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Nosema meligethi I. & R. (Microsporida) in Populations of *Meligethes* spp. in Europe

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Samples of rape blossom beetles, Meligethes *spp., from 12 countries in Eastern and Western Europe were inspected for the occurrence of* Nosema meligethi *I. & R. infection. From most countries about a dozen samples and a few hundred beetles were inspected. A total of 13 910 individual beetles were checked under a compound microscope, and of these, 561 were found to be infected (4.03%). Despite careful examination, infection was not detectable in samples from the UK (1039 beetles inspected), Germany (694), Sweden (489), or Switzerland (280). One infected beetle was found among 444 beetles inspected from Austria, and one from Denmark (1/85). In contrast, the parasite was found rather regularly in samples from Finland and from Eastern European countries. No obvious reason for this pattern of infection is known, but pesticide usage at or close to the sampling sites may play a role, because most samples from which* Nosema *was detected originated from areas where few pesticides are used. Spore size measurements were made from seven* N. meligethi *isolates, and they appeared relatively uniform. It appears that artificial spread of the disease might be worthwhile in an effort towards the integrated control of* M. aeneus *F.*

Keywords: *microsporidia, protozoan pathogens,* Meligethes aeneus, Meligethes viridescens, Nosema meligethi

INTRODUCTION

In a recent paper (Hokkanen & Lipa 1991a), we reported on the occurrence of the parasitic protozoan *Nosema meligethi* I. & R. in Finland. Surprisingly, this pathogen occurred only in a few populations of the rape blossom beetle (*Meligethes aeneus* F.), and practically only in regions where rape is not intensively grown, while in the main growing areas the infection level was extremely low or the parasite was not present. Other *Meligethes* species were not collected in that survey.

N. meligethi was originally discovered and described in the Leningrad region (now St Petersburg), where it is considered to play an important role in the winter mortality of

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M. aeneus (Issi & Raditscheva 1979). Therefore, its introduction and artificial dissemination to regions where it is not present—or where the infection level is very low—could play an important part in establishing pest management programmes for this pest (Hokkanen *et al.*, 1988; Hokkanen 1989).

To verify the pattern of occurrence and to assess the European distribution of *N. meligethi*, we requested members of the IOBC/WPRS Working Group on 'Integrated Control in Oilseed Rape' to supply us with samples of *Meligethes* spp. from various countries. In addition, we also made collections during 1989–90 of *M. aeneus* from various regions of Europe.

Here we report the results of our survey for the presence of *N. meligethi* in *Meligethes* spp. in Europe during 1989–90. In two other papers we report on safety of *N. meligethi* with respect to beneficial insects (Lipa & Hokkanen, in press), and on a new parasitic protozoan *Haplosporidium meligethi*, recorded in *M. aeneus* (Lipa & Hokkanen, 1991).

MATERIALS AND METHODS

In response to our request, samples of overwintered ('old') and/or summer generations ('new') of *M. aeneus* were received from Austria, Denmark, UK, Germany, Norway, Sweden and Switzerland. In addition, specimens of the overwintered 1989 generation of *M. viridescens* F. were received from Switzerland. During 1989 and 1990 we additionally collected specimens of *M. aeneus* in Czechoslovakia, Denmark, Finland, Germany, Hungary, Poland and the (then) Soviet Union.

Sampled Localities

Austria.
Overwintered (1989) generations: Durnkrut 11.4.90; Marchegg 11.4.90; Korneuburg 18.4.90; Neulengbach 18.4.90; Petzenkirchen 18.4.90; Halbturn 23.4.90; Sebersdorf 3.5.90.

Czechoslovakia.
Summer (1989) generation: Ceske Budejovice 26.9.89.

Denmark.
Overwintered (1989) generation: Copenhagen 25.4.90, 30.4.90; Lyngby 27.4.90; Mikkelborg 29.4.90; Rusland 29.4.90; Gilleje 29.4.90. Summer (1990) generation: Arhus 25.7.90.

Finland.
A total of 10 066 beetles were examined during 1989-1990, collected from over 100 localities in southern and central Finland (Hokkanen & Lipa, in press).

Germany.
Overwintered (1989) generation: Neuengeseke 3.7.90; Oberense 3.7.90; Kappeln 7.7.90; Renmark 8.7.90; Varel Tange 9.7.90; Bad Zwischen-Wehnen 9.7.90; Fanlueck 11.7.90; Rostock 12.7.90; Gross Potrems 15.7.90; Mittergars a. Inn 16.7.90; Hassleberg 18.7.90; Gottingen-Northeim 18.7.90; Hannover-Wuelferode 18.7.90; Travemünde 18.7.90; Kiel-Levensan 25.7.90; Kiel-Nettelsee 16.7.90; Trappenkamp 27.9.90; Kiel Surendorf 28.7.90; Travemünde 29.7.90.

Hungary.
Summer (1989) generation: Budapest 22.9.89.

Norway.
Overwintered (1989) generation: Grimstad 10.7.90; Summer (1990) generation: Stavanger 3.8.90; Kapp 8.8.90; Ås 21.8.90.

Poland.
Summer (1989) generation: Poznan 17.9.89, 22.9.89, 2.10.90; Winnagora 25.9.89. Overwintered (1989) generation: Minikowo 4.4.90, Poznan 4.4.90, 31.5.90. Summer (1990) generation: Poznan 12.6.90, 18.6.90, 20.6.90, 22.6.90; Konarzewo 22.6.90; Winnogora 25.6.90, 1.10.90; Edwardowo 12.10.90.

Soviet Union.
Summer (1989) generation: Gancevivhi-Brest 1.8.89; Priluki-Minsk 15.8.89. Overwintered (1989) generation: Priluki-Minsk 11.4.90.

Sweden.
Overwintered (1989) generation: Alnarp 5.6.90. Summer (1990) generation: Alnarp 20.7.90; Björnstorp 20.7.90; Vasaholm 20.7.90; Horjel 20.7.90; Ramsåsa 20.7.90; Flyinge 20.7.90; Fulltofta 20.7.90; Uppsala 30.8.90.

Switzerland.
Summer (1990) generation: Vitters 30.5.90; Trimmis 305.90; Bad Ragaz 30.5.90; Buchberg 15.6.90; Thayngen 15.6.90; Flaach 15.6.90. In addition, samples of *Meligethes viridescens* (F.) were obtained from Flaach 15.6.90; Buchberg 15.6.90; Thayngen 15.6.90.

UK.
Overwintered (1989) generation: Coven WM 19.4.90; Clifton Campville (S) 19.4.90; Rosemaund (H) 19.4.90; Burton-on-Trent (S) 19.4.90; Wrottesley (S) 19.4.90; Checkenden (0) 16.5.90; Batford (H) 23.5.90; Hemel Hempstead (H) 25.5.90; Boxworth (C) 28.5.90; High Mowthorpe (NY) 28.5.90. Summer (1990) generation: High Mowthorpe (NY) 19.7.90; Rosemaund (H) 5.7.90; Wrottesley (S) 5.7.90; Tettenhall (W) 7.7.90; Schifnal (Sh) 11.7.90; Gleadthorpe (N) 12.7.90; Wolverhampton (WM) 12.7.90; Albrighton (WM) 14.7.90; Whibourne (W) 15.7.90; Wolverhampton (WM) 16.7.90; High Mowthorpe (NY) 19.7.90; Selby (NY) 20.7.90; Batford (H) 27.9.90; Hemel Hempstead (H) 27.7.90; Harpenden (H) 28.7.90. (WM = West Midlands; S = Staffordshire; H = Herefordshire; O = Oxfordshire; C = Cambridgeshire; NY = North Yorkshire; SH = Shropshire; N = Nottinghamshire; W = Worcestershire).

Microscopical Techniques
Specimens supplied for our studies were killed in a freezer at about −20°C, and air-dried at room temperature before mailing, to avoid growth of saprophytic fungi. On arrival, the beetles were microscopically examined according to the method used in our earlier studies (Hokkanen & Lipa, 1991a, b).

Beetles were placed individually into a small drop of water on a microscope slide and their bodies were crushed with a rounded glass rod, in order to release the spores of *N. meligethi* from infected tissues. The slides were then examined with a compound microscope at magnifications from 160 × to 640 ×. The spores of *N. meligethi* are easily recognizable due to their uniform size and refractile wall, as compared with the frequently present yeast cells, which are variable in size and shape, and are often observed budding. In doubtful cases, or in the case of a low-level infection—when only a few spores were present in the microscope field—the water-mounted preparation was dried, fixed in methanol for 2 min, and stained with 0.1% Giemsa solution for 3 to 12 h. On the stained preparations the spores of *N. meligethi* have a very characteristic appearance (deep-blue stained sporoplasm surrounded by unstained area) and they can be easily noticed and identified from yeast cells or fungal conidiospores, which are uniformly and evenly stained.

In order to find out whether there were morphological differences between *N. meligethi* isolates from various European populations of *M. aeneus*, the dimensions of 50 spores of several isolates were microscopically measured.

RESULTS

1. Distribution of *N. meligethi* in Europe

In Table 1 we summarize the results of the microscopic examination of *M. aeneus* and *M. viridescens* adults, conducted during 1989–90. *N. meligethi* was found only in some populations of *M. aeneus* in Austria, Czechoslovakia, Denmark, Finland, Hungary, Poland and the Soviet Union. In spite of examining a relatively large number of samples and specimens from the UK, Germany, Norway, Sweden and Switzerland, infection by *N. meligethi* was not recorded either in overwintered adults or summer generation beetles.

In a broad review conducted in Finland we found that in the areas without intensive rape cultivation the pathogen was observed in 56.1% of *Meligethes* samples, while in areas where rape was cultivated *N. meligethi* was found only in 15.4% of the samples (Hokkanen & Lipa, in press). Infection levels up to 25% could be found outside the rape growing area, but only up to 10% within them (Hokkanen & Lipa, in press).

TABLE 1. Occurrence of *Nosema meligethi* in *Meligethes* spp. populations in Europe in 1989–1990

Country and generation	Number of samples	Number of beetles examined	Number of infected beetles	Per cent infection
Austria				
OV-1989	6	444	1	0.2
Czechoslovakia				
SU-1989	1	13	3	23.0
Denmark				
OV-1989	6	85	1	1.2
Finland				
SU-1989	50	1 575	20	1.3
OV-1989	69	3 812	128	3.4
SU-1990	100	4 679	361	7.7
Germany				
SU-1990	16	694	0	0
Hungary				
SU-1989	1	10	2	20.0
Norway				
SU-1990	4	180	0	0
Poland				
SU-1989	4	112	17	15.2
OV-1989	3	126	4	3.2
SU-1990	8	298	13	4.4
Soviet Union				
SU-1989	2	58	11	18.9
OV-1989	1	16	0	0
Sweden				
OV-1989	1	85	0	0
SU-1990	7	404	0	0
Switzerland				
SU-1990	6	172	0	0
SU-1990[a]	3	108	0	0
UK				
OV-1989	9	480	0	0
SU-1990	14	559	0	0
Total	311	13 910	561	4.03

SU = new generation beetles of the given calendar year.
OV = overwintered beetles of the 1989 generation.
[a]Data refer to *Meligethes viridescens*.

2. Spore Dimensions of *N. meligethi*

In Table 2 we give the data characterizing the length and width of spores of *N. meligethi* from some localities, visualized in Figure 1. Although the mean size values show some differences between various isolates, the data suggest that all isolates form a relatively uniform group. Only the *N. meligethi* isolate from Hartola, Finland, had spores somewhat longer and wider than the other isolates. The biological significance of this difference requires further studies.

TABLE 2. Dimensions of *Nosema meligethi* spores from different locations in Europe

Location	Spore length (μm)	S.D.	Spore width (μm)	S.D.
SU 1	3.89	0.38	2.33	0.14
SU 2	3.68	0.43	2.37	0.17
SF*	4.17	0.41	2.48	0.13
HU	3.90	0.27	2.32	0.13
PL	3.91	0.40	2.36	0.12
CS	3.91	0.33	2.30	0.15
A	3.93	0.36	2.39	0.11

Origins of the samples: SU1 = Soviet Union, Leningrad; SU2 = Soviet Union, Minsk; SF = Finland, Hartola; HU = Hungary, Budapest; PL = Poland, Poznan; CS = Czechoslovakia, Ceske Budejovice; A = Austria, Sebersdorf. N = number of measurements; CI = confidence interval. All measurements were carried out by the same person, at the same time and with the same equipment. For each sample 50 spores were measured.
*Data concerning the SF sample presumably refer to a sample of mixed spores of *N. meligethi* and *Haplosporidium meligethi*.

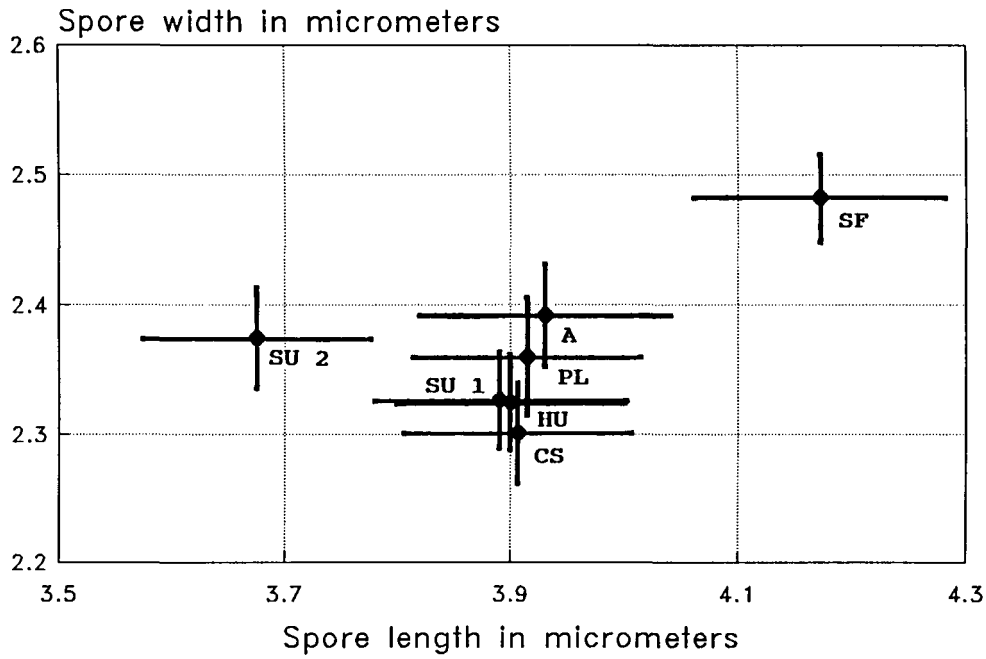

FIGURE 1. Spore dimensions for *Nosema meligethi* from different geographical locations. The data and symbols are from Table 2. The lines represent 95% confidence intervals of the measurements. For each location N = 50. Note: the data for SF refer to a mixed sample of *N. meligethi* and *H. meligethi*.

DISCUSSION

According to the information supplied to us by the scientists providing the insect samples, the adults of *Meligethes* spp. were always collected in regions with intensive rape cultivation nearby. This may partially explain the absence of *N. meligethi* infection in those samples, as was the case observed in Finland between rape-growing versus non-growing areas (Hokkanen & Lipa, in press). The possible reasons for the uneven distribution of *N. meligethi* in Finland are discussed in detail by Hokkanen & Lipa (in press), and they can be due to the principles of spread of the parasite, or due to the effect of use of insecticides in regions with intensive rape cultivation. In fact, as was first demonstrated by Rosicky (1951) and Rosicky & Weiser (1951) in the case of DDT interaction with *Nosema otiorrhynchi* Weiser infection in *Otiorrhynchus ligustici* L. (see Bell & McLaughlin 1970 and Brooks 1988 for further references), insects infected with microsporidians are more susceptible to insecticides than healthy ones. This phenomenon evidently leads to significant, or virtually complete elimination of infected *M. aeneus* adults from populations in regions with intensive use of insecticides.

The strikingly higher infection levels of *N. meligethi* observed in populations of *M. aeneus* in Czechoslovakia, Hungary, Poland and the Soviet Union area, can easily be explained by the origin of the samples, which were collected by us on experimental plots of research institutes (Hungary and Soviet Union), or on wasteland (Czechoslovakia and Poland). In all these cases, *M. aeneus* populations were not treated with insecticides, and evidently this situation allowed *N. meligethi* to build up relatively high infection levels. The same factors may govern the pattern of distribution and occurrence of *Haplosporidium meligethi*, which was observed in populations of *M. aeneus* in Byelorussia, Finland and Poland (Lipa & Hokkanen, 1991).

The full potential of *N. meligethi* in the management of *M. aeneus* populations is currently under intensive investigation. However, it seems quite obvious already that the introduction of *N. meligethi* to *M. aeneus* populations in countries or regions, in which this parasite is not present, could contribute to the natural mortality of this important rape pest without negative impact on the beneficial insects (Lipa & Hokkanen, in press). In addition, such an attempt would be justified by several examples of the role of microsporidians in biological control, or in the population dynamics of noxious insects (Anderson, 1982; Brooks, 1988). The best possibilities for a success in such an introduction would appear to be at locations where an integrated control programme for rape pests has already minimized the use of chemical insecticides.

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