OVERWINTERING OF *ERYSIPHE NECATOR* SCHW. IN SOUTHERN ITALY

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SUMMARY

Erysiphe necator overwinters as mycelium or conidia in dormant buds and/or as cleistothecia, but the importance of the two forms as sources of primary inoculum varies in different viticultural areas. The present study summarizes the observations made over 2001-2003, in 29 vineyards of southern Italy, on the occurrence and frequency of the two overwintering forms. Flag shoots were found in one-third of the vineyards, with a frequency highly variable between year and vineyard, being more frequent in vineyards for wine-grape than for table-grape production. An average of 20% flag shoots were present on the same vine and arm as the previous year, and 75% of them originated from the three proximal buds of the canes. Cleistothecia were found in 46% of monitored vineyards, in the leaves and bark, but not in the soil. In spring there were between 3 and 856 cleistothecia/g of leaf tissue with a viability of 7%, whereas in the bark between 2 and 464 overwintering cleistothecia/g of bark were found, with an average viability of 14%.

Key words: Erysiphe necator, grape powdery mildew, flag shoot, cleistothecia.

INTRODUCTION

Grapevine powdery mildew, caused by *Erysiphe necator* Schw. [syn. *Uncinula necator* (Schw.) Burr.] (Braun and Takamatsu, 2000) is one of the most important grapevine fungal diseases worldwide. In southern Italy, *E. necator* causes heavy yield losses because the warm and dry climate of the Mediterranean basin allows the fungus to persist in the vineyards and is optimal for the development of epidemics. In this area, crop protection relies on an intense preventative usage of fungicides from bud break to veraison, or later in table-grape vineyards, to control late infections on rachis.

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The possibility of establishing new environment- and consumer-friendly strategies for controlling the disease and reducing the input of fungicides in the vineyard is hampered by the paucity of information on the pathogen's biology and disease epidemiology. *E. necator* overwinters both as mycelium/conidia in dormant buds (Sall and Wrysinsky, 1982; Gemmrich and Seidel, 1996) and/or as cleistothecia (Bulit and Lafon, 1978; Pearson and Gadoury, 1987; Cortesi *et al.*, 1995, 1997), but their presence varies in the different grapevine-growing areas, and the relative importance of the two forms as sources of primary inoculum is still not clear.

During winter the fungus remains dormant in bud scales until the following season. Shortly after the bud break it reactivates, shoots become covered with white mycelium and abundant sporulation, and appear short with wrinkled, deformed and folded up leaves (Sall and Wyrsinki, 1982; Pearson and Gartel, 1985). These infected shoots, known as "flag shoots", carry abundant conidia that cause secondary infections to neighbouring leaves and vines (Boubals, 1961; Cortesi *et al.*, 1997).

Cleistothecia form on all green tissues as the result of the sexual process, which is controlled by a bipolar heterothallism (Smith, 1970; Gadoury and Pearson, 1991; Miazzi *et al.*, 1997). In spring, cleistothecia discharge ascospores that give rise to scattered fungal colonies predominantly on the basal leaves of shoots (Diehl and Heintz, 1987; Pearson and Gadoury, 1987). The role of inoculum from cleistothecia has long been debated, because of the great differences in their diffusion in the viticulture areas. For instance, in New York State, cleistothecia are the main source of primary inoculum (Pearson and Gadoury, 1987) while they were shown to serve as an additional source of inoculum in California (Stapelton *et al.*, 1988) and in Australia (Magarey *et al.*, 1997). In Europe, as well as in other viticultural areas of the world, they were neglected for a long time, but recently they have been found more and more frequently and have been reconsidered as important additional sources of inoculum (Wicks and Magarey, 1985; Banihashemi and Parvin, 1995; Munshi *et al.*, 1996; Steinkellner, 1998; Halleen and Holz, 2001; Abu Blan and Khalil, 2001; Ovadia *et* *al.*, 2006). In Italy, cleistothecia are frequent in the northern regions (Viccinelli and Brunelli, 1993; Cortesi *et al.*, 1995, 1997), while in the south their role in the epidemiology of the disease and in the pathogen's life cycle has not been studied.

The objective of this research was to determine the incidence and distribution of *E. necator* overwintering forms (mycelium/conidia in dormant buds and cleistothecia) in vineyards of southern Italy, highlighting the role of the two overwintering forms and their potential contribution to the onset of epidemics in this important viticultural area.

MATERIALS AND METHODS

In 2001-2003, 29 vineyards located in Apulia, Basilicata, Calabria, and Molise were surveyed for the occurrence of flag shoots and cleistothecia. Table- and winegrape vineyards were selected as representatives of the different grapevine-growing areas, cultivars and training systems. In each vineyard, plots ranging from 400 to 4000 m^2 were arbitrarily selected for observations and samplings (Table 1).

Observations on flag shoots. The presence and distribution of flag shoots were surveyed at the beginning of each growing season (April-May). Vines presenting one or more flag shoots were marked with plastic flagging tape for further observations. Plots of 100-200 vines were established in 9 vineyards, and the number and the position of the flag shoots were recorded in detailed maps, each year, to follow the evolution of powdery mildew infections presumably initiated from flag shoots. In 4 vineyards, 10 vines with flag shoots and 10 random vines without flag shoots were selected and, one month after the appearance of flag shoots, 4 leaves (from 3rd to 6th) on all shoots of each selected vine were examined and the percentage of infected leaves (disease incidence) and of infected leaf surface (disease severity) was estimated.

Table 1. Vineyards monitored for the presence of flag shoots and cleistothecia.

Vineyard No.	Location	Cultivar	Observed area (m^2)	Training system	Year		
					2001	2002	2003
1	Acquaviva (Bari)	Italia	3000	Arbour vineyard	C[a]	C	C
2	Alberobello (Bari)	Verdeca	2000	Bilateral cordon	--	--	--
3	Fasano (Brindisi)	Verdeca	2000	Bilateral cordon	--	--	--
4	Mola di Bari (Bari)	Victoria	3500	Arbour vineyard	C	--	--
5	Mola di Bari (Bari)	Victoria	3500	Arbour vineyard	C	C	--
6	Monopoli (Bari)	Verdeca	1000	Head-trained vine	--	--	--
7	Turi (Bari)	Italia	1000	Bilateral cordon	--	--	--
8	Valenzano (Bari)	Malvasia nera	1000	Head-trained vine	C	C	C
9	Valenzano (Bari)	Negroamaro	400	Head-trained vine	C	C	C
10	Brindisi	Malvasia nera	2000	Bilateral cordon	--	F[b]	F-C
11	Brindisi	Negroamaro	2500	Bilateral cordon	--	F	F-C
12	Brindisi	Negroamaro	2000	Bilateral cordon	--	--	--
13	Oria (Brindisi)	Malvasia nera	2000	Arbour vineyard	F-C	F	F
14	Trinitapoli (Foggia)	Michele Palieri	3600	Arbour vineyard	F	F	--
15	Ginosa (Taranto)	Italia	2000	Arbour vineyard	C	C	--
16	Ginosa (Taranto)	Victoria	2000	Arbour vineyard	C	C	C
17	Ginosa (Taranto)	Victoria	4000	Arbour vineyard	C	C	C
18	Manduria (Taranto)	Cabernet	1600	Arbour vineyard	--	F-C	F-C
19	Manduria (Taranto)	Montepulciano	600	Arbour vineyard	--	F-C	C
20	Manduria (Taranto)	Merlot	600	Arbour vineyard	--	F-C	F-C
21	Sava (Taranto)	Chardonnay	600	Arbour vineyard	--	F-C	C
22	Cirò (Crotone)	Greco Bianco	1500	Bilateral cordon	--	--	--
23	Cirò (Crotone)	Greco Bianco	1500	Bilateral cordon	--	--	--
24	Cirò (Crotone)	Greco Bianco	1500	Head trained vine	--	--	--
25	Guglionesi (Termoli)	Montepulciano	1200	Arbour vineyard	F-C	F-C	F-C
26	Guglionesi (Termoli)	Chardonnay	600	Arbour vineyard	F-C	F-C	F-C
27	Campomarino (Termoli)	Pinot bianco	600	Arbour vineyard	F-C	F-C	F-C
28	Campomarino (Termoli)	Aglianico	600	Arbour vineyard	F-C	F-C	F-C
29	Campomarino (Termoli)	Pinot-Chardonnay	600	Arbour vineyard	--	F-C	F-C

[a]Cleistothecia on the leaves; [b]Flag shoot; (--)neither

Observations on cleistothecia. The occurrence of cleistothecia was assessed through observation of leaves in 29 vineyards (Table 1). In 16 of them, in autumn, senescent leaves still on vines or fallen to the ground were collected near 100 randomly selected vines, placed in plastic boxes covered with a polyethylene net, and kept in the vineyard during winter to study the variations in number of cleistothecia and their viability. Samples of 5 kg of leaves were kept both in the darkness at controlled temperature (4, 11, 22 and 25°C), and outdoors on the premises of the Faculty of Agriculture of the University of Bari, to assess the effect of different temperatures on the viability of cleistothecia.

In spring, just before bud break, twenty samples (100 g each) of bark were collected, separately, from the upper trunk or branches of randomly selected vines located along the diagonals of the vineyard, brought to the laboratory and processed. Soil was sampled collecting ten samples from the upper 2-cm layer of soil beneath vines. Soil samples were air-dried in the laboratory at room temperature (25±1°C) for 24 h before use.

Cleistothecia were separated from the substrate, according to Cortesi *et al.* (1995). Three samples of 30 g of bark or leaves or 100 g of soil were placed in 2 l Erlenmeyer flasks containing 1.5 l of water (1 l for soil). The flasks were shaken vigorously by hand for 3 min and the suspension was filtered through 120-mesh (125 µm) and 150-mesh (106 µm) Cobb sieves. The process was repeated three times, but shaking time was reduced to 1 min. After the fourth rinse, cleistothecia collected on the 150-mesh sieve were recovered in 25 ml of water. Three replicated aliquots of 1 ml of each suspension were spread on 10 cm^2 filter paper discs in Petri dishes. Cleistothecia on the paper were observed and counted with the aid of a dissection microscope. To evaluate viability, 50 cleistothecia from each of three replicates of each substrate were observed. Cleistothecia were considered viable when they contained at least one viable ascospore. Viability of ascospores was evaluated using fluorescein diacetate staining (Widholm, 1972).

Meteorological data from weather stations at Valenzano (Bari), Guglionesi (Campobasso) and Manduria (Taranto) were used to assess the occurrence of permissive conditions for ascospore release (at least 2.5 mm rainfall and daily mean temperature above 4°C) and infection (at least 2.5 mm rainfall and daily mean temperature above 10°C (Gadoury and Pearson, 1990).

RESULTS

Flag shoots. In spring, flag shoots are easily recognised because of their typical wrinkled and deformed leaves and the abundant sporulation. Vines were observed from the phenological phase BBCH11 1 to BBCH19 and flag shoots were found in 6, 13 and 10 of 29 vineyards, respectively in the three years of observations (Table 1).

The number of flag shoots was highly variable from vineyard to vineyard and year to year, being present on 3 to 27% of the vines in 2001, and on 3 and 54% of the vines in 2002. In 2003, flag shoots were absent in vineyards No. 18, 19 and 21, and between 3 and 81% of the total observed vines in the remaining vineyards (Table 2). They were more frequent in vineyards for wine-grape production (Brindisi, Taranto and Termoli provinces)

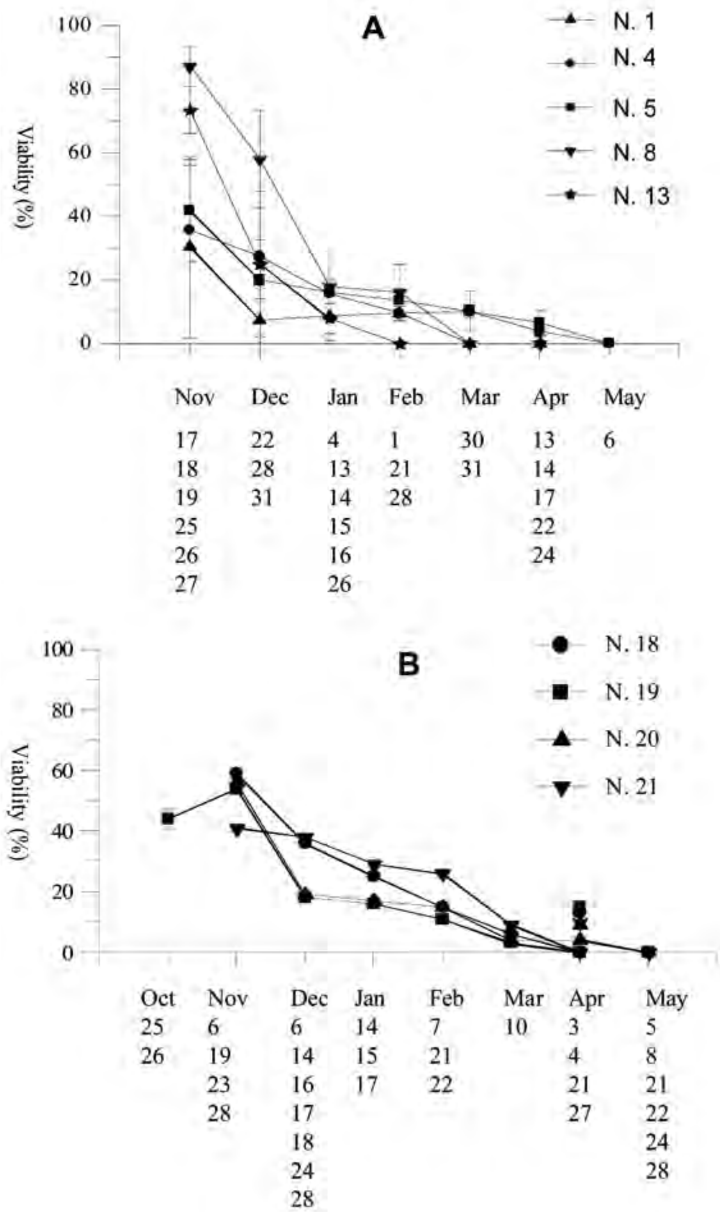

Fig. 1. Viability of cleistothecia on leaves conserved in plastic boxes on Bari (**A**) (2001) and Campobasso (**B**) (2002), during the winter; bars represent the standard deviation. Numbers indicate the date of occurrence of minimal conditions conducive to ascospore release. Numbers in legend indicate different vineyards.

Table 2. Variation in number and position of flag shoots in the years 2001-2003 in vineyards located in southern Italy.

Vineyard No.	No. of observed vines	Cultivar	2001		2002				2003			
			Flag shoots per ha	Vines with flag shoots (%)	Flag shoots per ha	Vines with flag shoots (%)	Vines with flag shoots on the same vine of 2001 (%)	Vines with flag shoots on the same arm of 2001 (%)	Flag shoots per ha	Vines with flag shoots (%)	Vines with flag shoots on the same vine of 2002 (%)	Vines with flag shoots on the same arm of 2002 (%)
14	850	Michele Palieri	8	3	12	4	34	28	-	-	-	-
18	560	Cabernet	-	-	17	5	-	-	1	0	0	0
19	100	Montepulciano	-	-	5	3	-	-	0	0	-	-
20	120	Merlot	-	-	95	27	-	-	10	4	50	33
21	100	Chardonnay	-	-	5	3	-	-	0	0	-	-
25	100	Montepulciano	1	3	8	7	0	0	20	11	33	33
26	140	Chardonnay	7	18	45	14	33	11	28	9	53	5
27	100	Pinot Bianco	19	27	140	54	24	11	455	81	17	17
28	100	Aglianico	4	10	27	13	56	19	10	3	66	67
29	10	Pinot	-	-	25	12	-	-	33	16	30	10
Average							29.4	17.2			35.6	23.5

- not monitored

Table 3. Distribution (%) of flag shoots along one-year-old canes.

Year	No. of observed vineyards	Nodes of one-year-old canes [a] I	II	III	IV	V	VI	VII	IIX	IX	X	XI	XII	XIII
2001	6	40	41	14	4	0	0	1	0	0	0	0	0	0
2002	11	29	37	11	8	5	3	3	1.5	2	0.5	0	0	0
2003	10	26	21	25	5	5	7	5	3	1	0	1	1	0

[a] From basal (I) to apical node (XIII).

than in vineyards for table grapes (Bari province) (Table 2). In the second and third year of observation (2002 and 2003), an average of 29.4% and 35.6% flag shoots were present on the same vine of the previous year (except in vineyard No. 25) and 17.2% and 23.5% respectively, were present also on the same arm (Table 2).

Observations made on the position of the flag shoots along 1-year-old canes showed that 95, 77 and 72% of them arose from the three basal buds , respectively, in the three years (Table 3). In four vineyards, powdery mildew symptoms on 10 vines with flag shoots and 10 without flag shoots were assessed in May, about one month after their appearance. Data reported in Table 4 show a clear delay in the appearance of disease symptoms on vines without flag shoots compared to those bearing flag shoots.

Cleistothecia. In autumn (September-November) of each year of the survey, cleistothecia were observed on leaves and/or bunches. They were detected in 13 vineyards out of 29 in 2001 and in 16 vineyards in 2002 and 2003 (Table 1). Cleistothecia were not detected in soil samples in 2001, hence soil was not further investigated in the following years.

The number of cleistothecia formed on the leaves at the end of the season was highly variable between years and vineyards. In 2000, their number ranged between 4 (vineyard No. 13) to 2096 (vineyard No. 5) cleistothecia g^{-1} of leaves and from 2 (vineyard No. 15) to 1700 (vineyard No. 25) in 2001 (Table 5). A broad variability was observed also within single vineyards for the number of cleistothecia ranged from 231 to 1339 in vineyard No. 18, and from 337 to 774 in vineyard No. 26, indicating a remarkable irregularity in the differentiation of cleistothecia (Table 6).

During winter, the number of cleistothecia on the leaves decreased slowly and had a sudden reduction in spring (Table 5); their viability decreased suddenly between November and December, and in March (Fig. 1). Although the rain events, necessary for ascospore discharge and infection, were recorded at these times, it was not possible to clearly relate them with the decreased viability of cleistothecia (Fig. 1). At the time of bud break, in March-April, cleistothecia were still present on the leaves and they had an average viability of 6% and 8%, respectively in 2001 and 2002 (Table 5).

Table 4. Comparison of powdery mildew symptoms on leaves of vines with or without flag shoots one month after their appearance in different vineyards (a).

Vineyard N.	Flag shoots	Incidence %	Severity %
14	With flag shoots	11.5 A	41.4 A
	Without flag shoots	0 B	0 B
25	With flag shoots	6.8A	69.9 A
	Without flag shoots	0 B	0 B
26	With flag shoots	9.2A	87.5 A
	Without flag shoots	0 B	0 B
28	With flag shoots	6.6A	68.7 A
	Without flag shoots	0 B	0 B

(a) Mean values refer to 10 vines. For each vineyard, mean values followed by different letters are statistically different at the probability level P=0.01, according to the Duncan's multiple range tests.

On vine bark, cleistothecia were not found in 2001, while in 2002 they were present in 7 vineyards out of 16, at a number between 2 and 464 cleistothecia g^{-1} of bark, and with a viability of 14% (Table 7). The effects of temperature on cleistothecia were observed in experiments under controlled storage. Cleistothecia stored at 22 or 25°C lost their viability very fast, becoming completely non-viable after 4 months; cleistothecia stored at 4 or 11°C retained 10% of viability even after 7 months of storage (Fig. 2).

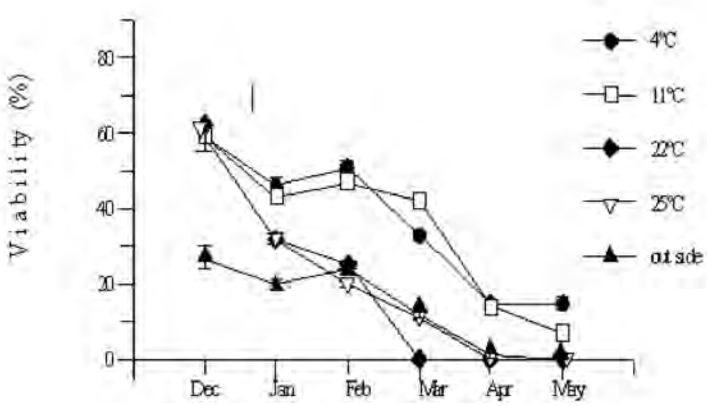

Fig. 2. Viability of cleistothecia on leaves from the vineyard N. 8 maintained at different temperatures; bars represent the standard deviation.

Table 5. Evolution of number (No.) and viability (V) of cleistothecia on leaves during the winter.

	Vineyard No.	November		December		January		February		March		April		May	
		No.	V (%)	No.	V (%)	No.	V (%)	No.	V (%)	No.	V (%)	No.	V (%)	No.	V (%)
2000-2001	1	160	51	28	20	22	9	21	9	15	0	14	0	-	-
	4	1298	39	1065	23	795	16	262	9	347	10	138	4	-	-
	5	2096	41	1810	19	1377	17	1475	13	1491	12	664	6	-	-
	8	8	87	6	58	5	19	4	20	4	0	3	0	-	-
	13	4	70	3	25	3	14	2	10	2	8	0	0	-	-
2001-2002	1	82	27	0	-	0	-	0	-	0	-	0	-	-	-
	8	206	30	295	26	388	23	176	22	174	0	144	0	-	-
	9	709	23	662	27	690	20	633	24	662	12	248	1	107	0
	15	2	-	0	-	0	-	0	-	0	-	0	-	0	0
	16	37	10	10	-	30	-	9	-	0	-	0	-	-	-
	17	561	67	225	31	248	27	217	3	278	0	101	0	-	-
	18	1025	60	1998	36	1152	25	1003	14	689	6	725	0	-	-
	19	876	54	1444	18	984	16	889	11	971	3	810	0	-	-
	20	289	56	193	19	307	19	500	15	285	4	453	0	-	-
	21	133	41	142	22	89	29	43	26	36	9	19	0	-	-
	25	1700	22	1284	59	997	47	596	37	1056	14	856	10	708	0
	26	423	45	77	41	88	23	58	31	94	24	38	8	18	0
	27	212	26	245	39	625	28	254	27	348	14	177	6	182	1
	28	6	-	40	20	2	-	11	-	13	-	0		0	0

Table 6. Variation in number and viability (%) of cleistothecia within single vineyards (October).

Plot No.	Vineyard No.18		Vineyard No. 26	
	No.[a]	Viability[b]	No.	Viability
1	798	58	495	44
2	805	52	393	49
3	711	51	280	42
4	939	58	337	48
5	231	51	608	48
6	780	51	774	52
7	1339	55	439	42
8	656	53	338	48
9	973	57	424	41

a Number of cleistothecia per g of leaves.
b Percentage of viable cleistothecia.

Table 7. Number and viability of cleistothecia on the bark of vines in spring (15 April 2002).

Vineyard No.	Bark of arms		Bark of trunk	
	No. (a)	V. (b)	No. (a)	V. (b)
8	0	0	2	11
18	20	10	16	13
19	22	14	12	15
20	2	9	3	9
21	3	6	3	10
25	464	28	21	23
26	6	15	2	15
Average		13		14

(a) Number of cleistothecia per g of bark
Percentage of viable cleistothecia.

DISCUSSION

The overwintering mode of the grape powdery mildew fungus *E. necator*, as mycelium in dormant buds or as ascospores in cleistothecia, is a key element in the epidemiology of the disease, since it determines the amount of the primary infection and the dynamics of epidemics. Moreover, infections from ascospores depend strongly on rainfall, whereas mycelium overwintering in buds is less dependant on environmental conditions (Pearson and Goheen, 1988).

In northern Italy it was shown that cleistothecia are the prevailing form of overwintering (Viccinelli and Brunelli, 1993; Cortesi *et al.*, 1995; Cortesi and Ricciolini, 2001), even if their amount and distribution varies between vineyards and years. In southern Italy, a three-year field survey of the two overwintering forms in 29 vineyards, indicates that both mycelium and cleistothecia can be sources of primary inoculum and can play an important role in the onset of *E. necator* epidemics.

Flag shoots were found very early in the season (April-May) in one-third of vineyards, appearing to be more dispersed than in vineyards of northern Italy (Cortesi *et al.*, 1997). This is probably due to the environmental conditions of the Mediterranean area, which are more permissive to the pathogen overwintering as mycelium in dormant buds, compared to the rigid winter temperatures in northern Italy (Cortesi *et al.*, 1997).

Flag shoots were more frequent in wine-grape (Brindisi and Campobasso provinces) than in table-grape vineyards (Bari province). The wine-grape vineyards are very well protected during the first phases of vine development, and these frequent treatments probably prevent the newly formed buds from being infected from the precocious inoculum that would perpetuate in the flag shoots (Diehl and Heintz, 1987; Rügner *et al.*, 2002). Our survey showed that 30% of the flag shoots reappear on the same vine in the next spring, and 20% on the same arm, also in vineyards where the growers removed them soon. This persistence of flag shoots, and the fact that most of them arise from the basal buds of one-year-old canes, suggests that flag shoots perpetuate probably through a very early infection of the new buds. This would be in agreement with the findings of Rademacher *et al.* (2002) and Rügner *et al.* (2002) that indicate that the bud's highest susceptibility to infection from *E. necator* is at the prebloom stages of grapevine development.

Cleistothecia were found in almost half of the surveyed vineyards and their occurrence seems to be totally dependant on the occurrence and severity of late infections, as highlighted by their high variability in numbers even within single vineyards. As for flag shoots, they were more frequent and abundant in the wine-grape area (Molise) than in table-grape areas (Bari and Taranto), as a result of the containment of the fungal development deriving from the intensive spray-schedules applied against powdery mildew in table-grapes.

Cleistothecia were never detected in the soil, as reported for northern Italy (Cortesi *et al.*, 1997). On the leaves, at the bud break, they were still present at a high number but their viability seemed to be strongly dependent on the environmental conditions in the different vineyards. In the favorable climate of southern Italy, that allows the survival of the leaf litter until bloom, leaves seem to be a potential efficient substrate for overwintering cleistothecia, as reported for northern Italy (Cortesi *et al.*, 1997), but only if winter temperatures are not too mild, because the high temperatures have detrimental effects on the viability of cleistothecia.

Cleistothecia overwintering in the anfractuosities of the bark, although less numerous, had a double viability respect to cleistothecia overwintering on the leaves, and this was similar in all vineyards, indicating a contained influence from the winter climate factors. Therefore, cleistothecia overwintering in the bark can be postulated as being responsible for primary infections, in southern Italy, as was also the case reported for northern Italy by Cortesi *et al.* (1997).

In southern Italy, both mycelium and cleistothecia are present and widespread. They can be sources of primary inoculum and play an important role in the onset of epidemics of powdery mildew. Despite the reduction of the importance of mycelium in dormant buds in the overwintering of *E. necator* recorded in the last years, in southern Italy there are vineyards where flag shoots are numerous and persistent during years. In these vineyards they are important sources of inoculum for the neighboring shoots and vines and, being present in the vineyard very early, they can be a premise for precocious and severe epidemics (Cortesi *et al.*, 2004). In this case, strategies of chemical control in the very early stages of shoot development could prevent colonization of buds by the pathogen, and then reduce the risk of epidemics from the vegetative overwintering form.

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