Further pathogenicity studies of Eutypa lata (::E. armeniacae) on almond

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Further pathogenicity studies of *Eutypa lata* (= E. armeniacae) on almond

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RESUME

Le champignon Eutypa lata **(Syn.** E. Armeniaca, **Stat. con.** Cytosporina **sp, syn.** Libertella blepharis) **est associé aux chancres des plaies de taille se produisant au niveau de l'insertion de la branche sur la charpentière. Des essais d'inoculation ont été faits sur des plaies sur le cultivar « Texas ». Ils ont induit des chancres plus importants que dans les conditions naturelles allant jusqu'à la mort des branches latérales. Vingt variétés différentes inoculées ont montré de faibles différences de sensibilité. La majorité des chancres cesse de se développer après 2 à 6 mois étant ceinturés par du tissu cicatriciel sain. D'autres essais doivent être faits en utilisant la technique d'inoculation sur plaies de taille avant de conclure définitivement.**

ABSTRACT

The fungus Eutypa lata (syn. E. armeniacae, **stat. con.** Cytosporina **sp., syn.** Libertella blepharis) **was found associated with pruning wound cankers, originating at the point of the junction of the branches and the trunk. Inoculation tests carried out on almond trees cv. «Texas» showed that those tests made on freshly exposed wood resulted in higher infection levels and larger cankers with eventually killing of lateral shoots, than those made on the cambial zone. Twenty almond cultivars inoculated in the cambial area with** E. lata **gave indications of small differences in their susceptibility to the fungus. The majority of the cankers ceased to enlarge after 2-6 months, and were surrounded by healthy callus tissue. The fungus weas not easily recovered from discolored tissues. Further pathogenicity test must be carried out using the pruning wood inoculation technique before definite decisions can be drawn.**

INTRODUCTION

The ascomycetous fungus *Eutypa lata* (Pers: Fr.) Tul. (syn. *E. armeniacae* Hansf & Carter; anamorph: *Cytosporina* sp. syn. *Libertella blepharis* A. L. Smith) has been initially recorded as the cause of «gummosis» or «diebak» disease of apricot (*Prunus armeniaca* L.) in many countries of the world (Carter 1957, Carter and Bolay 1972, Carter and Moller 1974, Carter et al. 1964, English et al. 1963, Matthee et al. 1973). The pathogen invades pruning wounds, and causes cankers which usually result in the death of the affected apricot branches.

Similar cankers and diebacks are found also on grapevine (*Vitis labrusca* L. and *V. vinifera* L.). The typical symptoms of «dying arm» disease of grapevine, reported to occur in several grape growing areas of the world (Bolay and Moller 1977, Carter 1975, Dye and Carter 1976, Kouyeas et al. 1976, Lehoczky and Moller 1979, Moller and Kasimatis 1978, Teliz and Valle 1979) include stunting of spring shoot growth, yellowing and cupping of young leaves, shedding of blossom clusters, vascular discolorations, cankers around old pruning wounds, and death of vine arms (Trese et al. 1980).

Beside apricot and grapevine, investigations in different countries showed that the host range of the fungus includes also lemon (*Citrus limon*) (Kouyeas 1978), apple (*Malus domestica*) (Glawe et al. 1983), Japanese plum (*Prunus salicina* Lindl.) (Carter 1982), pear (*Pryrus communis* L.) (Carter 1982), walnut (*Juglans regia*) (Rumbos 1984) and almond (*Prunus dulcis* [Miller] D. A. Webb) (Carter 1982, Rumbos 1983). More recent work has revealed a very wide host range of *E. lata*, encompassing some 80 species in 27 families (Carter et al. 1983, Bolay and Carter 1985).

The pathogen on almond was initially recorded as a saprophyte (Carter and Talbot 1974), found on a dead stump in Australia (Carter 1960). Pathogenicity studies on almond in Australia (Carter and Moller 1971) and North America (English and Davis 1978) showed that infection could occur, but the fungus did not produce the symptoms of cankering and dieback seen in apricot. In 1981, severely cankered branches were recorded on two almond trees (cvs. «Nonpareil» and «Strouts») in Australia (Carter 1982). During the last decade several almond plantations were found affected in Greece (Rumbos 1983). Cankering and dieback symptoms have been observed only on the cvs. «Texas» and «Retsiou». The cankers, always associated with pruning wounds, originated at the point of the junction of the branches and the trunk and extended downwards to the graft union or upwards to one or usually more branches (Fig. 1). The affected bark was externally dark, depressed and malformed (Fig. 2). Cracking and gum exudation were often observed. Preliminary pathogenicity studies on apricot and almond resulted in cankering or dieback of the shoots (Rumbos 1984).

This study gives more information on a) the susceptibility of different almond cultivars to infection; and b) the susceptibility of almond branch tissues to infection.

MATERIALS AND METHODS

Experiment Nr 1. On 30 october 1982, 2-year-old almond trees cv. «Texas» growing near the Institute, were used for inoculations. Inoculum consisted of discs of mycelium cut from PDA plates; inoculum in control treatments consisted of sterile PDA discs. Inoculations were made by placing inoculum directly onto the 1.0-2.0 m. diameter freshly wood, exposed after pruning off the apex of the main stem. Inoculation wounds were covered with aluminum foil to prevent rapid desiccation. Aluminum foil was removed after 1 month. Ten-day-old cultures of *E. lata* isolated from almond, pear, apricot and grapevine were used. Five trees were inoculated with each of the eight treatments. The severity of external canker symptoms was assessed on 29.2.1984 by measuring the length of the discoloration in the bark tissues. The results were again evaluated on 18.3.1984 by splitting inoculated stems longitudinally and measuring the total length of xylem discoloration. Isolations were made from all the affected tissues of the bark and wood, including discolored streaks of the xylem extended 70 to 100 cm. beyond the inoculation site.

Experiment Nr 2. On 2 December 1983, 4-year-old almond trees of 20 different cultivars growing in the field, were inoculated with ten-day-old cultures of the fungus isolated from almonds trees (AT5). Four trees of each cultivar were inoculated. Inoculation points were selected on branches 1.0-1.5 cm in diameter. A 7 mm diameter disc of bark was removed from each inoculation site using a stainless-steel hollow punch and a transverse V-shaped notch was cut through the cambium in a depth of 1.0-1.5 mm into the sapwood. On each inoculation point was then inserted a 7 mm diameter inoculum disc, with the mycelium towards the wood surface. In control treatments inoculum consisted of sterile PDA discs. Inoculation sites were coovered with aluminum foil for 1 month.

Assessments were made on 30 May 1984, 30 September 1984 and 13 April 1985 by measuring the total length of necrotic bark.

Experiment Nr. 3. On 4 april 1984, 5-year-old almond trees of 20 different cultivars growing in pots were inoculated with six-day-old cultures of *E. lata* isolated from almond trees (AT2). Four trees of each cultivar were used for inoculations. The method of inoculation was the same as in the experiment Nr. 2. Assessments were made on the 27 June 1984 by measuring the total length of necrotic bark.

RESULTS

Results of pathogenicity tests of three isolates of *E. lata* from Australia and four from Greece are presented in tables 1 and 2. All seven isolates were pathogenic in our experiments. Xylem discoloration extending downwards from wounds inoculated with isolate Almond-AG2 was significantly smaller than that produced by the isolates Grape-VG and Almond AA (table 1). Bark cankers produced by the isolate Almond-AA, were significantly greater than those resulting after inoculating wounds with the isolates Apricot-BG, Apricot-BA, Grape—VG and Almond-AT5 (table 2). From the 35 almond trees cv. Texas which were inoculated with the different fungus isolates, 60 % showed dead shoots 16 months later (Fig. 3) while the length of the bark cankers fluctuated between 2-19 cm. The extension of internal discoloration was, in 80% of the trees, as long as the length of the whole main stem of the tree including the rootstock.

The fungus was not isolated from all cankers. Isolations made from necrotic phloem and xylem near the advancing margin of the canker usually yielded the pathogen, while it could not be recovered from tissues which had been dead for some time. More difficult was the isolations of *E. lata* from discolored wood in a distance of 30 to 100 cm. from inoculation point. From the 120 isolations made from necrotic bark and xylem tissues only 14 yielded the pathogen.

In contrast to the inoculations made on freshly exposed pruning wood, which in many cases resulted in the death of shoots, cambial inoculations made on almond shoots of 20 different varieties resulted in low infection levels and little canker formation (Fig. 4). Almost all cankers have ceased to enlarge 2 to 6 months after inoculation and many have been surrounded by healthy callus tissue. In general, cambial inoculations made on almond trees growing in the field resulted in cankers of greater length, which in some cases caused the death of the inoculated shoot, than those made on almond trees growing under stress conditions in pots, which appeared inactive 2 months after inoculation.

The results of the pathogenicity tests made on 20 almond cultivars are presented in the tables 3, 4 and 5. Almost all cultivars growing in the field gave indications of small differences in their susceptibility to *E. lata* (table 3, 4). «Anonymous Italian» has proved to be the most tolerant cultivar while cvs «42-16-88» and «Phyllis» the most susceptible, when statistical analysis was based on the measurements of the external barkcanker length (table 4). When analysis was based on the measurements of the length of the internal wood discoloration, cv «Ai», was the most tolerant and cv. «Ferraduel» the most susceptible (table 3). From the test on the cultivars growing in pots the most tolerant ones resulted to be the first 13 presented in table 5, while the most susceptible were «42-16-68» and «Retsiou». From the 90 isolations made near the advancing margin of the canker only four yielded the fungus.

DISCUSSION

During the early 1980's our attention was drawn on a canker disease of almonds in a commercial 10-year-old plantation of cv. «Texas» showing a high incidence of cankers. The disease, which manifested itself by severe cankering of the scaffold branches and occasionally by craking and gum exudation, was attributed to the fungus *E. lata* (Rumbos 1983). Cankers, always associated with pruning wounds, were quite long, extending downwards to the graft union and upwards to some branches. Cross section through the canker often revealed only a narrow strip of live wood.

Nervertheless, affected branches continued to be productive. This fact, together with the infrequent occurence of the disease, minimize its economic importance. Complete girdling of the affected branch was not usually observed. Usually, as the infection grew older, the wood became drier, more brittle, and the branch broke up easily, especially after a strong wind.

Inoculation tests carried out on almond trees of the cv. «Texas» showed that those tests made on freshly exposed wood resulted in higher infection levels and larger cankers with eventually killing of lateral shoots, than those made on the cambial zone. Seventeen months after inoculation, would-pruning originating cankers continued to enlarge, and the fungus could be isolated from bark and xylem coextensive with the canker. In contrast, cankers produced after cambial inoculation ceased to enlarge after 2-6 months, and were surrounded by healthy callus tissue. The fungus was not easily recovered. Very difficult was also the isolation of the pathogen from xylem discolorations extended several centimeters from the canker margin.

Our results are in agreement with those of similar experiments carried out by other workers. English and Davis (1978) reported that apricot pruning wounds were highly susceptible to infection of *E. lata* and most pruning-wound cankers continued to enlarge 2 years after inoculation, in contrast to cambial inoculations which resulted in small cankers that were surrounded by healthy wound callus after the first year. The ability of the fungus to infect almond, causing extensive xylem invasion but not producing cankering and dieback symptoms, was shown by Carter and Moller (1971) by inoculating pruning wounds with ascospores. Further inoculations of almond wounded tissues with mycelia of the fungus resulted in canker development and dieback symptoms, although in this case symptoms were not so much pronounced as in apricot, and most cankers showed healthy callus development (English and Davis 1978).

Isolations from naturally developed almond cankers yielded the pathogen only occasionally, indicated that some of them were inactive. Similar isolations from

bark and xylem discolored wood from artificially induced cankers provided again evidence that most of the cankers, especially those originating from cambial inoculations, were inactive. The pathogen was also not easily isolated from discolored xylem extended above or below of inactive or active cankers. It appears probable that the fungus was dead in some cankers, because of the accumulation of toxic metabolites or of the action of a defense mechanism of the plant. It was also shown that the fungus was inactivated faster when it was inoculated on almonds growing in pots under stress conditions than on plants growing under field conditions. Furthermore, in many cases of faster grown trees, the fungus produced larger cankers, which were still active 1 year after inoculation, than on trees growing in pots. In the last case the cankers were soon surrounded by healthy callus tissue.

An apparent tendency of *E. lata* to die in apricot infections was shown by English and Davis (1978). They attributed this death of the fungus to unfavorable temperature or/and moisture conditions, or to the accumulation of toxic metabolites. However, it seems probable that the pathogen reacts in a similar way in infected apricot or almond tissues.

Although not all of the inocula used in our pathogenicity experiments were originated from single ascospore cultures, this study provides considerable evidence about the existence of different pathotypes in *E. lata*. Significant differences in virulence among isolates originating from ascospores were also observed (Carter et al. 1983, English et al. 1983). Carter et al. (1985) reported about the existence of virulent and hypovirulent isolates of the fungus, which were identified on the basis of the response obtained after inoculation of single ascospore isolates to four cultivars of apricot.

Pathogenicity test on 20 almond cultivars presented in this paper give only an indication of the existence of tolerant and susceptible cultivars. Inoculation experiments must be made again avoiding the cambial inoculation method and placing the inoculum on freshly exposed wood.

Figure 1. Appearance of the cankered area of an almond tree cv. Texas infected by fungus **Eutypa lata.**

Figure 2. Cankered almond branch cv. Texas showing depressed, darkened area, bark cracking and malformation.

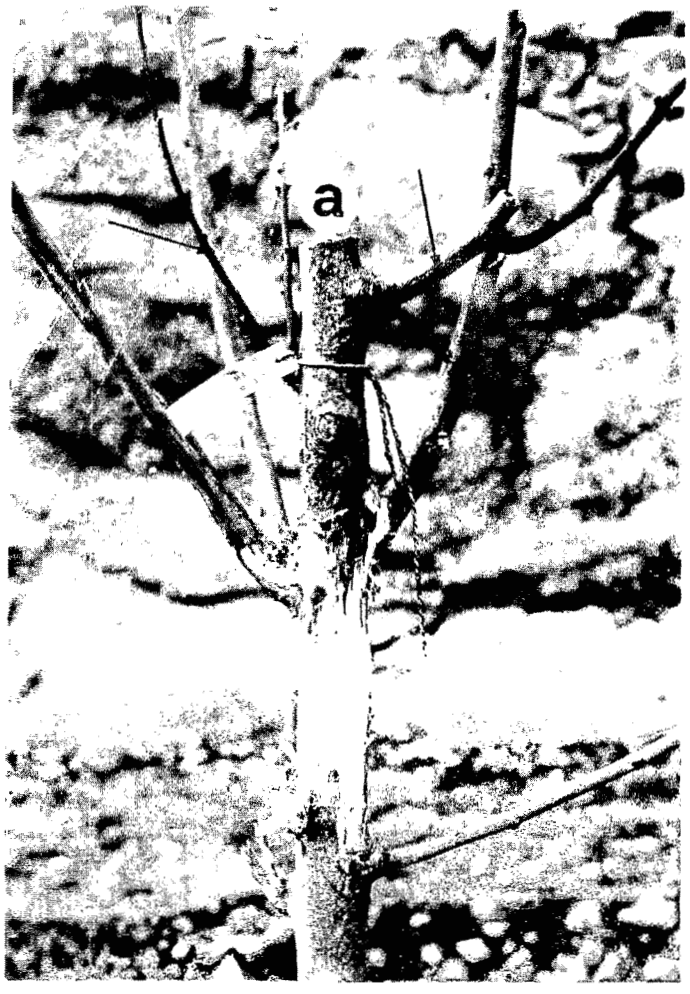

Figure 3. Pathogenicity test of **E. lata** *on almond seedling cv. Texas showing point of inoculation (a), dead shoots (arrows) and external canker development.*

Figure 4. Pathogenicity test of **E. lata** *on almond shoots (cambial inoculation). Inoculation points are surrounded by healthy callus tissue. Note extensive wood discolorations.*

Table 1

Pathogenicity of seven isolates of **Eutypa lata** *on almond (***Prunus dulcis** *cv. Texas) 17 months after inoculation.*

a/a	Isolate	Average length internal discoloration (cm)[y]
1.	Grape-VG	101 a[x]
2.	Almond-AA[z]	93 a
3.	Apricot-BA[z]	88 ab
4.	Pear-PA[z]	87 ab
5.	Apricot-BG 1	87 ab
6.	Almond-AT 5	85 ab
7.	Almond-AC	69 b
8.	Control	1 c

[x] Values followed by the same letter are not significantly different from each other (P = 0.05).
[y] There were five replications of each isolate.
[z] Australia isolates.

Table 2

Pathogenicity of seven isolates of **Eutypa lata** *on almond (***Prunus dulcis** *cv. Texas) 16 months after inoculation.*

a/a	Isolate	Average length internal discoloration (cm)[y]
1.	Almond-AA[z]	15 a[x]
2.	Pear-PA[z]	13 ab
3.	Almond-AC 2	11 ab
4.	Apricot-BG	10 b
5.	Apricot-BA[z]	10 b
6.	Grape-VG	10 b
7.	Almond-AC.A 5	8 b
8.	Control	3 c

[y] There were five replications of each isolate.
[z] Australia isolates.
[x] Values followed by the same letter are not significantly different from each other (P = 0.05).

Table 3

Susceptibility of 20 almond cultivars growing in the field to infection of **Eutypa lata**

a/a	Cultivar	Average length internal discoloration (mm)[z]
1.	Aï	160 a[x]
2.	Nonpareil	170 ab
3.	Anonymous Italian	179 ab
4.	Merced	189 ab
5.	Pagrati	190 ab
6.	Vavatsikou	191 ab
7.	Marcona	191 ab
8.	Truoïto	204 ab
9.	Phyllis	204 ab
10.	Ferragnes	215 ab
11.	4-17-67	216 ab
12.	Bellou	223 ab
13.	Texas	233 ab
14.	10-21-67	238 ab
15.	Syllogistos	244 ab
16.	Fournat de Brezenaud	253 ab
17.	Retsiou	253 ab
18.	42-16-68	270 b
19.	Tricioni	271 b
20.	Ferraduel	all four shoots dead

[x] Values followed by the same letter are not significantly different from each other (P = 0.05).
[z] There were four replications of each isolate.

Table 4

Susceptibility of 20 almond cultivars growing in the field to infection of **Eutypa lata**

a/a	Cultivar	Average length internal discoloration (mm)[z]
1.	Anonymous Italian	107 a[x]
2.	Truoïto	120 ab
3.	10-21-67	121 ab
4.	Texas (= Mission)	128 abc
5.	Nonpareil	137 abc
6.	Aï	140 abc
7.	Merced	140 abc
8.	Ferraduel	140 abc
9.	Vavatsikou	148 abc
10.	Retsiou	153 abc
11.	Bellou	155 abc
12.	Marcona	157 abc
13.	Tricioni	157 abc
14.	Fournat de Brezenaud	160 abc
15.	Ferragnes	160 abc
16.	4-17-67	163 abc
17.	Pagrati	165 abc
18.	Syllogistos	167-abc
19.	Phyllis	185 c
20.	42-16-68	205 c

[x] Values followed by the same letter are not significantly different from each other (P = 0.05).
[z] There were four replications of each isolate.

Table 5

Susceptibility of 20 almond cultivars growing in pots to infection of **Eutypa lata**

a/a	Cultivar	Average length internal discoloration (mm)[z]
1.	4-17-67	21,8 a[x]
2.	Tricioni	22,0 a
3.	Syllogistos	23,3 a
4.	5-3-67	27,8 a
5.	Marcona	28,8 a
6.	Bellou	34,0 a
7.	Aï	34,0 a
8.	Ferraduel	34,5 a
9.	Nonpareil	37,8 a
10.	Vavatsikou	40,3 a
11.	Ferragnes	41,5 a
12.	10-21-67	45,0 a
13.	4-2-67	51,0 a
14.	Texas	53,0 ab
15.	47-17-69	59,0 ab
16.	Phyllis	59,3 ab
17.	Truoïto	60,8 ab
18.	Pagrati	79,0 abc
19.	Retsiou	153,0 c
20.	42-16-68	166,8 c

[x] Values followed by the same letter are not significantly different from each other (P = 0.05).
[z] There were four replications of each isolate.

BIBLIOGRAPHY

1. BOLAY, A. ; CARTER, M.V. 1985. *Newly recorded hosts of* **Eutypa lata (E. armeniacae)** *in Australia.* **Plant. Protection Quartely I** (in press).
2. BOLAY, A. ; MOLLER, W.J. 1977. **Eutypa armeniacae**, *agent d'un dépérissement de vignes en production.* **Rev. Suisse Vitic, Arboric. Hortic.,** 9: 241-251.
3. CARTER, M.V 1957. **Eutypa armeniacae** *Hansf. et Carter, sp. nov., an airborne vascular pathogen of* **Prunus armeniaca L.** in *southern Australia.* **Austral. J. Bot.,** *5: 21-35.*
4. *CARTER, M.V. 1960. Further studies on* **Eutypa armeniacae** *Hansf. et Carter.* **Austral J. Agric. Res.,** 11: 498-504.
5. CARTER, M.V. 1975. *«Dying arm» disease of vines —A world wide problem.* **Austral. Grape Grower and Winemaker,** Dec. 7-8.
6. CARTER, M.V. 1982. *Additional hosts of* **Eutypa armeniacae** *in Australia.* **Austral. Pl. Pathol.,** 11: 46-48.
7. CARTER, M.V., ; BOLAY, A. 1972. *Eutypa dieback of apricot is prevalent in Switzerland.* **Phytopathol. Z.,** 75: 187-189.
8. CARTER, M.V. ; MOLLER, W.J. 1971. *The quantity of inoculum required to infect apricot and other Prunus species with* **Eutypa armeniacae. Austral. J. Exp. Agric. Anim. Husb.,** 11: 684-686.
9. CARTER, M.V. ; MOLLER, W.J. 1974. *Eutypa canker of apricot in Spain.* **Plant Dis. Reptr.** 58: 442-443.
10. CARTER, M.V. ; TALBOT, P.H.B. 1974. **Eutypa armeniacae.** *C.M.I. Descriptions of pathogenic fungi and bacteria.* No. 436, 2pp.
11. CARTER, M.V. ; BOLAY, A. ; RAPPAZ, P. 1983. *An annotated host list and bibliography of* **Eutypa armeniacae.** *Rev. Pl.* **Pathol.** 62: 251-258.
12. CARTER, M.V. ; MORVAN, G.S. ; CASTELAIN, C. 1964. *An extension of the known distribution of* **Eutypa armeniacae Nature.** 202: 1.134-1.135.
13. CARTER ; M.V. BOLAY, A. ; ENGLISH, W.H. ; RUMBOS, I. 1985. *Variation in the pathogenicity of* **Eutypa lata (=E. armeniacae). Austral. J. Bot.,** 33: (in press).
14. CARTER, M.V. ; BOLAY, A. ; RAPPAZ, P. ; ENGLISH, W.H. ; RUMBOS, I. 1983. *Pathogenic variation in* **Eutypa armeniacae.** 4 th. Intern. Congr. Pl. Pathol., Melbourne Abstr. No. 795, p. 199.
15. DYE, M.H. ; CARTER, M.V. 1976. *Association of* **Eutypa armeniacae** *and* **Phomopsis viticola** *with a dieback disease of grapevines in New Zealand.* **Austral. Plant Pathol. Soc. Newsl.** 5 (1): 6-7.
16. ENGLISH, H. ; DAVIS, J.R. 1978. **Eutypa armeniacae** *in apricot: pathogenesis and induction of xylem soft rot.* **Hilgardia,** 46: 193-204.
17. ENGLISH, H., ; O'REILLY, H.J. ; MCNELLY, L.B. ; DeVAY, J.E. ; RIZZI, A.D. 1963. *Cytosporina dieback of apricot.* **Calif. Agric.,** 17: 2-4.
18. GLAWE, D.A. ; DILLEY, M.A. ; MOLLER, W.J. 1983. *Isolation and identification of* **Eutypa armeniacae** *from* **Malus domestica** *in Washington state.* **Mycotaxon,** 18: 315-318.
19. KOUYEAS, H. 1978. **Eutypa armeniacae** *on lemon in Greece* **Phytopath. Z,** 91: 235-237.
20. KOUYEAS, H.; CHITZANIDIS, A. ; RAPPAS, A. ; CARTER, M.V., 1976. *Eutypa armeniacae on apricot and grapevine in Greece. Phytopath. Z.,* 87: 260-263.
21. LEHOCZKY, J. ; MOLLER, W.I. 1979. *Eutipás rák és tökeelhalás a szölö Magyarországon most felismert súlyos betegsére. (Eutypa canker and dieback, a newly recongnized and serious grapevine disease in Hungary).* **Kertgazdaság** 11 (2): 37-52.
22. MATTHEE, P.N. ; THOMAS, A.C. ; SMIT, A. 1973. *Eutypa dieback in apricot.* **The Deciduous Fruit Grower,** 23: 230-232.
23. MOLLER, W.J. ; KASIMATIS, A.N. 1978. *Dieback of grapevines caused by* **Eutypa armeniacae. Plant Dis. Rep.,** 62: 254-258.
24. RUMBOS, I. 1983. *Eutypa canker and dieback of almond in Greece* **Z. PflKrankh. PflSchutz,** 90: 99-101.
25. RUMBOS, I. 1964. *Occurence of* **Eutypa armeniacae** *on almond in Greece. Proc.* 6th Congr. Un. Phytopath. Mediterr. Cairo, Egypt. Oct. 1-6.
26. RUMBOS, I. 1984. *Damages caused by the fungus* **Eutypa armeniacae** *Hansf. & CARTER.* **Geotechnica,** 4: 118-125 (Gr.).
27. TELIZ, D. ; VALLE, P. 1979. *Eutypa dieback in Mexican vineyards.* **Pl. Disc. Rep.,** 63: 312-314.
28. TRESE, A.T. ; BURTON, C.L. ; RAMSEDELL, D.C. 1980. **Eutypa armeniacae** *in Mishigan vineyards: Ascospore production, survival, Host infection, and fungal growth at low temperatures.* **Phytopathology,** 70: 788-793.