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HISTOPATHOLOGICAL AND CONTROL OF GRAPEVINE DEAD-ARM DISEASE

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ABSTRACT

Light microscope used in histopathological investigation of *Phomopsis viticola* Sacc. on artificially inoculated grapevine shoots cv. Thompson seedless (highly susceptible) revealed the investigated sections immediately after inoculation, exhibited similar structure in both inoculated and non-inoculated shoots. Seven days after inoculation, *Phomopsis viticola* induced disorganized epidermal and cortical cells with a dark brown color. Fungal hyphae were clearly noticed 14 days after inoculation in both xylem parenchyma and xylem vessels causing necrosis in this tissues. Not only the pathogenic fungus caused necrosis in xylem parenchyma and xylem vessels but also colonized the tissues with their hyphae and dark inclusion bodies as well as abundant production of tylosis were clearly noticed. Twenty one days after inoculation, all tested shoot tissues were colonized with fungus hyphae causing completely break down.

INTRODUCTION

Dead-arm disease of grapevine (*Vitis vinifera* L.) caused by *Phomopsis viticola* Sacc. can lead to losses up to 50% of the normal yield in many of the vine growing area of the world (Person and Goheen, 1994 and Atia and Saber, 1995). Diseases symptoms can be seen as angular, small tiny black spots on the leaves, stem, canes and flower cluster. Most of the spot have yellowish margins and dark centers. Spots grow and kill the buds. In some cases the fungus grow back into the woody part where it gradually attacks the water conducting system of the plant and kill the arm, thus giving the disease this name. The dead arm disease of grapevine occurs on American varieties in eastern section of North America, South Africa, Japan, Italy and Holand (Pine, 1958). In Egypt, the disease was first reported by (Atia and Saber, 1995) isolated *Phomopsis viticola* from grapevine entire dried and this was a companied by die-back of shoots. Aly, *et al.*, 2002 isolated *Phomopsis viticola* from grapevine and proved their pathogenic capabilities shoot died-back and/or cankered in different governorate in Egypt. The importance of dead-arm disease has increased in Egypt during the last few years and become a serious problem to grapevine growers. Because of this increased activity of the pathogen, it become important to study histological changes in the structure of *grapevine* shoot tissues after artificial inoculation.

MATERIALS AND METHODS

1. Source of *Phomopsis viticola* isolates:

Five virulent isolates of *Phomopsis viticola* were obtained kindly from Fruit and Woody trees Diseases Research Department, Plant Diseases Research Institute, Agricultural Research Center, Giza, Egypt. Their

pathogenic capability were proved in previously research work by Aly *et al.*,(2002) the details of *Phomopsis viticola* isolates were tabulated below:

Code No.	Governorate	Symptoms
N-13	North Siena	Phomopsis canes and leave spots
I-14	Ismaelia	Phomopsis canes and leave spots
I-15	Ismaelia	Die-back and canker
Sh-16	Sharkia	Gummosis and die-back
Sh-17	Sharkia	Die-back

2. Virulance evaluation of *Phomopsis viticola* isolates

2.1 Plant material:

Green shoot of one year-old rooted vines produced from cutting of cv. Thoposon seedless, the highly susceptible cv. (Saber, 1998 and Aly *et al.*, 2002) were planted in pots (40 cm/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.

2.2 Pathogenicity test:

Pathogenicity test was carried out in the basal part of shoots treated by macking disks using cork borer 3 mm.in diameter and then removed with sharp cuter. Disks 3mm. Taken from two weeks old culture were transfered to the wounds and sealed with parafilm for 48 h.before being removed. PDA free from fungus as a control. Five replicates were used for each treatment. The average of lesion length and width were estimated after 7, 14 and 21 days. At the end of vegetative growth, the shoots were prunded to 3 buds/plant in january. Symptoms were observed during the following vegetative growth cycle (March and April) by calculating the number of mortal buds .

2.3-Histopathological studies:

Tissues blocks from either healthy shoot or artificially inoculated shoot by Ismaelia isolate (I-14), the most pathogenic isolate, were fixed in formalin-alcohol acetic acid solution, then dehydrated with increasing concentration of ethanol and imbedded in wax according to Sass (1961). Section were cut into 15 and 20 µm thick using a rotary microtome and fixed on a series of glass slides, then stained using the methods described by Yilum *et al.*, (1993) and mounted in Canada balsam. The stained sections were examined by a light microscope and photomicrographed.

3- Evaluation of some Different Fungicides Against *Phomopsis viticola*:

Eleven different fungicides i.e. Fostyle- Aluminium as Aliette 80 %, Cyproconazole+ Sulphate as Atemi (100) 10% SI., Copper hydroxide as Champion 77% W.P., Mancozeb as Diathane M 45, Triadminal as Bayfidan 25% EC., Thiophanate methyle as Topsin M 70, Diniconazole as Sumi-eight, Fenarimol as Rubigan 12% EC., Iprodione as Roural 50% W.P., Flusalazol as Bunch and Penconazol as Topas 100 EC. were tested either *in vitro* and *in vivo* against *Phomopsis viticola.*

4. in vitro effects:

In vitro tests were conducted using isolate No. 14. Stock solution of the commercial fungicides products were made by suspending them directly in 1000 ml distilled water, while the technical grade chemicals (Fenarimol and Thiophanate methyl) were first dissolved in 10 mL acetone before adding them to 990 mL. water as recommended by Marlene V. Jaspers (2001)

4.1. Conidial germination Percentag:

For each fungicide, different concentrations (0, 5 , 10, 25, 50, 100and 200 ppm were prepared by adding stock fungicides solutions in mL according to required conc. prepared as to melton (50 °C) PDA Ten mL.of it were poured into three Petri dishes for each treatment and inoculated within 2-4 h. after pouring. For inoculation, conidia were harvested by adding 10 ml sterile distilled water containing 0.1% Tween 20, then a camel hair brush was used to release the spores within water. The conidial concentration was adjusted to $1x10^6$ conidia per ml. One ml of conidial concentration was spread onto plats of fungicide amended agar. Three replicates for each plates concentration were used. Inoculation plate were inocubated at 20°C for 48 and 72 h. before determining the frequency of spore germination. For each plate, three 1-1 cm. agar squares were cut out, mounted on slides and examined with a compound microscope at high magnification. One hundred conidia were assessed for germination in each agar square and mean percent of germination relative to control was calculated for each fungicide.

4.2 Effect of fungicides on linear growth

Different concentrations (0, 1, 5, 10, 25,50, 100, 200,300, 400,500 and 400 ppm) of each tested fungicide were prepared according to their active ingredient and mixed with autoclaved PDA medium before solidification to study their effect on the linear growth of *Phomopsis viticola* (isolate No. 14). Three replicates of each concentration were inoculated with an equal disc (7 mm. in diam.) for 7 days old culture. The inoculated dishes were incubated at 25°C until fungal growth completely covered the surface of the check treatment fungicides free medium. The two perpendicular diameter of the linear growth were measured and their mean was calculated.

4.2 Field Experiments:

A commercial vine yard (*Vitis vinifera* L. cv. Thompson seedless with super pruned top wire cardon trained vines located in exhibited typical symptoms and confirmed the causal agent as *Phomopsis viticola* were used as tested plant in two experimental seasons in Badr Center, Behiera Governorate. Twelve fungicides were sprayed separately with the recommended dose for each. Five replicates for each treatment were arranged in a randomized complete block, each subplot consisted of single row of 40 vine. Each treatment, was sprayed twicethe first one followed pruning directly and the other at bud burst. The number of buds/vine were calculated after pruning and before fungicide application. Decrease in disease incidence were calculated 30 days for mortality in buds % as well as mean of necrotic area on new shoots relative to control.

RESULTS AND DISCUSION

Disease symptoms were recorded as dark brown to black necrosis that extended from inoculation point up and down along the inoculation part. Shoot tips of the control plants, inoculated with un-colonized agar plugs remained vigorously green colored and erect. The inoculated shoots were parler, less vigorous and less rigid. These results agree with those obtained by Latham *et al.,* (1992), Kuo *et al.,* (1999) and Atia *et al.,*(2003). Data I Tables (1 and 2) show that, all *Phomopsis viticola* isolates were pathogenic on grape shoots cv. Thompson seedless. Ismaelia isolate (I-14) were more aggressive isolate recorded both the highest infected parts and percentage of mortality in buds followed by North Siena isolate (N-13) while, Sharkia isolate (Sh-17) recorded the lowest effect either in buds mortality or infected part. These result agree with those obtained by Latham *et al.,*(1993), Kuo *et al.,* (1999) and Atia *et al.,*(2003).

Table 1: Virulence of five *Phomopsis viticola* isolates in the current growth season

Code No. of tested isolates	Average length and width of necrosis part (mm) after days			Mean
	7	14	21	
N-13	11	19	31	20.33
I-14	14	23	40	25.70
I-15	6	14	25	15.00
Sh-16	7	13	22	14.00
Sh-17	7	11	19	12.33
Control	0	0	0	

Table 2: Virulence of five *Phomopsis viticola* isolates as % mortality in buds in the next growth season

Code No. of tested isolates	N-13	I-14	I-15	Sh-16	Sh-17	Control
% mortality in buds	60	86.7	60	46.7	40	100

Light microscope observations of Paraffin sections of inoculated shoots showed that, immediately after inoculation, the investigated sections exhibited similar structure in both inoculated and non-inoculated shoots. This structure consists of intact epidermal, cortical, xylem and phloem cells (Fig.1. A). Seven days after inoculation, *Phomopsis viticola* induced disorganized epidermal and cortical cells with a dark brow in color were seen. The cells showed plasmolysis and most of phloem and cambial cells were destroyed. It is also seen that, xylem rays (XYR) were a suitable path way for rapid and easy spreading of the hyphae to the other tissues (Fig.1. B). Transverse sections revealed 14 days with *Phomopsis viticola* that, the disease had rapidly progressed and therefore different reactions were manifested by the various tissues of the infected shoots. Necrotic area in both xylem parenchyma and xylem vessels, colonized by hyphae. The dark inclusion

bodies inside the xylem as well as abundant production of tylosis were observed, the pathogen spread to various tissues causing completely breakdown (Fig.2.A and B). No hyphae and tylosis were noticed in the non-inoculated tissues as previously mentioned in Fig.1.A. Twenty one days with *Phomopsis viticola*, microscopically examined infected shoot sections appeared, all tissues breakdown and non-distinguished (Fig.2.C). These results agreement with those obtained by Sepulveda, *et al.*,(1979); Giorbdize (1984) and Atia *et al.*,(2003). Thus the appearance of dead-arm symptoms might be due to presence of necrotic vessels, fungal hyphae, induction of inclsion bodies, plugging with gum and/or tylosis which cause disfunction of xylem with destruction in flow water. Similar results were obtained by Atia *et al.*,(2003).

Evaluation of twelve different fungicides against *Phompsis viticola In vitro*

The effect of different concentration of twelve fungicides were tested against the linear growth as well as spore germination. Data in table (3&4) show that, the tested fungicides differed in their inhibitive effect against both mycelial growth and spore germination. It is clear that, the mycelial growth and spore germination inhibition generally increased with increasing the fungicides concentration except the fungicide Aliett was ineffective at reducing mycelial growth and spore germination, Data in table (3) reveal that, the most inhibitory effect against linear growth of *Phompsis viticola* was obtained by Punch which recorded complete inhibition at 1 ppm followed by Topsin and Rubigan 12 % recorded complete inhibitionat 5ppm followed by Topas 100 Ec. Which recorded complete inhibition at 50 ppm .While linear growth was also completely inhibited by Sumi – eight and Bayfidan at 100 ppm. It is evident from data presented in table (4) that, the most inhibitory effect against spore germination obtained by Punch 40% Ec ,Rubigan 12% ,Topas 100 Ec and Topsin M 70 % which recorded complete inhibition of spore germination at 5 ppm followed by Sumi–eight and Bayfidan recorded complete inhibition at 40 and 50 ppm respectively while Champion 77%, Diathane M 45 and Ridomil plus 50% recorded complete inhibition at 200 ppm

The result of this project provide mangers with more retinal basis for treatment decisions. This result agreement with Kuropatwa(1994) who reported that, Flusilazole as Punch 40% and Fenarrmol as Rubigan 12 Ec gave the greatest inhibition of *Phompsis viticola* at the lowest concentration tested also in harmony with Lizel Mostert *et al* (2001) whose reported that, Flusilazole, Penconazol and Trifloxystrobin gave better inhibition at lower concentration than Folpet and Fosetyle AL + Mancozeb.

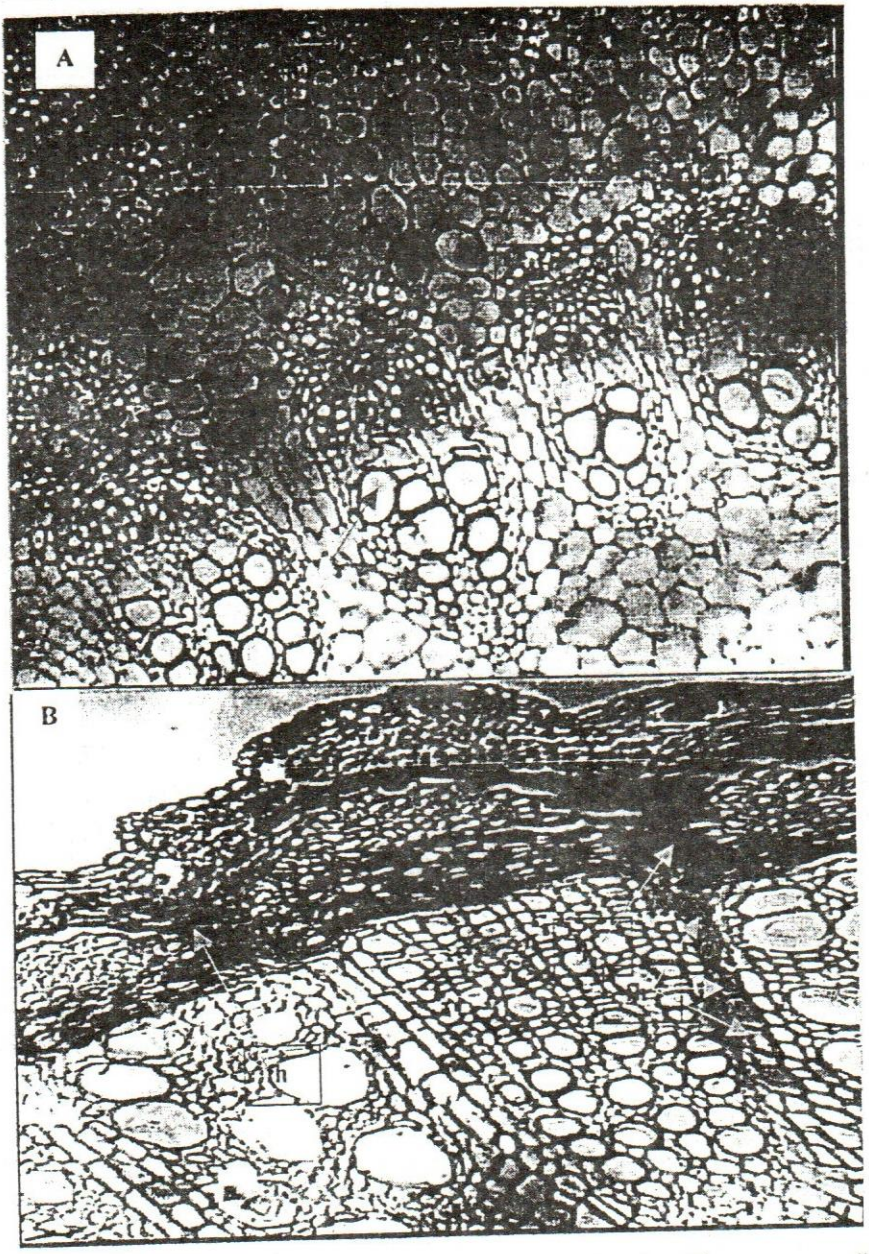

Fig. 1: Light micrographs showing cross sections in the Thompson seedless grapevine cv. internodes area of non inoculated (A) and inoculated (B) shoots with *Phomopsis viticola* Sacc. A, showing, normal epidermis (Ep), Cortex (Co), Phloem (Ph), Cambium (Ca), xylem (Xy), xylem rays (XR) and Pith (P) X200. B, Cross section 7 days after inoculation showing, disorganized epidermal and cortical layers, fungus hyphae (fh)extending through xylem rays indicated by arrows. X400.

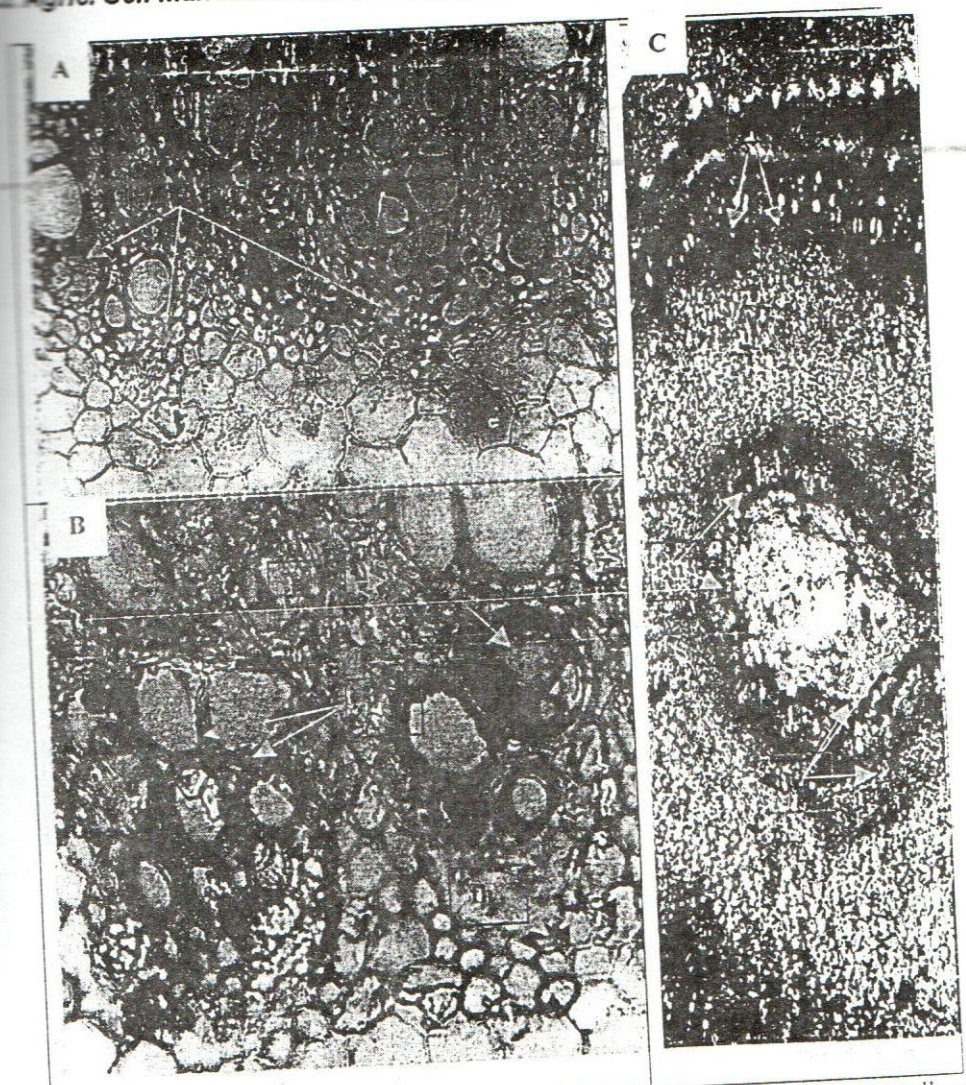

Fig. 2: Light micrographs showing cross sections in the Thompson seedless grapevine cv. Shoots, 14 days after inoculation. A, showing fungal mycelium (fh) present in xylem parenchyma and xylem vessels also in intercellular spaces as indicated by arrows. X 250 B, showing xylem vessels blocked with big tylosis(t). also there are partial necrosis (n) in xylem parenchyma and xylem vessels as indicated by arrows. X 250 C: Microscopic photographs of transverse sections 21 days after artificially inoculation with *Phomopsis viticola* Sacc. showing, mycelium mass (fh) completely colonized pith. Notice, pith cells were destroyed, vascular bundles completely necrosis(n). Also, phloem was destroyed and separated from rest tissue (indicated by arrows. X100

Table 3: Effects of different concentrations of tested fungicides on the linear growth of *Phomopsis viticola*.

Tested Fungicides	Linear growth (mm) at different concentrations (ppm)											
	0	1	5	10	25	50	100	200	300	400	500	600
Aliette 80%	90	90	90	90	90	90	90	74	70	61	57	51
Atemi 10% SL	90	90	90	90	90	70	21	0.0	0.0	0.0	0.0	0.0
Bayfidan	90	90	90	65	45	16	0.0	0.0	0.0	0.0	0.0	0.0
Champion 77% WP	90	90	90	90	90	90	83	71	63	35	30	0.0
Diathane-M 45	90	90	90	90	90	90	81	67	50	0.0	0.0	0.0
Topsin-M 70%	90	70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sumi- eight	90	80	74	61	40	13	0.0	0.0	0.0	0.0	0.0	0.0
Rovral 50%	90	90	90	90	90	90	90	60	57	39	21	16
Ridomil Plus 50%Wp	90	90	90	90	90	90	79	62	47	0.0	0.0	0.0
Topas 100 EC	90	90	90	79	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rubigan 12% EC	90	80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Punch 40% EC	90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	90	90	90	90	90	90	90	90	90	90	0.0	0.0

L. S. D. value at 5% : Fungicides (F) : 0.5208 Concentration (C) : 0.1796 F x C : 1.727

Table 4: Effects of different concentrations of tested fungicides on spore germination of *Phomopsis viticola*.

Tested Fungicides	% spore germination at different concentrations (ppm) after 42 h. from inoculation						
	0	5	10	25	50	100	200
Aliette 80%	37	37	37	37	37	37	37
Atemi 10% SL	40	35	33	20	13	0.0	0.0
Bayfidan	42	42	29	14	0.0	0.0	0.0
Champion 77% WP	38	38	38	38	33	28	0.0
Diathane-M 45	40	40	40	40	40	20	0.0
Topsin-M 70%	44	0.0	0.0	0.0	0.0	0.0	0.0
Sumi- eight	37	37	37	0.0	0.0	0.0	0.0
Rovral 50%	38	38	38	38	38	38	38
Ridomil Plus 50%Wp	45	45	45	45	39	20	0.0
Topas 100 EC	46	0.0	0.0	0.0	0.0	0.0	0.0
Rubigan 12% EC	40	0.0	0.0	0.0	0.0	0.0	0.0
Punch 40% EC	40	0.0	0.0	0.0	0.0	0.0	0.0

L. S. D. value at 5% : Fungicides (F) : 1.186 Concentration (C) : 1.136 F x C : 3.935

Effect of different fungicids on disease reduction (field obsorvation)

The ability of different fungicides treatment to control disease differed significantly when compared with control (Tab. 5). Punch 40% EC, Rubigan 12% EC and Topas 100 EC caused the greatest reduction in disease severity recorded 75.67, 72.97% and 70.27% as reduction in mortality of buds followed by Sumi-eight and Topsin-M 70% recorded 62.16 and 54.45% respectively. Also, no visible symptoms appeared on a new growth shoot when treated with the previous fungicide. It is also clear that, the differences between Punch 40% EC and Rubigan 12% EC were not significant, also no significant between Sumi-eight and Topsin-M 70%, while Punch 40% EC was significantly more than the other fungicides. These results are in agreement with those obtained by Pscheidt (1989) and Kuropatwa (1994). In the light of

the obtained findings, it may be suggested that, one or two sprays at 15 days intervals are required to eliminate the entire infection. Kuropatwa (1999) reported that, Flusizole as Punch40% EC and Fenarimol as Rubigan 12 EC gave the greatest reduction. Future research shoud center on inoculum production and disease forecasting to fine time spray recommendation.

Table 5: Effect of different fungicides on disease reduction (field observation)

Tested Fungicides	Average number of mortality in buds	Reduction of mortality in buds	Average canker length (*) on new growth shoots		Means
			Minimum	Maximum	
Rovral 50%	27	27.02	12	30	24
Atemi 10% SL	16	56.75	9	27	20
Aliette 80%	30	18.91	14	34	32
Sumi- eight	14	62.16	#	#	#
Bayfidan	22	40.54	11	23	22
Diathane-M 45	18	51.35	8	28	27
Champion 77% WP	29	21.62	14	38	35
Punch 40% EC	9	75.67	#	#	#
Topas 100 EC	11	70.27	#	#	#
Ridomil Plus 50%Wp	25	32.43	13	37	30
Rubigan 12% EC	10	72.97	#	#	#
Topsin-M 70	15	59.45	7	29	25
Control (water treatment)	37	00.00	19	44	33

(*) linear extension of canker after two month (mm)
(#) no visible symptoms on growth shoots
L. S. D. values at 5%
Fungicides (F) = 5.64 **F x R = 6.73**
Reduction of mortality in buds (R) = 4.33 **F x C = 5.93**
Canker length (C) = 4.14 **F x R x C = 4.34**

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التشريح المرضى و المكافحة لمرض الزراع الميت فى العنب

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أظهر الفحص الميكرسكوبى لقطاعات أفرع عنب صنف تومسين سيدليس سبق عدواها صناعيا بفطر فومبسيس فيتيكولا حدوث تدمير وعدم انتظام الخلايا فى طبقة البشرة و القشرة مع ظهور لون بنى داكن فى هذة الانسجة وذلك بعد سبعة أيام. اما بعد ١٤ يوما من العدوي لوحظ ميسليوم الفطر بوضوح فى بارانشيمة و اوعية الخشب مع وجود مساحات متكرنزة. ولم تكن هذة الأعراض فقط التى ظهرت ولكن ظهر بوضوح استعمار الفطر للأنسجة داخل و بين الخلايا بجانب تكوين تيلوزات فى أوعية الخشب. و قد لوحظ استعمار الفطر لجميع أنسجة الفرع مع حدوث تدمير شامل لها وذلك بعد ٢١ يوما من العدوى. ثبط النمو الخطى و انبات جراثيم الفطر عند أقل تركيز من مبيد فلوسيلازول فى صورة المستحضر التجارى بانش ٤٠ % فى حين اعطى المبيد فوسيتيل ألومنيوم فى صورة المستحضر التجارى اليت حتى عند استخدامه بتركيز ٦٠٠ جزء فى المليون. وفى اختبارات الحقل أعطت المبيدات التالية بانش، روبيجان و توباز أعلى نسبة اختزال للمرض وذلك فى صورة اختزال نسبة الموت فى البراعم وكذلك اختزال نسبة التقرحات على الأفرع وكانت الاختلافات بين هذة المبيدات غير معنوية.