Acta Phytopathologica et Entomologica Hungarica 38 (1–2), pp. 53–60 (2003)

Natural Parasitism of *Uncinula necator* Cleistothecia by *Ampelomyces* Hyperparasites in the South-Western Vineyards of Hungary

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The natural occurrence of *Ampelomyces* hyperparasites was determined in *Uncinula necator* cleistothecia in nine south-western vineyards of Hungary between 1997–2000. During this study, a total of 1600 grape leaf samples were collected and examined from seven grapevine cultivars. The results showed that *Ampelomyces* spp. were widespread in that area and parasitized 0–55.7% (mean: 8.3%) of the cleistothecia on the adaxial surface of the leaves and only 0–4.8% (mean: 0.8%) on the abaxial leaf surfaces. The incidence of *Ampelomyces* in *U. necator* cleistothecia was the highest on the leaves covered more than 60% with powdery mildew mycelia. The results suggested that the naturally occurring *Ampelomyces* hyperparasites play a role in the natural control of grapevine powdery mildew and may reduce the overwintering inoculum of *U. necator* in the studied area.

Keywords: *Uncinula necator,* grape powdery mildew, cleistothecia, *Ampelomyces,* hyperparasitism, natural control.

Ampelomyces spp. are the most common, naturally occurring hyperparasites of powdery mildew fungi (Kiss, 1997a; 1998). Their hyphae penetrate the hyphae of their fungal hosts, grow from cell to cell, and produce their intracellular pycnidia in the hyphae, conidiophores, conidium initials and immature cleistothecia of powdery mildew fungi. Conidia of *Ampelomyces* are released from intracellular pycnidia in the presence of water. They germinate on host plant surfaces and penetrate again the hyphae of powdery mildew colonies. Thus, the sporulation of powdery mildew mycelia parasitized by *Ampelomyces* is stopped and the infected colonies are killed in 7–10 days after the penetration of the hyperparasite (Sz. Nagy and Vajna, 1990; Falk et al., 1995a, b; Kiss, 1998).

The taxonomy of the pycnidial fungi belonging to the genus *Ampelomyces* is still controversial. During the past 15–20 years, most authors considered that the genus contains only one single species, *Ampelomyces quisqualis* (Kiss and Vajna, 1995). However, molecular studies showed that there is a high genetic variability in the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) in different *Ampelomyces* isolates suggesting that *'A. quisqualis'* should be regarded as a name of a problematic species complex (Kiss, 1997a). Phylogenetic analysis of rDNA ITS sequences showed that the ten *Ampelomyces* isolates studied represent at least six different species (Kiss and Nakasone, 1998). So, the use of the name '*Ampelomyces* spp.' was recommended when referring to the pycnidial hyperparasites of powdery mildew fungi (Kiss and Nakasone, 1998).

Ampelomyces hyperparasites were used in many biological control experiments of powdery mildews infecting different crops. The first biological control experiment using an

Ampelomyces isolate was carried out by Yarwood (1932). These hyperparasites were used successfully against powdery mildew infections of various greenhouse crops (Jarvis and Slingsby, 1977; Vajna, 1987; Sztejnberg et al., 1989). *Ampelomyces* hyperparasites were also used against powdery mildew infections of field crops including grapevine (Falk et al., 1995a, b; Moncheiro et al., 1996; Daoust and Hofstein, 1996; Santomauro et al., 1997).

Since the late eighties, natural occurrence, biology and genetic variability of *Ampelomyces* hyperparasites and their use in the biological control of powdery mildew fungi have been studied thoroughly in Hungary (Vajna, 1987; Sz. Nagy and Vajna, 1990; Sz. Nagy, 1992; Kiss, 1997a, b, 1998, 2001; Kiss and Nakasone, 1998; Fischl, 2000). The natural occurrence of *Ampelomyces* in the grape powdery mildew pathogen, *Uncinula necator,* in Hungary was reported for the first time by Sz. Nagy and Vajna (1990). Later, Kiss (1998) reported the rate of incidence of *Ampelomyces* spp. in *U. necator* and also the intensity of mycoparasitism in grape powdery mildew mycelia. Füzi (1999a, b, c) studied the parasitism of *U. necator* cleistothecia by *Ampelomyces.*

Cleistothecia of *U. necator* are one of the sources of the primary inocula of grape powdery mildew in Hungary (Füzi, 1999b, c). The aim of this study was to determine the number of *U. necator* cleistothecia naturally parasitized by *Ampelomyces* in the south-western vineyards of Hungary.

Materials and Methods

A total of 1600 leaf samples were collected in mid-October in 1997, 1998, 1999 and 2000. Each sample consisted of one full-grown vine leaf representing a total of seven *Vitis vinifera* cultivars (Blue Frankish, Nosztori Riesling, Muscat Ottonel, Kadarka, Leányka, Portugieser and Zweigelt). Leaves were collected from both sides of the vine rows and also from the upper, middle and lower parts of the vine stocks. Samples were collected in the vineyards of six localities (Csötönyi-völgy, Görögszó, Kajmád, Pécs, Sióagárd and Szentgálpuszta) in south-western Hungary *(Table 1).* These vineyards belonged to the Szekszárd vine district and to the Vinicultural and Viticultural Research Institute of the Hungarian Ministry of Agriculture in Pécs. The ways of cultivation of the vines and the use or lack of chemical control against grape powdery mildew in these vineyards were indicated in *Table 1.*

In each sample, the percentage of powdery mildewed area was determined on the adaxial leaf surface. In order to determine the number of parasitized and nonparasitized cleistothecia belonging to different stages of development, five discs were cut out from each leaf. Leaf discs measured 1.13 cm^2. The number of immature, half mature and mature cleistothecia, parasitized or not by *Ampelomyces,* was counted on both the adaxial and abaxial surfaces of each leaf disc using a stereomicroscope. Cleistothecia were confirmed to be parasitized when conidia of *Ampelomyces* were detected in them under a light microscope. Yellow cleistothecia with a diameter less than 50 μm were considered as immature, orange ones measuring 50–100 μm were considered as half mature while dark brown or black ascocarps with or without well-developed appendages and measuring 100–150 μm were considered as mature.

Table 1

Natural occurrence of *Ampelomyces* spp. hyperparasitic fungi in *Uncinula necator* cleistothecia in the south-western vineyards of Hungary in October 1997–2000

Location *Vitis vinifera* variety Way of cultivation	Year	On the adaxial surface of leaves			On the abaxial surface of leaves		Chemical control*
		Proportion of mildewed area %	Total cleistothecia per cm²	Parasitized cleistothecia per cm²	Total cleistothecia per cm²	Parasitized cleistothecia per cm²	
1 Csötönyi-völgy	1997	6.6	0.6	0.0	0.0	0.0	–
Blue Frankish	1998	0.7	0.0	0.0	0.0	0.0	–
umbrella	1999	1.6	0,5	0.0	1.7	0.0	–
	2000	73.2	60.2	16.7	51.6	2.5	–
2 Görögszó	1997	38.5	20.9	0.0	2.1	0.0	+
Blue Frankish	1998	11.0	6.6	0.0	0.2	0.0	+
umbrella	1999	53.9	57.9	3.4	1.8	0.0	+
	2000	49.0	28.0	0.1	3.6	0.0	+
3 Görögszó	1997	29.7	23.5	0.0	0.7	0.0	–
Nosztori Riesling	1998	39.2	28.2	3.0	22.6	0.0	–
umbrella	1999	81.8	116.4	15.4	95.0	0.0	–
	2000	68.8	69.6	0.2	48.6	0.0	–
4 Kajmád	1997	0.0	0.0	0.0	0.0	0.0	+
Muscat Ottonel	1998	0.3	0.0	0.0	0.0	0.0	+
umbrella	1999	46.7	19.8	3.0	2.7	0.1	+
	2000	30.4	9.1	0.2	0.0	0.0	+
5 Pécs	1997	0.6	0.0	0.0	0.0	0.0	+
Kadarka	1998	40.0	32.9	8.6	0.1	0.0	+
Moser	1999	18.8	10.1	0.5	0.0	0.0	+
	2000	27.9	13.3	0.0	0.1	0.0	+
6 Pécs	1997	0.3	0.0	0.0	0.0	0.0	+
Leányka	1998	24.8	16.7	9.3	0.1	0.0	+
Moser	1999	19.3	10.6	0.0	0.3	0.0	+
	2000	38.6	32.4	0.2	6.6	0.0	+

Table 1 (cont)

Location	Year	On the adaxial surface of leaves			On the abaxial surface of leaves		Chemical
Vitis vinifera variety		Proportion of	Total	Parasitized	Total	Parasitized	control*
Way of cultivation		mildewed area	cleistothecia	cleistothecia	cleistothecia	cleistothecia	
		%	per cm^2	per cm^2	per cm^2	per cm^2	
7 Pécs	1997	3.8	0.1	0.0	0.0	0.0	+
Portugieser	1998	49.0	57.7	16.1	10.6	0.0	+
Moser	1999	45.6	68.1	2.4	2.4	0.1	+
	2000	50.0	47.7	0.1	4.5	0.1	+
8 Sióagárd	1997	29.9	7.5	0.0	0.0	0.0	+
Blue Frankish	1998	19.4	8.2	0.0	0.5	0.0	+
umbrella	1999	61.0	89.2	5.4	30.7	0.0	+
	2000	78.3	140.5	2.0	35.8	0.0	–
9 Szentgálpuszta	1997	1.2	0.1	0.0	0.0	0.0	+
Zweigelt	1998	8.0	4.3	0.0	0.0	0.0	+
GDC	1999	8.8	5.7	0.1	0.0	0.0	+
	2000	74.3	95.8	3.0	48.5	0.0	–
	Mean	**31.42**	**30.06**	**2.49**	**10.30**	**0.08**	

* + = regularly sprayed with fungicides against powdery mildew
– = non sprayed with fungicides against powdery mildew

Results

The data on the natural occurrence of *Ampelomyces* hyperparasites in the cleistothecia of *U. necator* in the studied area are shown in *Table 1.* In 1997, parasitized cleistothecia were not found in any samples, but they were detected in a number of samples between 1998–2000. The first parasitized *U. necator* cleistothecia appeared in late August or early September each year. Hyperparasitism was much more intense on the adaxial leaf surfaces. The results showed that *Ampelomyces* parasitized 0–55.7% (mean: 8.3%) of the *U. necator* cleistothecia on the adaxial surface of the leaves and only 0–4.8% (mean: 0.8%) on the abaxial leaf surfaces. Parasitized cleistothecia were dull, fawn-coloured, measuring 50–140 µm and bearing rudimentary appendages. Apparently mature, dark brown or black cleistothecia that were, in fact, parasitized by *Ampelomyces* were frequently found in October 1998 (Füzi, 1999a). They were recognized based on their rudimentary appendages and parasitism was confirmed using light microscopy. Most probably, in these cases the maturation of the cleistothecial wall was completed, but, instead of asci and ascospores, *Ampelomyces* pycnidia were developed in the cleistothecium. This was also observed by Sz. Nagy and Vajna (1990).

The distribution of immature, half mature and mature cleistothecia, parasitized or not by *Ampelomyces,* was studied in function of the coverage of leaves with powdery mildew mycelia *(Fig. 1).* When the powdery mildew coverage was less than 50% on the leaves, the cleistothecia were generally free of *Ampelomyces.* The more infected the leaves were the more parasitized cleistothecia occurred on them. The incidence of *Ampelomyces* pycnidia in *U. necator* cleistothecia was the highest on leaves covered more than 60% with powdery mildew mycelia *(Fig. 1).* As the coverage of leaves with powdery mildew increased, the number of immature cleistothecia decreased. At the same time, the number of parasitized cleistothecia increased.

Discussion

This four-year study showed that *Ampelomyces* hyperparasites parasitized on average 8% of the *U. necator* cleistothecia on the adaxial surface of the leaves and only 0.8% on the abaxial leaf surfaces in the studied area. So, these fungal antagonists were able to naturally destroy on average 5% of the total number of grapevine powdery mildew cleistothecia produced in autumn to serve as primary sources of inocula for the next season. However, in some years and in some sample places the activity of *Ampelomyces* hyperparasites was very intense parasitizing up to 55.7% of grapevine powdery mildew cleistothecia *(Table 1).*

In this study, the role of *Ampelomyces* in reducing the primary inoculum of *U. necator* was estimated based on the number of parasitized cleistothecia on the leaves in autumn. However, in spring, the major sources of primary inocula are the cleistothecia overwintered on the bark of the grapevine stocks. So, the role of *Ampelomyces* in the natural control of *U. necator* would have been more accurately determined if the number

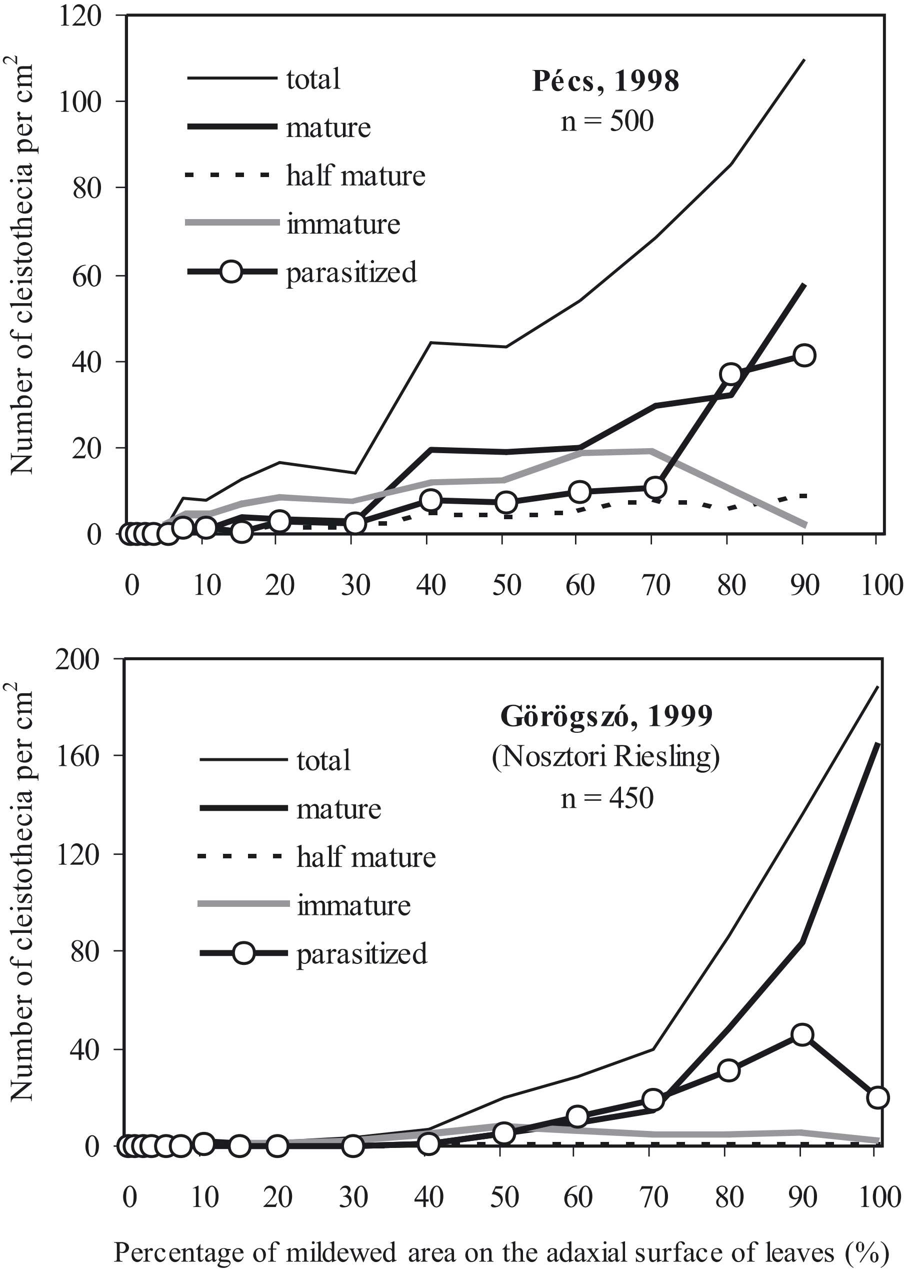

Fig. 1. Distribution of parasitized and nonparasitized *Uncinula necator* cleistothecia at different stages of development on the adaxial surface of grapevine leaves in function of the intensity of powdery mildew infection

of overwintered cleistothecia, parasitized or not by hyperparasites, had been counted on the bark in spring. Falk et al. (1995a, b) studied the impact of *Ampelomyces* on the number of grapevine powdery mildew cleistothecia found on the bark of the grapevine stocks. However, many cleistothecia overwintered on the bark originate from leaves from where they are washed off in autumn. So, the data obtained in this study on the number of parasitized and nonparasitized cleistothecia on the grapevine leaves also show the role of *Ampelomyces* in the natural control of the primary inoculum of *U. necator.*

Parasitism of cleistothecia was approximately ten times more intense on the adaxial leaf surfaces than on the abaxial surfaces of grapevine leaves *(Table 1).* Two observations might explain this data: (i) grapevine powdery mildew attacks especially the adaxial leaf surfaces in autumn (Füzi, 1999b), and (ii) splash dispersal of *Ampelomyces* conidia is much more enhanced by rain on the adaxial surfaces of leaves compared to the abaxial leaf surfaces.

According to a mathematical model of parasite-hyperparasite interactions (Shaw, 1994; Shaw and Peters, 1994), hyperparasites might cause apparently random fluctuations in the abundance of parasites from year to year, even in an absolutely constant environment. The data obtained in this study showed a considerable variation in the degree of parasitism of *U. necator* cleistothecia by *Ampelomyces* hyperparasites. In 1997, no parasitized cleistothecia were found in any samples while in 1998 *Ampelomyces* parasitized up to 55.7% of *U. necator* cleistothecia *(Table 1).* All these data suggest that the naturally occurring *Ampelomyces* hyperparasites play a considerable role in the natural control of grapevine powdery mildew.

Acknowledgements

The author is grateful to Dr. Levente Kiss (Plant Protection Institute of the Hungarian Academy of Sciences, Budapest) for his help in identifying *Ampelomyces* and also to Prof. Géza Fischl (Plant Protection Institute, Georgikon Faculty of Agricultural Sciences, Veszprém University, Keszthely) for comments on the manuscript.

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