remaining isolates were moderately aggressive. Based on the above-mentioned results, it could be concluded that no specific date palm isolate of *B. theobromae* is present in Egypt. The presented data indicate that most of *B. theobromae* isolates had a wide host range and able to cause several diseases to a large number of fruit trees. Such results are in agreement with those reported by Barakat *et al.,* (1990) who revealed that *B. theobromae* isolated from peach died-back twigs could infect apple, apricot, Japanese persimmon and mango. Similarly, Ammar (1998) found that one isolate of *B. theobromae* produced die-back symptoms on grapevine and was able to produce die-back and canker symptoms on shoots of apple and peach, but unable to infect mango shoots. Such a non – host specific presence of the pathogen provides a variety of sources for inoculum in nature.

Histopathological studies:

Histopathological investigation using light microscope of sections carried out on healthy and artificially infected rachis tissues of date palm Zaghloul cv. with *B. theobromae* and grapevine isolate showed the typical monocot anatomy as shown in Fig, 1.A. In this section vascular bundles surrounded by paranchymatous cells which are delimited by fibers. Sections through tissues infected with *B. theobromae*, 7 days after inoculation, revealed that fungal hyphae were clearly noticed in parenchyma surrounding vascular bundles and spread intra and intercellularly towards phloem, xylem parenchyma and a few necrotic area were noticed (Fig.1. C and D).

Fourteen days after artificial inoculation, fungal hyphae spread towards meta-xylem and necrotic area were seen in the proto-xylem (Fig.2. E, F and G) and most of the phloem cells were destroyed. Transverse and longitudinal sections, 21 days after inoculation, revealed that the fungus had rapidly progressed and therefore different reactions were manifested by various tissues of the infected leaves, disorganization and destroyed cells with necrotic area (Fig.2. H and I). Also, vascular bundles were undistinguished and some parenchyma cells were filled with brown material. These results are in harmony with those recorded by Aly *et al.* (2004), Atia *et al.* (2003) and El.Morsi (2004) who reported cell plasmolysis, disorganization and dark brown

discoloration of paranchymatous cells surrounding vascular bundles caused by hyphae of *B. theobromae*, the causal pathogen of deterioration of date palm off-shoot.

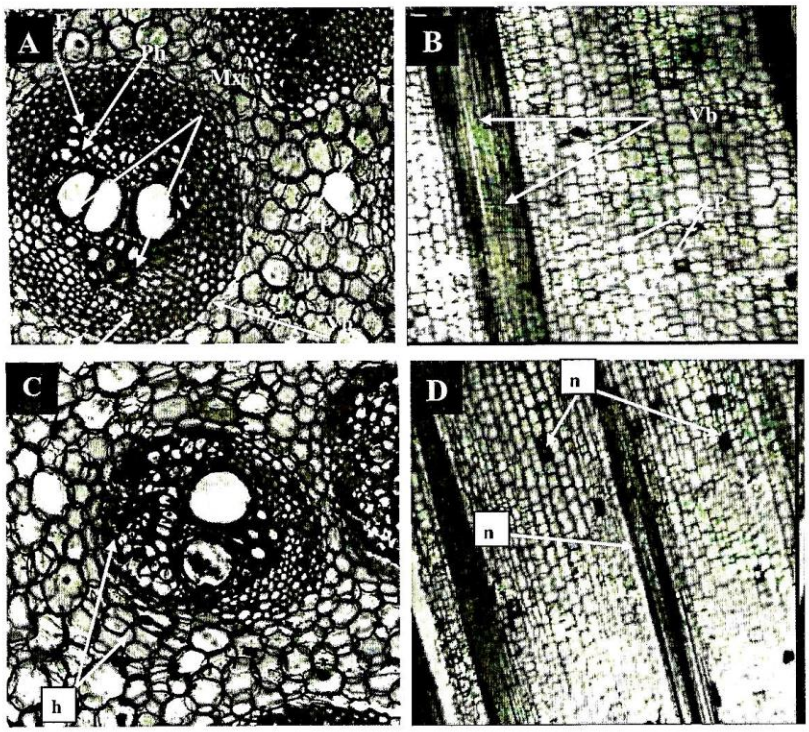
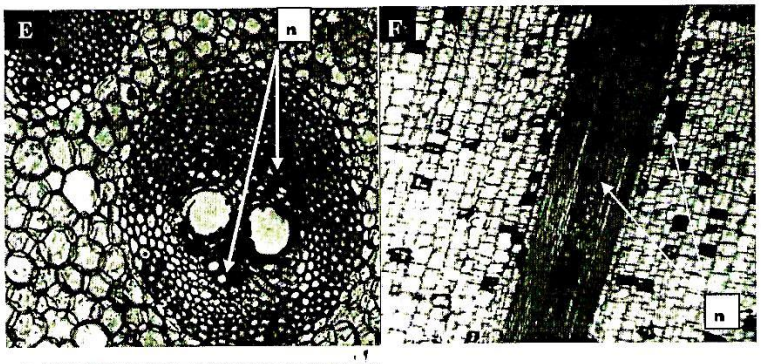


Fig. 1. Light microscope photographs showing cross and longitudinal sections in the leaf base area inoculated with *Botryodiplodia theobromae* and non inoculated date-palm cv. Zaghloul (X. 250). A&B: cross and longitudinal sections in non inoculated parts showing normal Vb = Vascular bundle, P = Parenchyma cells, Xp = Xylem parenchyma, Mx = meta-xylem, Px = Proto xylem, Ph = Phloem, F = Fibers and Bs = bundle sheath C&D:sections in the leaf base ,7days after inoculation,C:cross section showing fngus hyphae (h) spread inter and intra-cellular with necrosis(n) of Px as well as phloem and Meta xylem. D: longitudinal section showing necrotic area(n) in Vb and xylem parenchyma (indicated arrows).



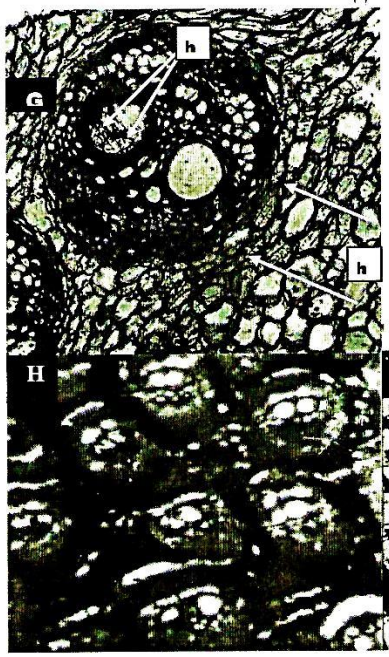


Fig. 2. Lightmicroscope photographs (X. 25) of date-palm off-shoot cv. Zaghloul, showing sections in the leaf base 14 days after inoculation with *Botryodiplodia theobromae*. E& F: showing increase of necrosis (n) in Px in E, as well as parenchyma cells in F, G: cross sections 14 days after inoculation showing hyphae (h) extending through parenchyma cells to the Meta xylem and the colonization by the fungus hyphae (indicated by arrows).

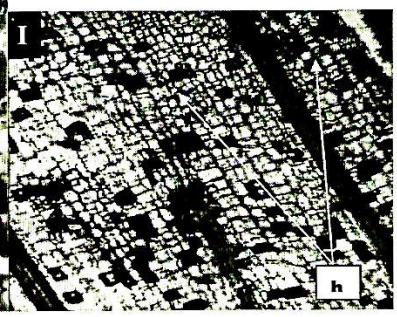


Fig. 3. . Light microscope photographs (X. 25) of date palm off-shoot cv. Zaghloul, showing sections in the leaf base 21 days after inoculation with *Botryodiplodia theobromae*. H: Transverse section. I: longitudinal section showing dark brown color of the Paranchymatous cells surrounding Vascular bundles and parenchyma cells was completely colonized by the fungus hyphae

***In vitro* evaluation of four different fungicides against the linear growth of *Botryodiplodia theobromae* isolates:**

Seven concentrations of four fungicides were used to evaluate their efficiency against *B. theobromae* isolates. Data in Table (3) reveal that the most inhibitory effect against linear growth of *B. theobromae* isolates was obtained by Hista 70% wp. which recorded complete inhibition at 5 ppm followed by Actazime 50% wp. Linear growth was also completely inhibited by Dorado 200 EC at 30 and 40 ppm as well as Cornazol at 50 ppm. These results are in agreement with those obtained by Ahmed *et al.* (1995) who reported that the fungal growth of *B. theobromae* was completely suppressed by Carbendazim (0.1 %) and Thiophanat-methyl (0.1%). The variations obtained in the effect of different fungicides on the fungus growth could be attributed to one or more of the following factors: (1) Degree of permeability of cell wall and/or plasma-lemma of the fungus for the uptake and passage of fungicides into fungal cell (Giffin, 1981).The majority of antifungal components act within the cell by the inhibition of vital processes including biosynthesis and activity of enzymes. (2) Degree of the antagonistic action of the fungal cell to a specific of fungicides. (3) The effect of environmental conditions as described by (Carregi *et al.,* 1990). (4) Chemical composition of fungicides.

Table 3. The effect means of different concentrations of five fungicides on mycelial growth of *B. theobromae* isolates under laboratory conditions and the lowest concentration completely inhibited the mycelium growth.

Codes of *B. theobromae* isolates	Means of mycelium growth (mm) on poisoned PDA				
	Fungicides				Mean
	Cornazol 10% EC	Hista 70%	Actazime 50% wp	Dorado 200 EC	
4	5.9 (50ppm)	1.3 (5 ppm)	1.7 (10 ppm)	3.5 (40ppm)	3.1
B2	6.2 (50 ppm)	1.3 (5 ppm)	1.3 (10 ppm)	3.1 (30 ppm)	3.0
900	4.2 (40 ppm)	1.6 (10 ppm)	1.3 (5 ppm)	3.7 (40 ppm)	2.7
777	4.0 (30 ppm)	1.3 (5 ppm)	1.5 (10 ppm)	3.4 (30 ppm)	2.5
334	5.0 (50 ppm)	1.3 (5 ppm)	1.5 (10 ppm)	3.4 (40 ppm)	2.8
410	5.6 (40 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	3.2 (30 ppm)	2.8
T11	4.0 (30 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	3.0 (20 ppm)	2.4
811	4.7 (50 ppm)	1.6 (10 ppm)	1.3 (5 ppm)	2.7 (20 ppm)	2.6
555	6.5 (50 ppm)	1.3) (5 ppm	1.6 (10 ppm)	2.5 (20 ppm)	3.0
880	4.4 (50 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	2.8 (20 ppm)	2.4
901	7.1 (50 ppm)	1.3 (5 ppm)	1.5 (10 ppm)	2.3 (30 ppm)	3.3
902	6.0 (50 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	3.3 (30 ppm)	2.9
590	4.5 (40 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	3.4 (30 ppm)	2.6
504	5.0 (50 ppm)	1.3 (10 ppm)	1.3 (5 ppm)	3.0 (20 ppm)	2.6
501	6.3 (50 ppm)	1.3 (5 ppm)	1.3 (5 ppm)	2.9 (20 ppm)	2.9
Mean	5.3	1.3	1.4	3.16	

L.S.D. at (0.05) for:
Fungicides (F) = 0.01405
Concentrations (C) = 0.01984
Isolates (I) = 0.02925
F x C = 0.0423
F x I = 0.0506
C x I = 0.07166
( ) = The concentration in ppm. Recorded complete inhibition

Evaluation of four different fungicides in controlling *B. theobromae* (*in vivo*).

The fungicides tested on the linear growth of tested isolates were used to study their effect on disease reduction at a recommended dose. It is evident from data presented in Table (4) that Actazime 50% was the best tested fungicide in controlling the disease (%80disease reduction) followed by Hista 70% and Cornazol 10% EC. (77.77 and 62-22% respectively) while Dorado was the least effective (37.77% reduction).

Table 4. Effect of different fungicides on disease reduction.

Fungicides	Recommended dose.	Disease incidence as infected area (mm^2)	% disease reduction
Hista70%w.p	2g/l	20	77.77
Actazime50%w.p	2g/l	18	80.00
Cornazof10%ec	0.5ml/l	34	62.22
Dorado20%EC	0.1ml/l	56	37.77
Water	100%	90	-

L.S.D values at 5%

Infected area (I) = 4.0

Disease reduction (D) = 4.14

I X D = 4.18

These results are in harmony with those recorded by Ahmed *et al.,* (1995) and (Shelar *et al.* (1977). Carbendazime and Thiophanat-methyl gave the best control on infection. It is also clear that the differences between the two tested fungicides Hista 70% and Actazime 80% were not significant, while it was significant with the other fungicides tested.

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التشريح المرضى ومكافحة عفن البتروديبلوديا لفسائل نخيل البلح صنف زغلول (*Phoenix dactylifera* L.)

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٢. *قسم الانتاج النباتي – معهد الكفاية الانتاجية – جامعة الزقازيق*
٣. *المعمل المركزي لابحاث و تطوير نخيل البلح – مركز البحوث الزراعية – الجيزة*

تمت دراسة ١٥ عزلة ممرضه من الفطر بتروديبلوديا ثيبرومي متحصل عليها من ٩ عوائل مختلفة هي (المانجو- العنب- التفاح- المشمش- الكمثرى- البرقوق- الخوخ – الكاكي- النخيل) من مناطق مختلقة في مصر علي خلفات نخيل البلح (صنف زغلول) وكانت العزلات المتحصل عليها من المانجو غير ممرضة على نخيل البلح صنف زغلول في حين كانت باقي العزلات ممرضة وكانت العزلة المتحصل عليها من العنب أكثرها شراسة. بعد ٧ ايام من احداث العدوي لخلفات الصنف زغلول بالفطر بتروديبلوديا ثيبرومي (المعزول من العنب) تلاحظ انتشار الميسيليوم بوضوح داخل الخلايا البرنشيمية متجها لبرنشيما اللحاء و الخشب محدثا موت لهذه الخلايا. و من ناحية اخري فقد استعمرالفطر خلايا نسيج الخشب تماما بعد ١٤ يوم من احداث العدوي. ثبط النمو الخطي للفطر تماما عند التركيز ١٠ جزء بالمليون للمبيدان ثيوفانات الميثايل في صورة المستحضر التجاري هيستا ٧٠% القابل للبلل و المبيد كاربندازيم في صورة المستحضر التجاري اكتازيم ٥٠% القابل للبلل و تبعهما المبيد بيريفينوكس في صورة المستحضر التجاري دورادو ٢٠٠ و الذي ثبط نمو الفطر تماما عند التركيز ٤٠ جزء بالمليون ثم المبيد بروبيكونازول في صورة المستحضر التجاري كورنازول ١٠% عند التركيز ٥٠ جزء للمليون بينما كان ترتيب المبيدات في اختزال المرض هو اكتازيم ٥٠%، هيستا ٧٠%، كورنازول .E.C 10 ثم دورادو .E.C 200 علي التوالي.