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Esca in Austria

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Summary. Esca, known from Austria since it was reported by Zweigelt (1933), has increased in importance in the last ten years. Investigations on the incidence of esca were therefore undertaken to assess its impact on Austrian viticulture. A visual assessment of 14,000 vines showed that about 1.3 percent of plants exhibited external esca symptoms. This incidence was correlated with vineyard age, but not with the cultivar grown. The spatial dispersal of esca, investigated by monitoring contaminated vineyards, seemed to be random. Investigations on the temporal incidence of infestation revealed that the number of diseased plants increased dramatically within the time span studied. The average annual increase of plants with external esca symptoms in monitored vineyards was approximately 2.7 percent. Further studies focused on certain phytopathological aspects, such as the effect of white-rot fungi and *Phaeoacremonium chlamydosporum* on disease incidence, and the control of *Stereum hirsutum in vitro*. The occurrence of basidiocarps of *S. hirsutum*, *Trametes hirsuta* and *T. versicolor* on diseased vines and the isolation of *Phaeoacremonium* spp. were documented in Austria for the first time. Inoculations of grapevines with *S. hirsutum* and *P. chlamydosporum*, either separately or in combination, failed to produce symptoms on leaves or berries. On the other hand, cross-sections of woody tissues showed a decayed zone in the pith when they had been inoculated with *S. hirsutum*, and *P. chlamydosporum* induced dark-brown necrosis in the wood. A combination of these two fungi caused only dark-brown necrosis, without wood decay, even though re-isolation corroborated the presence of both fungi. Various fungicides were evaluated for their efficacy against *S. hirsutum in vitro*. The most effective fungicide for inhibition of mycelial growth was the EBI fungicide fenarimol with an EC_{50} value of 0.002 µg a.i. ml^{-1}. The dicarboximide fungicide procymidone was less effective, with an EC_{50} value of 80 µg a.i. ml^{-1}. The effects of benomyl and hydroxychinolin did not differ significantly when compared with the control.

Key words: viticulture, grapevine, esca, *Phaeoacremonium chlamydosporum, Stereum hirsutum, Trametes hirsuta, T. versicolor.*

Introduction

Although esca has been known from Austria since it was first reported by Zweigelt in 1933, it has increased in importance within the last ten years (Nieder, 1991; Reisenzein, 1995). The first significant occurrence of esca in Austrian vineyards was reported early in the 1990s. From 1985 to 1987, severe winter frost damages presumably induced an increase in esca incidence. Esca now occurs in all Austrian vine-growing areas. The broad range of symptoms makes it difficult for grape growers to diagnose the disease. In Austria both forms of esca occur: the mild form with typical foliage deterioration and/or fruit symptoms, and the severe form with apoplexy. Fruit symptoms differ: in most cases berries exhibit a violet coloration and reduced turgor. Berries can also display dark spots on the epidermis (black measles). In one case black mea-

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sles was associated with the occurrence of basidiocarps of *Trametes gibbosa* (Pers. ex Fr.) on the trunks of diseased vines (Redl, 1987). There are no pesticides or chemicals approved to date for the control of esca in Austria except agents for the protection of large pruning wounds. Alternative methods include uprooting of diseased plants or replacing diseased trunks with base shoots. Preventive measures rely on cultural practices to avoid entry points such as large pruning wounds or wounds resulting from mechanical cultivation. This paper describes the incidence and the dispersal of the disease in Austria. One focus of the study was also the etiology of esca.

Materials and methods

Incidence, syndrome and dispersal of esca

The presence of plants exhibiting external esca symptoms was monitored in randomly chosen vineyards in grid squares of 2.5 km^2 in the Austrian vine-growing area of Kremstal. A total of 70 plots was inspected. The cultivar grown, symptoms shown by diseased plants, and the presence of fruiting bodies were recorded. The vines were classified according to age: class I comprised vines aged 5 to 10 years, class II vines aged 10 to 20 years, and class III vines older than 20 years. The infection rate of esca in each vineyard was quantified by counting diseased and healthy plants in 36 plots. Relationships between vines with external symptoms, vineyard age and cultivar were estimated by the Crosstabs and Bivariate Correlation procedures of the SPSS Advanced Statistics package (SPSS Inc., Chicago, IL, USA, 1998). The spread of esca at different locations was monitored over 6 years.

In one vineyard the precise spatial pattern of diseased vines was also traced. Plants showing symptoms were marked in each year of the investigation. In order to analyze the spatial pattern, the expected and observed distributions of diseased plants of each grid were compared using a chi-test. The expected distribution was calculated by assuming a random spatial distribution of diseased vines. In order to determine the cryptic infection with white-rot fungi in this vineyard, a core borer (Mora Core Tax, extracting a 5.15 mm diam., 150 mm long core, Corp. Haglöf, Grube-Forst, Vorchdor, Austria) was used to take samples from trunks near the cordon. Twenty % of all visually healthy vines were investigated. The drill cores were sliced into 3-mm sections, their surface sterilized in sodium hypochloride and the sections placed on potato dextrose agar in Petri dishes. These cultures were incubated at room temperature for 7 days. Fungal growth was examined microscopically and fungi identified according to morphological characteristics.

Pathogenicity and control of *S. hirsutum*

Pathogenicity was tested in the greenhouse on five-year-old potted plants. The cultivar Grüner Veltliner, grafted on Kober 5BB, was inoculated with *Stereum hirsutum* (Willd.: Fr.) S.F. Gray, and *Phaeoacremonium chlamydosporum* Gams *et al*. Inoculation was performed either with one fungus only or with a composite of both fungi. The fungi were the defined isolates: *S. hirsutum* (DSMZ 3281, CH. Thielke) and *P. chlamydosporum* (*P. parasitica*, IMI 34 96 16). This last strain, isolated from vines in South Africa by J.H.S. Ferreira and classified as *Phialophora parasitica*, was assigned, following Crous *et al*. (1996) to *P. chlamydosporum* (L. Mugnai, personal communication). Inoculation was conducted with plugs of mycelia inserted into drill holes on the trunks and shoots. Filled holes were sealed with molten paraffin. The incubation period was 4 years, after which cross-sections of trunks and shoots were prepared. In order to reisolate the fungi, these cross-sections were disinfected with sodium hypochloride, placed on malt extract agar and incubated at room temperature.

Different fungicides (fenarimol, procymidone, benomyl and hydroxychinolin) were evaluated for their ability to inhibit radial growth of *S. hirsutum*. They were tested at different concentrations (0.1, 1.0 and 10 µg a.i. per ml potato-dextrose agar). Five-mm diameter plugs of mycelium were transferred to Petri dishes with each fungicide and without fungicide. After an incubation period of 7 days at 22 °C in total darkness, the colony diameters were measured. The trial was conducted with 10 replicates for each fungicide concentration. The entire experiment was repeated three times. The EC_{50} values were calculated by probit analysis. The mean percent inhibition from three experiments was analyzed by one-way ANOVA and separated by Duncan's multiple range test.

Table 1. Esca incidence versus vineyard age. The relationship between incidence of esca and vineyard age was demonstrated by the Pearson chi-Square Test. The resulting chi-Square is 31.15, the *P* value < 0.0005.

Vineyard age	Plots without esca		Plots with esca		Total number of plots surveyed
	No.	%[a]	No.	%	
Class I (5-10 years)	24	100	0	0	24
Class II (10-20 years)	11	33.3	22	66.6	33
Class III (>20 years)	3	23.1	10	76.9	13
Total	38	54.3	32	45.7	70

[a] Percentage over total number of plots inspected in each age class.

Table 2. Esca incidence versus grapevine cultivar. The statistical analysis of esca incidence and grapevine cultivars revealed that there was no association between these variables (Pearson chi-Square is 9.07, the *P* value is 0.336).

Cultivar	Plots without esca		Plots with esca		Total number of plots surveyed
	No.	%[a]	No.	%	
Grüner Veltliner	20	52.6	18	47.4	38
Müller Thurgau	4	50	4	50	8
Spätroter Veltliner	1	25	3	75	4
Riesling	5	83.4	1	16.6	6
Blauer Portugieser	2	28.6	5	71.4	7
Blauburger	2	100	0	0	2
Neuburger	2	66.7	1	33.3	3
Zweigelt	2	100	0	0	2

[a] Percentage over total number of plots per cultivar.

Results

Incidence of esca in Austria

Visual inspection of 14,000 vines revealed that approx. 1.3 percent showed external esca symptoms. Diseased plants were found in all grid squares in the Kremstal vine-growing area. The percentage of vineyards with and without esca is shown in Table 1. In the first age group (5-10 years) all vineyards were visually healthy, in the second (10-20 years) diseased vines were found in nearly 70% of the plots, and in the third (>20 years) the percentage of plots infected with esca increased to about 80%. The statistical analysis of the data revealed a significant association between esca incidence and vineyard age (Pearson chi-Square = 31.15, significance level less than 0.0005). No cells had an expected count of less than 5. The minimum expected count was 5.94. The hypothesis that esca infection and vineyard age are uncorrelated must therefore be rejected. On the other hand, an association between esca incidence and cultivar could not be demonstrated (Pearson chi-Square = 9.07, $P = 0.336$) in this investigation (Table 2).

Both the incidence of esca and the infection rate were closely related to vineyard age. On the average, about 1% of vines in age class II showed external esca symptoms. In age class III the infection rate was about 4%, with a range of 0-20%. The ratio of infection rate to vineyard age is shown in Figure 1, where the infection rate is expressed as a percentage of plants with external symptoms in each vineyard. The Pearson correlation between infecion rate and vineyard age is 0.372, with a significance level of 0.026. Because the linear model did not provide the best fit for this correlation ($R^2 = 0.138$), the ratio was also demonstrated with a non-parametric test (Spearman-Rho). This gave a correlation coefficient of 0.583, significant at $P = 0.001$.

Table 3. Occurrence of basidiocarps of white-rot fungi on vines in Lower Austria.

Locality	No. of basidiocarps of *Stereum hirsutum* on vines		No. of basidiocarps of *Trametes* spp. on vines with external symptoms	
	Without external esca symptoms	With external esca symptoms	*Trametes hirsuta*	*Trametes versicolor*
Göttweig	5	1	1	0
Geißenberg	11	2	1	0
Gobelsberg	1	2	0	0
Stammersdorf	1	1	0	0
Rohrendorf	0	3	1	0
Fels am Wagram	0	1	0	0
Schönberg	0	1	0	2
Feuersbrunn	15	0	0	0
Spinzenberg	9	0	0	0
Rehbergberg	6	0	0	0
Total	51	8	3	2

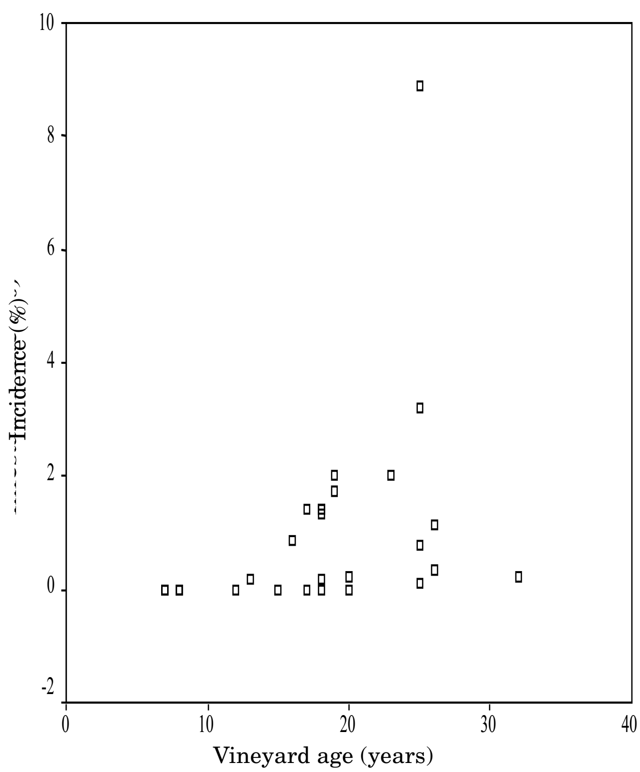


Fig. 1. Correlation between incidence and vineyard age. Incidence is expressed as the percentage of diseased vines (external symptoms) in each vineyard. The non-parametric Spearman-Rho – test showed a positive correlation between incidence and vineyard age (the correlation coefficient was 0.583, significant at *P*=0.001).

Fungi associated with esca in Austria and types of symptoms

The occurrence of basidiocarps of *S. hirsutum*, *T. hirsuta* (Fr.) Pil. and *T. versicolor* (Fr.) Pil. on vines is documented in Austria for the first time, while *Phaeoacremonium* spp. could be isolated from the discolored wood. Fructifications of other white rot fungi thought to be responsible for esca, such as *Phellinus igniarius* (L.: Fr.) Quél. or *Fomitiporia (Phellinus) punctata* (Karsten) Murrill, were not observed during this investigation. Basidiocarps of *T. hirsuta* were associated with diseased vines at three localities (Table 3). At the locality of Rohrendorf, the cv. Spätroter Veltliner showed foliar symptoms, and at the locality of Göttweig black measles was detected on the cv. Neuburger. Basidiocarps of *T. versicolor* were detected on vines with black measles at two localities (Fig. 2). Basidiocarps of *S. hirsutum* were found on 8 plants with external symptoms (Fig. 3), but in most cases the fruiting bodies of this fungus occurred on visually healthy plants (Fig. 4). Figure 5 shows the symptom expression of esca in the Kremstal vine-growing area in 1994. Twenty-two % of diseased vines showed only foliage deterioration, 10% only fruit symptoms, 68% had both. Symptom expression on vines with only fruit symptoms differed: about 7% of the diseased berries had a violet colour and reduced turgor, 3% had black measles.

Fig. 2. Basidiocarps of *Trametes versicolor* on the trunk of a diseased vine.

Temporal dispersal of esca

Records on the temporal spread of esca in several vineyards revealed that the infection rate increased dramatically with time (Fig. 6). The annual increase of vines with external esca symptoms in these vineyards had an average rate of about 2.7% yearly.

Fig. 4. Basidiocarps of *Stereum hirsutum* on the vine without esca symptoms on the leaves or berries.

Fig. 3. A diseased vine with external esca symptoms (top) and cross section of the trunk with wood decay (white rot) and brown wood streaking; basidiocarps of *Stereum hirsutum* on the bark (bottom).

Spatial dispersal of esca

The spatial dispersal of esca apparently did not start from pockets of infestation, but seemed to be random (Fig. 7). The calculated random distribution in one vineyard in Vienna agreed with the observed distribution to a significance level of 0.99. During six years the number of diseased

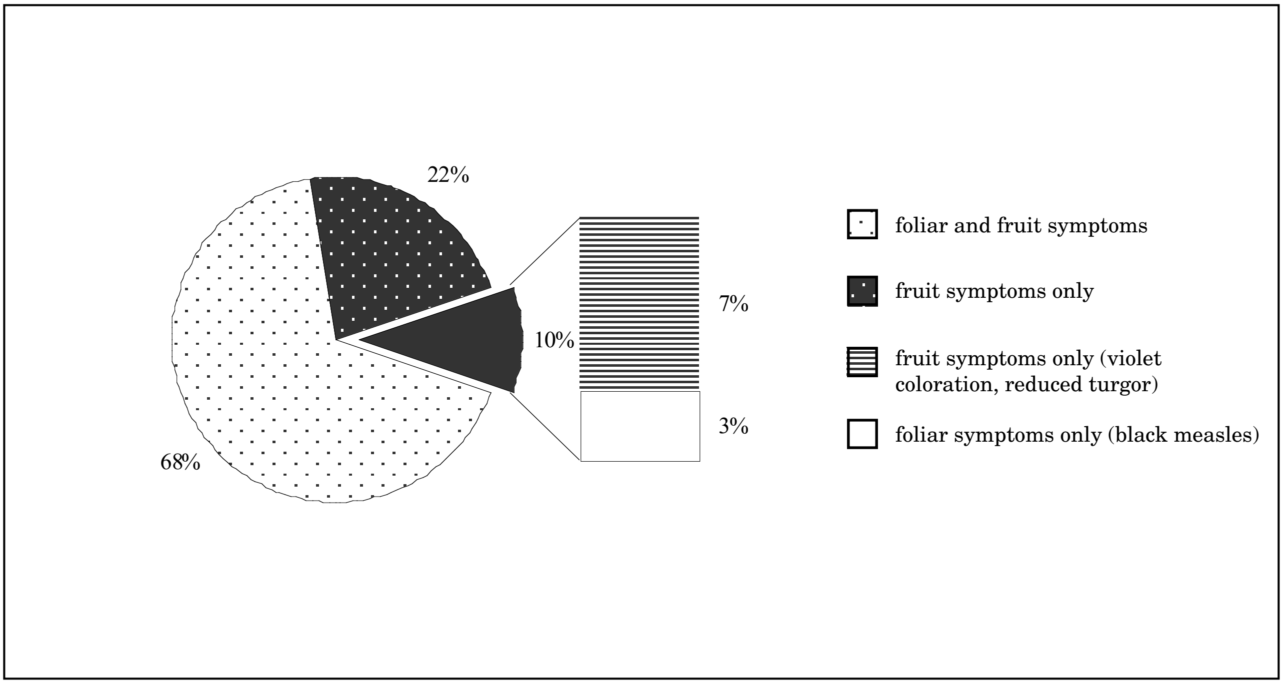


Fig. 5. Syndromes of esca in the Austrian vine-growing area of Kremstal in 1994. The symptoms of 134 diseased vines are classified by their mode of manifestation.

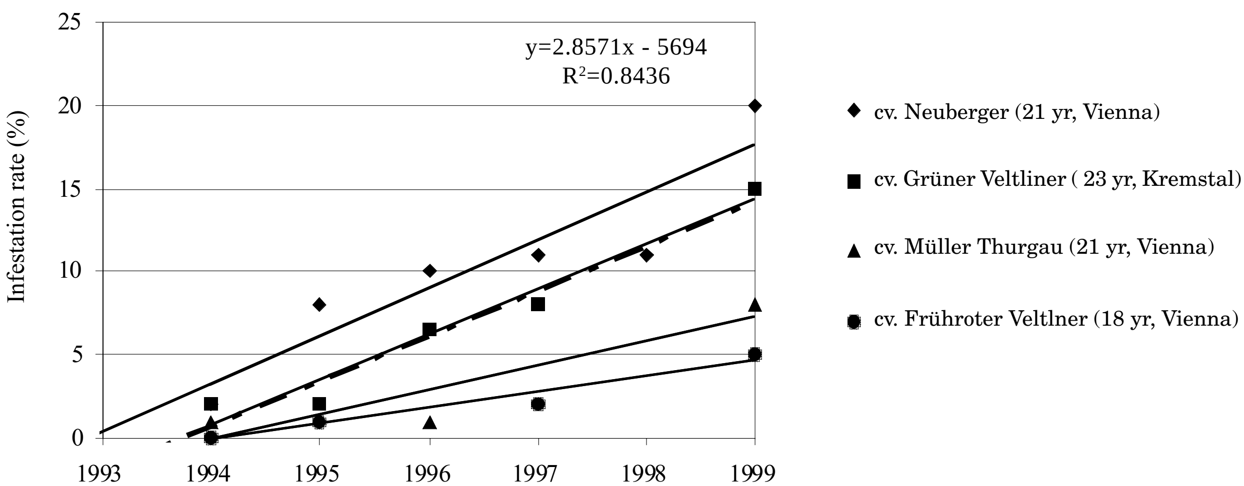
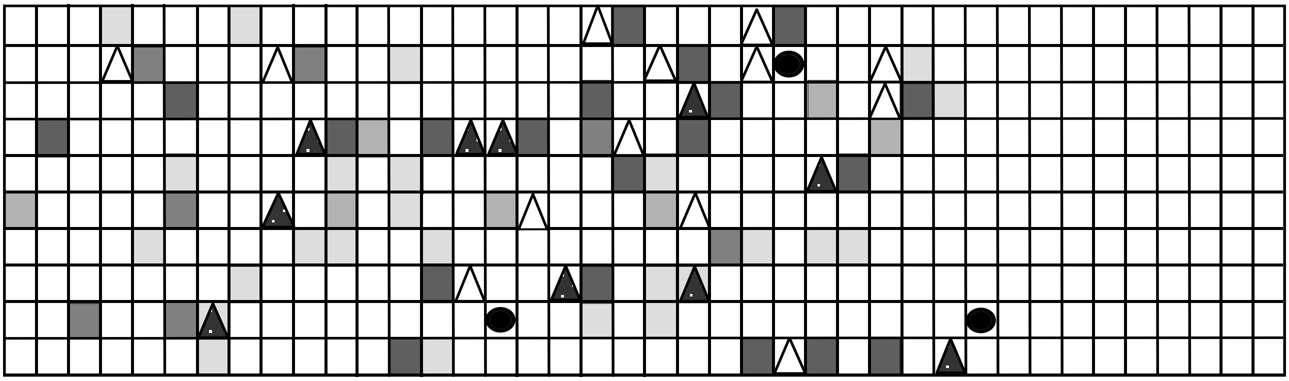


Fig. 6. The temporal dispersal of esca in four vineyards in the Vienna and Kremstal areas. The infection rate (percentage of vines with external symptoms per vineyard) increased annually by 2.7%.

vines in this vineyard increased from 2% to 20%. Although no symptoms were visible on the other vines, nearly half the tested plants were affected with white-rot. The white rot fungus was identified as *S. hirsutum*.

Pathogenicity and control of *S. hirsutum*

A pathogenicity test was performed with *S. hirsutum* and *P. chlamydosporum*, inoculated into vines either singly or in combination. After 4 years no symptoms appeared on leaves or berries, but symptoms



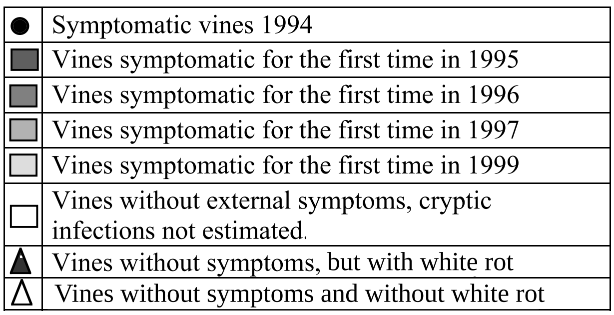


Fig. 7. Spatial pattern of esca distribution in a 24–year-old vineyard (cv. Neuburger grafted on Kober 5BB in the vine-growing area of Vienna 1994-1999). The round dots represent plants known to be diseased at the beginning of the investigation. In 1998 no newly diseased plants appeared. Cryptic infections with white-rot fungi were also estimated on a sample of visually healthy vines. Some plants without external symptoms, but affected with white-rot, exhibited external symptoms one year later. The analysis of the spatial pattern of diseased plants revealed a random distribution.

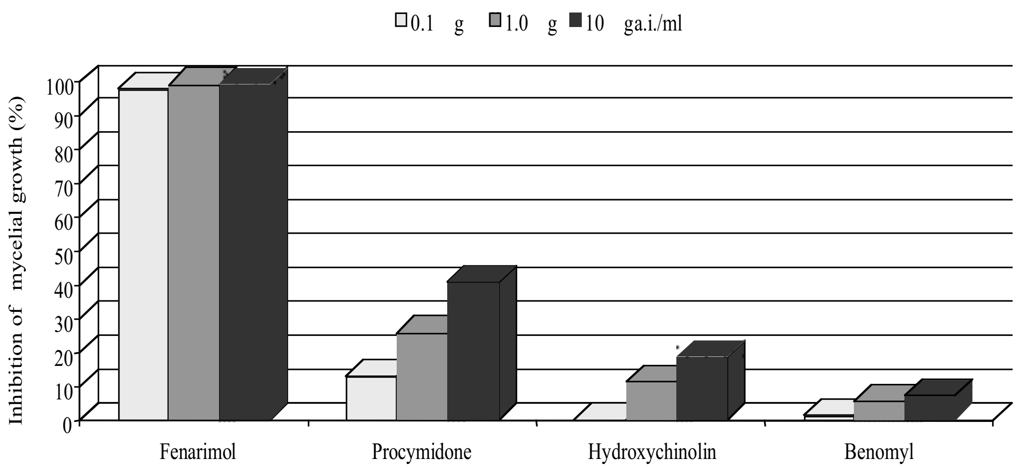


Fig. 8. Control of *S. hirsutum in vitro*. Inhibition of radial growth by fungicides is expressed as the mean percent of three experiments. The fungicides were tested at three different concentrations (0.1, 1.0 and 10 µg a.i. / ml). The most effective fungicide for inhibiting mycelial growth was the EBI fungicide fenarimol.

on the wood were likely common. Cross-sections of woody tissues inoculated with *S. hirsutum* showed a decayed zone in the pith. *P. chlamydosporum* caused dark-brown necrosis, the wood texture remaining hard. The combination of these fungi induced dark-brown necrosis, but no wood decay. Nevertheless, the re-isolation of both fungi was possible.

Different fungicides were evaluated for their efficacy against *S. hirsutum in vitro* (Fig. 8). The most effective fungicide for inibiting of mycelial growth was the EBI fungicide fenarimol, followed by the dicarboximide fungicide procymidone. The lowest concentration of fenarimol (0.1 µg a.i. ml^{-1}) inhibited radial growth by 100%. The EC_{50} for fenarimol was 0.002 μg a.i. ml^{-1}. All concentrations of procymidone tested also had a significant effect on mycelial growth. The EC_{50} for this fungicide was 80 μg a.i. ml^{-1}. The efficacy of benomyl and hydroxychinolin was very low. Even at the highest concentration used in this study (10 μg a.i. ml^{-1}), these fungicides did not differ significantly from the controls, as determined by Duncan´s multiple range test at a 0.05 level of probability.

Discussion

The incidence of esca in Austrian vineyards has increased continuously over the last ten years. The destructive potential and the widespread occurrence of esca have been demonstrated by field observations. In 1994 the incidence rate was about 1.3% in the Austrian vine-growing area of Kremstal. Examination of four vineyards showed that the incidence can increase dramatically. The number of vines with external symptoms increased by an average annual rate of 2.7% in 6 years, but in some vineyards the increase was between 2% and 20%. If it is born in mind that only plants with external symptoms were recorded each year and that this did not include infected pants without any symptom expression in a given year, one can postulate that the true infection rate in the investigated area is now many times higher than that estimated 5 years ago. This supposition corresponds to the visual impression in this and other Austrian vine-growing areas. These trends seemed to be important from an economic point of view. In contrast to other investigations (Minervini and Bisiach, 1995, Minervini *et al.*, 1996), esca incidence was correlated with vineyard age but not with the cultivar grown. In vineyards older than 20 years only 2 out of 10 were visually healthy. Analysis of spatial dispersal indicated that esca did not spread from pockets of infestation in the vineyard. Instead, it seemed to break out in a random pattern throughout the vineyard. The infection did not seem to disperse from vine to vine over short distances. Therefore, it could be assumed that the disease was dispersed by wind, insects or other agents.

The role of *S. hirsutum* in the disease complex is not yet clearly defined. Monitoring activities revealed that the occurrence of basidiocarps of *S. hirsutum* on vines was rather high in the vine-growing area of Kremstal. In almost all cases where basidiocarps of this fungus were found, however, vines showed no esca symptoms on the leaves or berries. One vineyard with basidiocarps of *S. hirsutum* was observed over a long period of time. Although basidiocarps were first detected here 8 years ago, the vines are still visually healthy. In some localities fruiting bodies were found saprophytic on wood vine-stakes, and these may have been the source of the inoculum. The pathogenicitiy test was undertaken to determine whether the simultaneous inoculation with *S. hirsutum* and *P. chlamydosporum* and a long incubation period would to induce foliar and/or fruit symptoms. *P. chlamydosporum* was chosen as one of the fungi for this artificial inoculations because at the beginning of this experiment, in 1996, this fungus (former name: *Phialophora parasitica*) had been associated with slow dieback of grapevines (Ferreira *et al.*, 1994). It should be pointed out that *P. parasitica* had never been isolated from vines in Austria. Another consideration in undertaking this test was that the fungi might need a long time to colonize the host tissue before the concentration of phytotoxic fungal metabolites become high enough to cause the typical symptoms. The results obtained give no support to this hypothesis however. The experiment performed with *S. hirsutum* and *P. chlamydosporum* in combination failed to induce esca symptoms on leaves or berries; only a dark-brown discoloration of the woody tissue was recorded. The lack of white-rot symptoms was attributed to a competitive inhibition of *S. hirsutum* by *P. chlamydosporum*. Inoculation with *S. hirsutum* alone induced rot decay in the pith. Inoculation with *P. chlamydosporum* alone also caused only the discoloration of the wood. These findings are consistent with literature data (Ferreira *et al.*; 1994, Larignon and Dubos, 1997). It can be summarized that the role of *S. hirsutum* in esca remains uncertain.

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