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Survey of pathogens and parasitoids in late instar *Lymantria dispar* larval populations in Sardinia, Italy

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Abstract

Gypsy moth (*Lymantria dispar* L.) larvae were collected in 20 oak stands in Sardinia to evaluate mortality factors. Collected larvae were reared in the laboratory on artificial diet until they died or pupated. Larval mortality ranged from 17.5 to 100%. Parasitoids that killed larvae and pupae were identified and the remaining larvae were evaluated for presence of pathogens. Of the five parasitoids species recorded, the dipteran tachinid *Blepharipa pratensis* Meigen was the most frequently observed and caused up to 57.5% mortality. The viral pathogen *Ld*MNPV caused mortality up to 37.5%. We recorded the presence of the fungus *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin as well as the microsporidium *Nosema portugal* Maddox et Vavra, which was previously identified as *Nosema lymantriae* Weiser. The fungal entomopathogen *Entomophaga maimaiga* Humber, Shimazu et Soper was not collected from the host populations surveyed. We suggest that an inoculative introduction of this pathogen into Sardinia could potentially reduce the need to control gypsy moth populations with microbial pesticides, which are expensive to apply and are toxic to many non-target organisms.

Key words: gypsy moth, pathogens, *Nosema*, *Entomophaga maimaiga*.

Introduction

The gypsy moth, *Lymantria dispar* L. (Lepidoptera Erebidae), is one of the most serious defoliating forest pests in Europe, capable of producing widespread outbreaks in temperate Holarctic regions (Keena *et al*., 2008). Although this univoltine defoliator can feed on more than 300 host plant species, populations intermittently erupt to outbreak densities in tree stands dominated by *Quercus*, *Salix*, *Populus*, and *Larix* species and can cause extensive damage. Damage tends to be more extreme in xeric sites where host trees may be under greater environmental stress (Liebhold *et al*., 1995; Muzika and Liebhold, 2000; Gray *et al*., 2008).

In Sardinia, a region of Italy where the most suitable habitats are forests dominated by cork oak, *Quercus suber* L., gypsy moth has cyclical fluctuations of population density with peaks every 8-9 years. Major phases within these cycles are defined as latency (endemic, typically low level or "innocuous" phase), progradation (population increase; also "release"), culmination (outbreak) and retrogradation (post outbreak, declining population) (Campbell, 1981; Elkinton and Liebhold, 1990). In degenerating oak woodlands, such as cork oak forests of Sardinia where animal grazing is especially intense, gypsy moth infestations are more frequent, occurring every 5-6 years (Luciano and Prota, 1995; Luciano and Roversi, 2001). During outbreaks, gypsy moth defoliation causes reduction of plant growth, both height and diameter. Cork production is also reduced, with nearly 60% reduction when defoliation is complete, and 40% reduction when the defoliation is approximately 50% (Cambini, 1971). During one season, up to 60% of the 100.000 ha of Sardinian cork oak stands may be defoliated. Moreover, in regions such as Sardinia where tourism is important, defoliation spoils the landscape at the beginning of the summer season, discouraging tourism and resulting in additional economic loss.

Pest management organizations in South and Central European countries currently use the microbial insecticide *Bacillus thuringiensis* Berliner subsp. *kurstaki* (*Btk*) and more broad spectrum insecticides such as Dimilin® and Mimic® to manage damaging gypsy moth larval populations (Pilarska *et al*., 2007). In Sardinia, the gypsy moth population has been monitored annually since 1980 in 282 sites, primarily in the major cork, holm (*Quercus ilex* L.) and pubescent oak (*Quercus pubescens* Willd.) areas (Luciano, 1989). The population density is monitored in each site by counting egg masses on 40 oak trees, consisting of 10 adjacent trees distributed linearly in the four cardinal directions from a common central reference point (Fraval *et al.*, 1978). The gradation phases are evaluated yearly for each of the Sardinian forest districts by analyzing the data recorded in the network of sites (Cocco *et al.*, 2010) and the need for *Btk* treatment is determined. Aerially sprayed *Btk* has been used since 1990 in Sardinia with satisfactory results and was applied on more than 100.000 hectares of cork oak forests between 2001 and 2010 (Luciano and Lentini, 2012). Use of pesticides, particularly chemicals but including *Btk*, is controversial, however, because they are not highly specific to the target pests, and impacts on non-target insect species have po-

tentially negative effects on forest ecosystem biodiversity (Miller, 1990; Sample *et al*., 1996; Luciano and Lentini, 1999; Boulton, 2004; Boulton *et al*., 2007.).
Previous research on natural enemies of the Sardinian gypsy moth focused on parasitoids (Delrio *et al*., 1978; Luciano *et al*., 1982; Luciano and Prota, 1982b; 1984; 1986) but only a few studies on *L. dispar* larval pathogens in Sardinia have been conducted. In the late 1970s, Purrini and Skatulla (1978) surveyed for *L. dispar* multicapsid nuclear polyhedrosis virus (*Ld*MNPV) and microsporidia in cork oak stands in Orune (Nuoro Province), and during the same time period, a *Nosema* sp. microsporidium was reported from the same forest sites (Luciano *et al*., 1982).

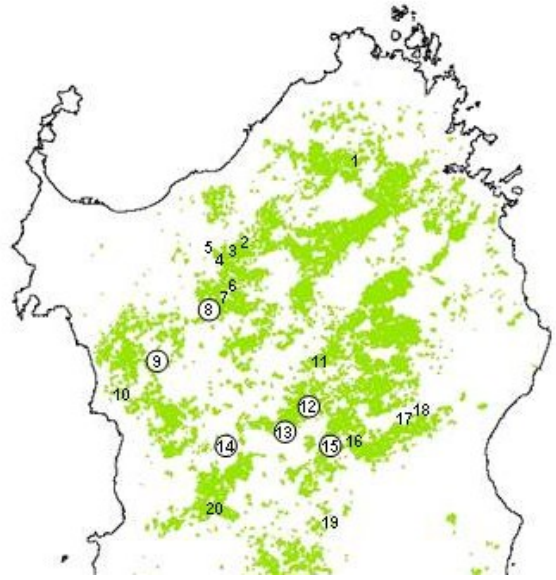

Figure 1. Map of North Central Sardinia with marked distribution of cork oak forests; numbers indicate the position of the study localities. Circled numbers refer to sites where gypsy moth larvae were collected in both years of study. Sites 17 and 18 are holm oak stands.

The aim of the present study was to document the current natural enemy complex of gypsy moth in Sardinia after 10 years of treatments with *Btk,* with a focus on determining whether the Asian fungal entomopathogen *Entomophaga maimaiga* Humber, Shimazu et Soper (Entomophtorales Entomophtoraceae) has spread to Italy, and specifically to Sardinia. This pathogen was originally described from Asian gypsy moth populations in Japan and now is well established in the USA. It was also recently established in Bulgaria where it was introduced at the end of 20th Century (Hajek, 1999; Pilarska *et al*., 2000; 2006; 2007; Georgiev *et al*., 2010). Because this pathogen is virulent and very host specific, and can cause epizootics in low density gypsy moth populations (reviewed by Solter and Hajek, 2009), there is considerable interest in its potential application for biological control (Hajek *et al*., 2000).

Materials and methods

Experimental sites

We monitored 15 sites in 2010 and 11 sites in 2011. Of these, 6 sites were monitored in both years. The cork oak stands were located in north central Sardinia, in the provinces of Nuoro (NU), Sassari (SS), Oristano (OR) and Olbia-Tempio (OT) (figure 1). Two sites were established in holm oak stands on Mount Ortobene (Nuoro I and II). Geographical coordinates were recorded for each site (table 1). Data recorded in the network of egg mass density monitoring sites showed that the gypsy moth population in Sardinian *Quercus* forests was generally in retrogradation or latency phase in 2010 and 2011, and no *Btk* treatments were necessary during that period.

Table 1. Localities, GPS coordinates and gypsy moth egg mass densities for Sardinian study sites in 2010 and 2011.

	Sites	Geographical coordinates	No. of egg masses on 40 cork oaks	
			2010	2011
1	Calangianus (OT)	40°56'13.62 N, 09°12'41.90 E	0	-
2	Chiaramonti I (SS)	40°45'30.91 N, 08°50'51.41 E	43	-
3	Chiaramonti II(SS)	40°43'50.88 N, 08°49'23.52 E	-	1
4	Chiaramonti III (SS)	40°42'39.42 N, 08°47'54.43 E	-	12
5	Chiaramonti IV (SS)	40°42'29.25 N, 08°48'01.34 E	21	-
6	Ardara I (SS)	40°37'58.22 N, 08°49'32.04 E	16	-
7	Ardara II (SS)	40°36'43.02 N, 08°48'31.26 E	-	12
8	Siligo (SS)	40°35'56.26 N, 08°46'47.26 E	0	3
9	Giave (SS)	40°29'22.58 N, 08°37'45.17 E	17	8
10	Montresta (OR)	40°23'28.85 N, 08°28'48.36 E	-	18
11	Bultei (SS)	40°27'36.31 N, 09°06'52.73 E	-	15
12	Bottidda (SS)	40°22'17.69 N, 09°03'18.04 E	6	2
13	Illorai (SS)	40°19'11.99 N, 09°01'27.01 E	0	0
14	Bortigali (NU)	40°16'35.71 N, 08°51'30.37 E	0	0
15	Oniferi (NU)	40°19'00.64 N, 09°10'35.31 E	18	7
16	Orani (NU)	40°18'17.75 N, 09°12'26.57 E	10	-
17	Nuoro I (NU)	40°19'31.00 N, 09°21'29.00 E	5	-
18	Nuoro II (NU)	40°19'28.00 N, 09°21'33.00 E	8	-
19	Ovodda (NU)	40°05'51.70 N, 09°10'26.00 E	10	-
20	Abbasanta (OR)	40°08'12.39 N, 08°47'50.31 E	4	-

Larval sampling and rearing

Forty 3rd to 5th instar gypsy moth larvae were collected per site. One collection was made per site each year, from May 21 to June 17, 2010, and from May 26 to June 1, 2011. Larvae were collected by beating randomly selected branches of oak trees onto an 80 cm^2 white sheet. A total of 600 larvae were collected in 2010 and 440 in 2011.

Larvae were reared individually on commercial meridic diet (Southland Products, Inc.) (Bell *et al.*, 1981; Solari *et al.*, 2002) in 100-ml cups at 23 ± 2 ºC, 16 h light/8 h dark until they died or pupated. They were checked daily for mortality and, if not killed by parasitoids, were held individually for 10 days post-mortem in a sterile Petri dish with moist filter paper to allow formation of mycelia or resting spores, and then were stored at 4 ºC in a household refrigerator.

Pathogen and parasitoid collection and analysis

Each dead larva was individually dissected and observed under light microscopy at magnification 100× and 400× for the presence of pathogens or parasitoids. Larvae that pupated were held at the same temperature and light conditions to evaluate the presence of parasitoids. We identified the parasitoids using published keys (Sabrosky and Reardon, 1976; Marsh, 1979; Simons *et al*., 1979).

When microsporidian spores were observed, smears were made on glass slides and stained with Giemsa. For molecular characterization, microsporidian DNA was sequenced using published PCR techniques, briefly described as follows. DNA was extracted from the spores using the Chelex method (Cordes *et al*., 2012). The ITS region of the DNA was amplified by PCR using primers HG4f (Gatehouse and Malone, 1998) and 580r (Baker *et al*., 1994). Platinum® Taq (Invitrogen) was used for PCR following the manufacturer's instructions using a standard 3-step PCR with an annealing temperature of 55 °C. The PCR product was sequenced using the same primers. The sequence was compared with known NBCI GenBank SSU-rDNA sequences of *Nosema* isolates from gypsy moth using ClustalX.

The presence of filamentous fungi was determined using the Biolog System (Biolog Inc., Hayward, CA, USA) and Filamentous Fungi Microplate. The Biolog System with GEN III Microplate was used to evaluate bacteria we detected in the larvae when we could not ascertain the cause of death.

Statistical analysis

The data (tables 2, 3 and 4) were analyzed by one-way ANOVA using SPSS software (IBM) to determine differences in mortality factors affecting gypsy moth larvae among sites and years.

Table 2. Percent mortality of gypsy moth larvae collected in study sites in Sardinia, Italy in 2010 and 2011. N = 40 larvae collected in each site.

Site	Year	Mortality			Total mortality
		Parasitoids	Pathogens	Unknown	
		%	%	%	%
Calangianus	2010	10.0	27.5	35.0	72.5
Chiaramonti I	2010	27.5	2.5	15.0	45.0
Chiaramonti II	2011	60.0	2.5	20.0	82.5
Chiaramonti III	2011	57.5	0	17.5	75.0
Chiaramonti IV	2010	50.0	2.5	17.5	70.0
Ardara I	2010	32.5	0	22.5	55.0
Ardara II	2011	35.0	2.5	12.5	50.0
Siligo	2010	52.5	10.0	27.5	90.0
	2011	62.5	7.5	5.0	75.0
Giave	2010	7.5	5.0	22.5	35.0
	2011	62.5	2.5	15.0	80.0
Montresta	2011	40.0	0	27.5	67.5
Bultei	2011	12.5	0	37.5	50.0
Bottidda	2010	10.0	2.5	72.5	85.0
	2011	15.0	2.5	12.5	30.0
Illorai	2010	0	22.5	37.5	60.0
	2011	25.0	2.5	65.0	92.5
Bortigali	2010	30.0	7.5	40.0	77.5
	2011	2.5	2.5	25.0	30.0
Oniferi	2010	0	12.5	82.5	95.0
	2011	5.0	2.5	10.0	17.5
Orani	2010	25.0	40.0	30.0	95.0
Nuoro I	2010	85.0	10.0	5.0	100.0
Nuoro II	2010	75.0	5.0	7.5	87.5
Ovodda	2010	32.5	37.5	7.5	77.5
Abbasanta	2010	7.5	5.0	80.0	92.5
Mean ± SE		31.63 ± 4.86	8.26 ± 2.18	28.84 ± 4.43	68.75 ± 4.54

Results and discussion

Total mortality rates of gypsy moth larvae collected from the Sardinian sites were variable among sites, ranging from 17.5% recorded in larvae collected in Oniferi in 2011 to 100% in Nuoro I in 2010 (table 2). Mortality was high in three sites in 2010 (85% in Bottidda, 92.5% in Abbasanta, 95% in Oniferi) and in one site in 2011 (92.5% in Illorai) but, unfortunately, we were not able to determine the mortality factor(s). Transfer of the larvae to the laboratory and transition to meridic diet could have resulted in stress and the high mortality we observed. Opportunistic bacteria, including *Enterococcus mundtii* and *Enterococcus casseliflavus* were identified in some of the dead larvae using Biolog.

Statistical analysis

Because of the small number of samples, collection sites in each province were grouped to represent four collection areas. There was no significant difference in overall mortality among sites or in total mortality caused by parasitoids or unknown factors, but total mortality due to pathogens was significantly different among areas (P = 0.013, collection year used as cofactor in the one-way ANOVA). Overall mortality and total mortality due to parasitoids and unknown factors is not significantly different between years, but total mortality due to pathogens was significantly different between years (P = 0.034, collection site as a cofactor). There was no significant difference in host mortality caused by each parasitoid species in different years or collection areas. Only mortality due to *Nosema portugal* Maddox et Vavra (Microsporidia Nosematidae) was significantly different among areas (P<0.01). Although a two-season collection is not sufficient to draw conclusions about correlations between pathogen and collection year, ANOVA analysis showed *Ld*MNPV mortality to be significantly higher in 2010 (P = 0.021), and found no difference in *N. portugal* mortality between collection years (P = 0.092).

Mortality due to parasitoids

Mortality due to parasitization by Diptera and Hymenoptera is presented in table 3. The data refer to ap-

Table 3. Parasitoids and percent parasitism of each species in gypsy moth larvae collected in Sardinia, Italy (parasitism of pupated larvae is reported in brackets).

		Percentage of parasitized larvae							
Site	Year	*Apanteles melanoscelus*	*Phobocampe unicincta*	*Parasetigena silvestris*	*Blepharipa pratensis*	*Exorista larvarum*	*P. silvestris B. pratensis*	*E. larvarum B. pratensis*	Total parasitism
		%	%	%	%	%	%	%	%
Calangianus	2010	5.0	5.0	0	0	0	0	0	10.0
Chiaramonti I	2010	2.5	0	0	25.0 (22.5)	0	0	0	27.5
Chiaramonti II	2011	0	0	2.5	57.5 (42.5)	0	0	0	60.0
Chiaramonti III	2011	0	0	5	50.0 (45.0)	0	2.5	0	57.5
Chiaramonti IV	2010	0	0	2.5	47.5 (37.5)	0	0	0	50.0
Ardara I	2010	15.0	0	0	17.5 (17.5)	0	0	0	32.5
Ardara II	2011	0	0	20.0 (2.5)	12.5 (10.0)	0	2.5	0	35.0
Siligo	2010	0	0	27.5	20.0 (5.0)	0	5	0	52.5
	2011	0	0	42.5 (2.5)	17.5 (17.5)	0	2.5	0	62.5
Giave	2010	2.5	0	0	5.0 (5.0)	0	0	0	7.5
	2011	0	0	12.5	50.0 (40.0)	0	0	0	62.5
Montresta	2011	0	0	7.5	32.5 (27.5)	0	0	0	40.0
Bultei	2011	0	0	0	12.5 (10.0)	0	0	0	12.5
Bottidda	2010	5.0	0	0	2.5 (2.5)	2.5 (2.5)	0	0	10.0
	2011	0	0	12.5	2.5 (2.5)	0	0	0	15.0
Illorai	2010	0	0	0	0	0	0	0	0
	2011	0	0	25.0	0	0	0	0	25.0
Bortigali	2010	0	0	2.5	22.5 (15.0)	0	5	0	30.0
	2011	0	0	0	2.5 (2.5)	0	0	0	2.5
Oniferi	2010	0	0	0	0	0	0	0	0
	2011	0	0	0	5.0 (2.5)	0	0	0	5.0
Orani	2010	0	0	0	20.0 (17.5)	2.5	0	2.5	25.0
Nuoro I	2010	0	0	40.0	22.5 (17.5)	0	22.5	0	85.0
Nuoro II	2010	0	0	25.0	40.0 (27.5)	0	10.0	0	75.0
Ovodda	2010	2.5	0	0	25.0 (25.0)	5.0	0	0	32.5
Abbasanta	2010	2.5	0	2.5	2.5	0	0	0	7.5
Mean ± SE		1.34 ± 0.62	0.19 ± 0.19	8.75 ± 2.55	18.94 ± 3.49	0.38 ± 0.22	1.92 ± 0.94	0.09 ± 0.09	31.63 ± 4.86

parent parasitism because emergence of parasitoids is, in part, affected by death of the hosts caused by diseases and other stresses (Herard *et al.*, 1979; Luciano and Prota, 1986). We identified the following parasitoids during the two years of study: *Apanteles melanoscelus* (Ratzeburg) (= *Cotesia melanoscelus*) (Hymenoptera Braconidae), *Phobocampe unicincta* Gravenhorst (Hymenoptera Ichneumonidae), *Parasetigena silvestris* Robineau-Desvoidy, *Blepharipa pratensis* Meigen and *Exorista larvarum* L. (Diptera Tachinidae). Overall parasitoid mortality ranged from 0% in the Illorai and Oniferi sites in 2010 to 85% observed in Nuoro I in 2010. Parasitism rates were also particularly high in samples collected in the holm oak stand in Nuoro II (75%) and in the Giave and Siligo cork oak stands in 2011 (62.5%). As documented in previous studies (Luciano and Prota, 1981), the oligophagous dipteran tachinids *P. silvestris* and *B. pratensis* were the most commonly recovered larval parasitoids of gypsy moth in Sardinia, especially during a retrogradation phase of the population.

The univoltine tachinid *B. pratensis,* which oviposits microtype eggs on foliage (Ticehurst *et al.*, 1978), has a cycle that is synchronized with the gypsy moth host larvae and causes greatest mortality when host population densities are in retrogradation (Luciano and Prota, 1986). *B. pratensis* parasitism was greatest in Chiaramonti II site, where mortality was 57.5% (table 3), confirming reports of previous studies conducted in Sardinia (Luciano and Prota, 1986) and in other European areas (Sisojevic, 1970).

P. silvestris is an effective univoltine tachinid that oviposits its eggs on the host exoskeleton posterior to the head capsule (Ticehurst *et al.*, 1978). In previous studies in Sardinia, it was recovered from only a few specimens and always when the gypsy moth population density was declining or in latency (Luciano and Prota, 1986). In our 2-year study, remarkable numbers of this tachinid were recorded. Mortality was 42.5% at the Siligo site in 2011, similar to the results of studies conducted in central Europe and former-Yugoslavia (Sisojevic, 1970; Fuester *et al*., 1981). In addition, in Nuoro I, we observed 40% mortality caused by *P. silvestris*, to which can be added 22.5% mortality due to multiple parasitism by *P. silvestris* and *B. pratensis* (table 3).

Superparasitism was observed in seven sites in both years of study. In 2010, 25 larvae were superparasitized (4.16% of the 600 collected larvae), 13 by *B. pratensis*, 11 by *P. silvestris* and 1 by *E. larvarum*. In 2011, 5 larvae were superparasitized by *B. pratensis* and 7 by *P. silvestris* (a total of 2.72% of the 440 collected larvae). Two individual larvae of *B. pratensis* were always obtained from each superparasitized host, as were two *E. larvarum* larvae*,* while four larvae of *P. silvestris* were recorded. These superparasitism rates were 3× lower than previous reports from Sardinian gypsy moth populations in progradation and culmination phases (Luciano and Prota, 1982a), and about 4× or 5× lower than those recorded for *B. pratensis* and *P. silvestris* in Pennsylvania in 1977 during a retrogradation phase of the gypsy moth population (Ticehurst *et al*., 1978). These values may be underestimated due to early host mortality caused by pathogens or other causes, which in 9 sites was approximately 50% (table 2). The greatest rate of superparasitism (12.5%) was recorded in Nuoro I and II sites where total parasitoid emergence was higher (to 85%) and mortality due to pathogens and unknown causes reached nearly 15% (table 2).

The parasitism rates of other parasitoid species recorded in this study were much lower, although the bivoltine *A. melanoscelus* caused 15% mortality in the Ardara I site, corroborating data previously recorded in Sardinia (Luciano and Prota, 1986) and in some parts of USA, where the highest levels of mortality were associated with sparse host densities, in the retrogradation phase (Ticehurst *et al*., 1978).

Mortality due to pathogens

Mortality caused by pathogens also varied, ranging from 0% in Ardara I to 40% in Orani in 2010. In 2011, the percentage of dead larvae that contained observable pathogens was lower than in 2010, and ranged from 0% (three locations) to 7.5% (Siligo) (table 2).

The fungus *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Deuteromycotina Hyphomycetes) was reported in 8 sites in 2010 and 7 sites in 2011, with prevalence 0-7.5%, except for the Illorai site where, during the 2010 season, the prevalence was 17.5% (table 4).

Prevalence of *Ld*MNPV was relatively high only in the Ovodda and Orani sites where we recorded a prevalence of 32.5% and 37.5%, respectively. *Ld*MNPV was not detected in any larvae collected in 2011 (table 4).

The microsporidium *N. portugal* was observed in 8 of 15 sites in 2010 and 1 site in the 2011. Prevalence ranged from 0 to 2.5% except in the Calangianus site where we recorded a prevalence of 27.5% (table 4). The SSU-rDNA sequence of this microsporidium was identical with that of *N. portugal* (Maddox *et al*., 1999). Described from gypsy moth larvae collected in Portugal (Jeffords *et al*., 1987), *N. portugal* is closely related to *Nosema lymantriae* Weiser described from the East Central European region (Maddox *et al*., 1999) and may be a genetic variation of that species (L. Solter and W-F. Huang, unpublished molecular data).

Purrini and Skatulla (1978) reported that in two years of study, 1976 and 1977, conducted in municipality of Orune (Nuoro Province), the prevalence of the microsporidium that they identified as *N. lymantriae* was very high, especially in 1977 when the prevalence was about 60%. They also reported the prevalence of *Ld*MNPV in the same area to be 1.2-10%.

Entomophaga maimaiga

No gypsy moth larvae collected and reared during the 2010 and 2011 seasons were infected with *E. maimaiga* and we suggest that preliminary studies be conducted before an introduction program is initiated. The complex of gypsy moth natural enemies in Sardinia has a significant impact when the host population density is in culmination and retrogradation phases but effectiveness is reduced during the years of latency and progradation (Luciano and Prota, 1981; 1986). In many sites where it has been introduced, *E. maimaiga* appears to be contributing to a lower gypsy moth population density

Table 4. Pathogens and percent mortality caused by each species in gypsy moth larvae collected in Sardinia, Italy.

Site	Year	*Ld*MNPV %	*Beauveria bassiana* %	*Nosema portugal* %	Total pathogen mortality %
Calangianus	2010	0	0	27.5	27.5
Chiaramonti I	2010	0	0	2.5	2.5
Chiaramonti II	2011	0	2.5	0	2.5
Chiaramonti III	2011	0	0	0	0
Chiaramonti IV	2010	0	2.5	0	2.5
Ardara I	2010	0	0	0	0
Ardara II	2011	0	0	2.5	2.5
Siligo	2010	10.0	0	0	10.0
	2011	0	7.5	0	7.5
Giave	2010	0	2.5	2.5	5.0
	2011	0	2.5	0	2.5
Montresta	2011	0	0	0	0
Bultei	2011	0	0	0	0
Bottidda	2010	0	2.5	0	2.5
	2011	0	2.5	0	2.5
Illorai	2010	2.5	17.5	2.5	22.5
	2011	0	2.5	0	2.5
Bortigali	2010	0	7.5	0	7.5
	2011	0	2.5	0	2.5
Oniferi	2010	5.0	7.5	0	12.5
	2011	0	2.5	0	2.5
Orani	2010	37.5	0	2.5	40.0
Nuoro I	2010	7.5	0	2.5	10.0
Nuoro II	2010	2.5	0	2.5	5.0
Ovodda	2010	32.5	2.5	2.5	37.5
Abbasanta	2010	2.5	2.5	0	5.0
Mean ± SE		3.84 ± 1.87	2.59 ± 0.75	1.82 ± 1.05	8.26 ± 2.18

(Elkinton *et al.*, 1991; Weseloh, 2003) because epizootics are not host density dependent and occur even when host density is low (Hajek, 1999). A successful introduction of *E. maimaiga* into Sardinia would potentially reduce the need for control with microbial and chemical pesticides.

Studies in the USA showed that *E. maimaiga* is highly host specific and, in the field, has been found to cause mortality in only a few individuals of three closely related lepidopteran species (reviewed by Solter and Hajek, 2009). Similar studies should be conducted in Sardinia to confirm that *E. maimaiga* is not infective to native phyllophagus Lepidoptera. Several of these native species are parasitized by the same complex of parasitoids that parasitize the gypsy moth (Delrio *et al*., 1983; 1988; Delrio and Luciano, 1985) and may, therefore, act as reservoirs. *E. maimaiga* could potentially augment control provided by hymenopterous and dipteran parasitoids that emerge from these species. Little is known about the effects of the fungus on other gypsy moth pathogens, but *E. maimaiga* provides a case for risk-benefit analysis due to its ability to cause significant host mortality at both low and high density population levels.

After the introduction and establishment of *E. maimaiga* in Bulgaria, the fungus expanded its range and has recently been recovered from gypsy moth populations in Serbia (Tabaković-Tošić *et al.*, 2012), the European part of Turkey (Georgiev *et al.*, 2012) and Georgia (Kereselidze *et al.*, 2011), demonstrating a remarkable ability to spread. Understanding the dynamics of *E. maimaiga* as it spreads to new environments and its potential interactions in different gypsy moth parasite and pathogen systems will allow foresters and growers to make better informed decisions about treatment.

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