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Occurrence and dynamics of *Nosema meligethi* (Microsporida) in populations of *Meligethes aeneus* (Coleoptera, Nitidulidae) in Finland

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A total of 221 samples of the beetle *Meligethes aeneus* Fabricius were collected from southern and central Finland in 1989–90, and 10 152 beetles were individually examined to determine the occurrence of the microsporidian disease *Nosema meligethi* Issi & Raditsheva. The dynamics of this disease was followed at seven sites throughout the season, and its ecological aspects were studied at one locality, Hartola. The level of *N. meligethi* infection steadily increased in *M. aeneus* populations during the season, with horizontal transmission appearing to take place in flowerheads, where the beetles aggregate to feed. Infected beetles migrated to overwintering sites earlier than did healthy beetles, and just prior to overwintering, infected beetles were lighter in weight than healthy ones. This fact may drastically affect the overwintering survival of infected beetles.

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1. Introduction

Meligethes aeneus, the rape blossom beetle, ranks as the number one pest of oilseed crucifers in Europe, occasionally accompanied by several other locally important insect pests such as the weevils *Ceutorrhynchus* spp., the flea beetles *Psylliodes chrysocephala* Linnaeus and *Phyllotreta* spp., or the gall midge *Dasyneura brassicae* (Winnertz) (Bromand 1990). Consequently, any natural antagonists of this pest are of great interest as potentially valuable biological control agents. The recent discovery of *Nosema meligethi*, a microsporidian disease of *Meligethes* (Issi & Raditscheva 1979), has led to studies of its distribution, life-cycle, and host range (Hokkanen & Lipa 1991, Lipa & Hokkanen 1991, 1992a, b).

Despite extensive surveys in different parts of Europe, *N. meligethi* has been recorded only from eastern Europe and Finland; one infected beetle has also been recorded from Denmark and one from Austria (Lipa & Hokkanen 1992a). In Finland *N. meligethi* was found almost exclusively in *M. aeneus* collected from non-rape-growing areas, which inspired speculations about *Nosema*'s noncompatibility with the regular use of insecticides on rape (Hokkanen et al. 1988, Hokkanen 1989, Hokkanen & Lipa 1991). Whether this blossom beetle disease is endemic in Finland, or possibly is at present spreading from the east, was not clear in our earlier study; for this assessment we therefore collected many more samples, particularly from the areas shown to be critical in our preliminary study (Hokkanen & Lipa 1991).

The aim of this paper is to report on 1) distribution and the level of infection of *Nosema meligethi* in the populations of *M. aeneus* in Finland, and 2) seasonal dynamics and ecological features of *N. meligethi*.

2. Material and methods

2.1. General

Samples of adult *M. aeneus* were collected from 145 different locations in Finland, covering the southern part of the country, including practically all the growing area of oilseed crucifers (Fig. 1). The westernmost sample was from the county of Houtskari, the easternmost from Värtsilä, the southernmost from Tenhola, and the northernmost from Ylistaro. Of the total of 221 different samples, 50 were taken in the autumn of 1989 (the new generation of beetles), 71 samples in the spring-summer of 1990 (overwintered, old generation beetles), and 100 samples in the autumn of 1990 (new generation beetles). Because from each sample approximately 30–60 beetles were examined, the individually examined adult beetles totalled 10 152.

Additionally, 40 samples of *M. aeneus* larvae were collected mainly from the rape-growing area, but also from all locations where *Nosema* had previously been detected.

To follow the seasonal dynamics of *Nosema* infection in a blossom beetle population, a series of samples throughout the season was collected at seven localities where the disease was known to occur.

In autumn, 1990, at Hartola, central Finland, the occurrence of the disease and its ecology were studied in greater detail. Here, in 1989, was an isolated turnip rape field of about 3 ha within a field complex of about 50 ha in total size. No other rape fields were within 20 km of this location. In 1990 no rape was grown in the area, but volunteer plants from the previous year's seeds grew as weeds among the cereals (mainly oats) sown there. Additionally the area has a relatively high population of cruciferous weeds.

Nosema meligethi occured at an infection level of about 3–21% in the Hartola population. In 1990 the dynamics of the disease was studied, and particularly its possible horizontal transmission in the autumn, as well as its effect on the overwintering migration of the host beetle.

In the autumn the beetles aggregate to feed on pollen in the flowers of *Sonchus arvensis*, a practice which may facilitate efficient horizontal transmission of the disease. Therefore, a total of 76 individual *Sonchus* flowerheads were collected, and all the beetles in each flower inspected for the presence of infection. For this analysis, samples were also collected from the study areas at Haukivuori and Pieksämäki, central Finland (where no rapeseed cultivation was practiced at the time of this study). A total of 1086 beetles were examined, and the distribution of the diseased individuals among the flowerheads was determined and tested for randomness vs. contagiousness.

At Hartola, three likely overwintering sites on the hills surrounding the fields were searched for migrating beetles. Two sites were about 1 km from the center of the rape field (north and southeast), and the third about 3 km from the field (southwest). Beetles were collected at these sites from the flowers of *Leontodon autumnalis* and *Hieracium* spp. These beetles were not yet overwintering, but had left the fields to enter the overwintering area. Samples were collected at 5- to 7-day intervals between August 31st and September 22nd. At the same time a sample of beetles was collected from the breeding and feeding site in the field.

To assess the effect of *Nosema* infection on the weight of adult beetles entering the winter diapause, beetles were collected from Hartola on September 17th, and air dried in the laboratory for 2 weeks to stabilize their weight (c.f. Hokkanen 1993). Then 243 beetles were individually weighed within an accuracy of 10^{-5} g (Mettler AE 163), and examined for the presence of infection.

2.2. Microscopical Techniques

Specimens in our study were usually examined live, within 1–2 days of collection. Each beetle was placed individually into a small drop of water on a microscopic slide and its body was crushed (ground) with a rounded glass stick, in order to release the spores of *N. meligethi* from infected tissues. Each slide was then examined under a compound microscope at a magnification from 160x to 640x. The spores of *N. meligethi* are generally easily recognizable due to their uniform size and refractile wall, as compared to the frequently present yeast cells, which are of variable size and shape and are in the budding phase. In doubtful cases, or in cases of weak 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 and can easily be noticed and identified from yeast cells or fungus conidiospores.

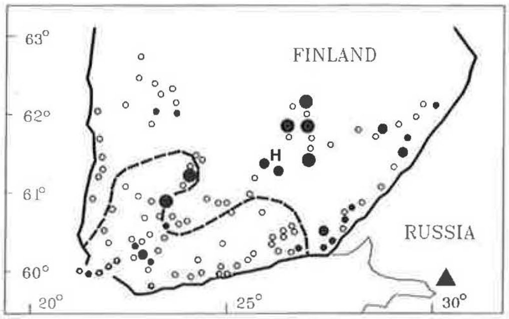

Fig. 1. Locations in Finland for *Nosema meligethi*. Maximum infection levels observed: large dots = >20%, medium dots = 10–20%, small dots = >0% but <10%. Open circles show locations where the samples did not contain infected beetles. South of the dashed line is the main region of oilseed crucifer cultivation (>5% of field area). H = Hartola, where more detailed investigations were carried out. The triangle = St. Petersburg (Leningrad), from where *N. meligethi* first has been reported.

3. Results and discussion

3.1. Geographical distribution and infection levels

Nosema meligethi has now been found at 25 locations in Finland, eight of which are within the main growing area of oilseed rape (turnip and swede rape), and 17 outside of it (Fig. 1, Table 1). Specifically there were 91 distinct sampling sites where rape was cultivated, and there the beetles apparently had been subjected to the normal agronomic practices related to rape growing. *N. meligethi* was found in 14 (15.4%) of these samples. In contrast, 41 sampling sites were areas where no rape was grown in the vicinity, so the beetles obviously subsisted on cruciferous weeds among the cereals, which seldom have to be treated with insecticides. Of these *Meligethes* samples, the disease was found in 23 (56.1%). This difference is highly significant ($x^2 = 23.22$, $p < 0.001$), and shows that *N. meligethi* is poorly compatible with the agronomic practices related to rape growing. The most apparent of such practices is the regular use of effective insecticides against the host beetle, *M. aeneus*. When the disease did occur, however, there was no significant difference in the level of infection per sample: 8.3% for rape-growing areas, and 8.6% for non-rape-growing areas.

The fact that *N. meligethi* was now detected even within the most intensive cultivation areas in Finland (cf. Hokkanen & Lipa 1991) is an indication that the disease potentially has a role in the natural control of *M. aeneus* in these areas as well. But the survey also shows that normally *Nosema* did not occur in cultivated areas (Table 2), the reasons for which are a clear target for future research.

As for the geographical distribution of *N. meligethi* in Finland, the infection is clearly dominant in eastern Finland, as our previous paper speculated (Hokkanen & Lipa 1991). It has spread further westwards than could be expected based on the 1989 sampling, but has yet to be detected from most of the western, as well as the southern coastal areas (Fig. 1). No doubt an intensive sampling effort would reveal its presence, but the fact remains that currently in most of the rape-cultivation area *N. meligethi* does not occur at levels of practical importance (Tables 1 and 2, Fig. 1).

The infection level normally remained rather low, with a mean of 1.5% over all the locations (Tables 1 and 2). Locally, however, the infection levels often reached 20–25% (up to 40%)in the non-rape-growing areas (Fig. 1). For an effective, permanent suppression of the host population this is too low — for pathogens such as

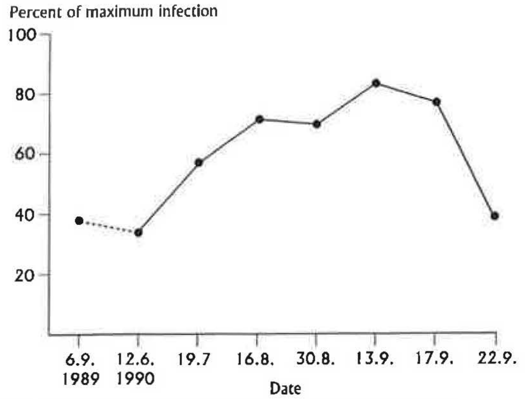

Fig. 2. Dynamics of *Nosema meligethi* infection over the season at seven locations as the proportion of maximum infection observed at each location. Based on data from Table 3.

Nosema the ideal infection level would be about 30–50% (see Andersson 1982). Artificial augmentation might be a solution, provided that suitable methods are found, and that compatibility of *Nosema* with pesticide treatments is achieved.

3.2. Seasonal dynamics

To facilitate detection of possible patterns, location-specific data on seasonal dynamics of *N. meligethi* (in adult beetles) from seven different locations were normalized by the maximum infection level observed at each location. In Fig. 2 the mean proportion for the seven locations on a given sampling date is shown. These data indi-

Table 1. Locations in Finland found for *Nosema meligethi* infection in *M. aeneus*. Infection percent for new generation adult beetles (NG 90) between 13.8.–27.9.1990. 'Major oilseed area' refers to locations where regionally >5% of field area is in oilseed crucifers (c.f. Figure 1). 'All 145 locations (mean)' also includes samples from other study collections at locations with no infection.

Location	% infection	N of examined beetles	Major oilseed area?
Kangasniemi	18.0	161	No
Haukivuori	17.0	364	No
Piikkiö	14.9	47	Yes
Mikkeli	14.3	63	No
Pieksämäki	13.6	310	No
Kerimäki	12.5	8	No
Miehikkälä	11.7	34	No
Hartola	11.1	1087	No
Kangasala	10.6	160	Borderline
Pertunmaa	10.0	40	No
Saari	10.0	40	No
Urjala	8.7	242	Borderline
Virrat	8.1	64	No
Forssa	7.5	40	Borderline
Kihniö	7.5	40	No
Lappeenranta	6.2	16	No
Ylämaa	5.1	39	No
Karhula	5.0	40	Borderline
Lieto	5.0	40	Yes
Virolahti	5.0	40	No
Kesälahti	2.5	40	No
Salo	2.5	40	Yes
Värtsilä	2.3	130	No
Vaalimaa	2.2	46	No
Korppoo	1.7	60	Borderline
Other 33 locations	0	1484	
All 145 locations (mean)	1.5	10152	

cate that during the season the level of infection roughly doubled from that of the spring (Table 3, Fig. 2). The increase was rather steady and was clear also among the old-generation beetles (the first two sampling dates in 1990), despite the likelihood of a differential mortality among healthy vs. infected beetles towards the end of their lives. This would indicate efficient horizontal transmission of the disease at that time. The same phenomenon seems also to occur after the emergence of the new-generation adults (16.8–13.9, Fig. 2); by that time all old-generation beetles have died. The last two sampling dates shown in Fig. 2 are at the time when migration to overwintering sites already has begun, and will be discussed below.

Overwintering mortality in *M. aeneus* due to the presence of *N. meligethi* can in theory be assessed by comparing the levels of infection in the autumn and in the spring at the same location. One difficulty in this assessment is the large amount of data required to approach statistical reliability. The combined data from all sampling sites does not reveal any differences in infection level between autumn 1989 and spring 1990 (Fig. 2). At the most intensively sampled areas, however, there was an approximately 50-100% decrease in infection level over the winter (Kangasala, Urjala, and Forssa; in Forssa the level dropped from 7% to 0%, cf. Hokkanen & Lipa 1991). This suggests that winter mortality of *M. aeneus* is greatly increased by the presence of *Nosema* infection in Finland, as also was reported by Issi & Raditscheva (1979) for Leningrad, and as is known for many other insects in similar situations (see Brooks 1988).

Despite our intensive efforts, *Nosema meligethi* was not detected in the larvae of *M. aeneus*. Over 3000 larvae were inspected, most from sampling sites where concurrently the infection level of adult beetles was 10–20%. At present we have no explanation for this. It is particularly puzzling, because immediately after the emergence of the new-generation adults, in-

Table 2. Overall infection level by *Nosema meligethi* in new generation adult beetles of *M. aeneus* in autumn 1989 and 1990, and in old generation beetles in spring 1990. Main rape growing area = area, where regionally >5% of field area is on oilseed crucifers; Reference area = all other areas.

	Main rape growing area			Reference area		
Time	Percent infection	N of beetles	N of samples	Percent infection	N of beetles	N of samples
Autumn 1989	0.9	926	29	1.9	649	21
Spring 1990	0.2	2463	57	8.6	1439	15
Autumn 1990	4.3	1249	31	8.6	3426	68
Total	1.4	4638	117	7.8	5514	104

Table 3. Fluctuations of infection by *Nosema meligethi* in adults of *M. aeneus* in Finland from autumn 1989 till autumn 1990. Maximum infection level at a location is indicated by underlining and boldface.

	Infection level in % on a sampling date in 1989–90							
Location	6.9.89	7–18.6.90	13–25.7.90	16.8.90	29–31.8.90	12–14.9.90	17.9.90	22.9.90
Hartola	3.3	14.6	11.1	10.3	7.0	**20.7**	14.7	10.5
Haukivuori	16.7	12.6	16.0	**25.0**	–	20.2	17.9	16.7
Kangasala	12.0	2.5	–	–	8.7	**25.0**	–	–
Kangasniemi	6.7	6.7	13.2	20.5	**27.8**	–	23.8	16.7
Mikkeli	3.3	3.8	15.0	**20.0**	–	–	–	0.0
Pieksämäki	10.0	14.1	10.5	5.0	–	**23.9**	19.4	9.1
Urjala	10.0	0.0	–	–	**20.0**	5.4	–	–

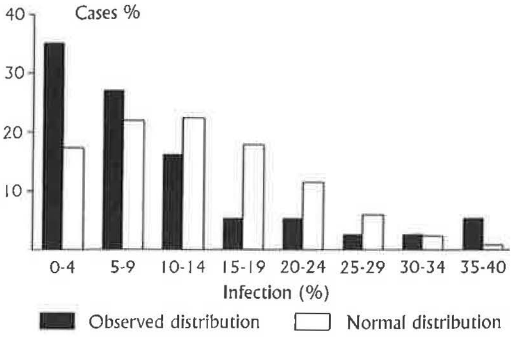

Fig. 3. Distribution of infection level in subpopulations of *Meligethes aeneus* on one flowerhead of *Sonchus arvensis* at Hartola, September 1990; overall infection level about 10%. 'Cases %' is proportion of *Sonchus* flowerheads with a *M. aeneus* subpopulation when sampled, with infection % in the range shown on the x-axis.

fection levels were "normal". Somehow the disease must be present in the larvae, but it will not replicate before the end of pupation. As such behaviour is not common for protozooan diseases, this should be studied separately in greater detail (cf. Weiser 1981, Brooks 1988).

3.3. Horizontal transmission of *Nosema*

An effort was made to determine whether any horizontal transmission could be detectable in a situation which would appear ideal: *M. aeneus* gathers in the spring as well as in the autumn in the flowerheads of yellow compositae, mainly *Taraxacum* and *Sonchus*. Dozens of beetles may appear in one single flowerhead to feed on pollen. Theoretically one infected beetle could easily contaminate the whole flowerhead, thereby efficiently spreading the disease.

Because the general level of infection at the study site has an influence on the distribution of healthy and infected beetles among the flowerheads, for this assessment only two kinds of samples were included: those from locations where the overall infection level at the sampling time was about 10%, and those where the level was 20%. Fig. 3 illustrates the situation at the 10% infection level.

In the autumn the situation appeared to be as expected: the disease distribution was contagious rather than normal for both two infection levels studied. At both levels in more flowerheads than expected infection was not present at all, or the level was very low; in contrast more flowerheads than expected showed high infection levels (Fig. 3). On the other hand, flowerheads where the infection level was approximately at the average level, were less numerous than expected. Both distributions differ significantly from the normal (chi-square = 19.93, df = 7, for the 10% infection level; and 4.46, df = 4, for the 20% level). The contagiousness parameter of Taylor's power law (Taylor 1961, 1965) is 1.69 for the distribution at 10% infection level, and 1.08 at the 20% level. In both cases the index of dispersion (see Southwood, 1978, p. 39) differs greatly from that produced by Poisson randomness ($I_D = 409.2$ and 404.7, respectively), indicating significant aggregation.

It is possible that the horizontal transmission is somewhat more effective than indicated by this comparison, because newly infected beetles are likely to escape detection in the microscopic assessment. In fact, most of the beetles examined were only lightly infected, indicating that the infection was rather recent.

3.4. Effects on overwintering beetles

A comparison of the infection levels in the autumn between beetles that remain in the fields still feeding on *Sonchus* flowerheads and beetles that have migrated to the overwintering sites, shows that levels at the beginning of the migration period (late August) are higher at the overwintering sites than at the breeding/feeding site (Fig. 4). This suggests that infected beetles fly to overwintering sites earlier than healthy beetles. The jump in the level later on at the breeding site may indicate effective horizontal transmission (c.f. 3.3, above) (Fig. 4).

Infected beetles appear to migrate about 1–2 weeks earlier than healthy beetles. This behaviour may be a direct response to the infection: it is probably better for survival to enter the winter diapause before the newly acquired disease has a chance to replicate excessively, thus further lessening chances of survival during the winter. Even a light, but detectable, infection in the autumn significantly reduces the weight of the beetles (t = 2.62,

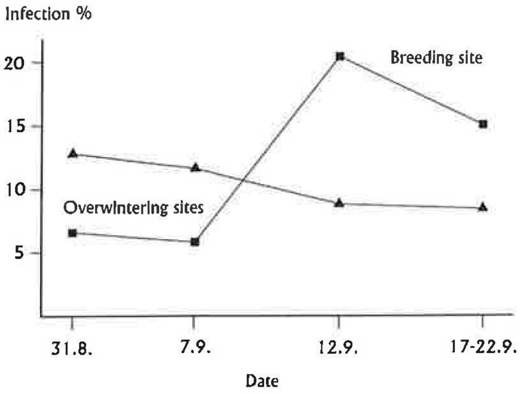

Fig. 4. Comparison of overall infection levels of *Meligethes aeneus* subpopulations at the date given at their breeding site (fields), and at their overwintering sites in nearby woods; Hartola).

p = 0.013, df = 34), whereas weight is crucial for winter survival (Hokkanen 1993). The reduction in weight is about 13%, which depending on the general nutritional status of the beetles, may have a great effect on their winter mortality.

As for the healthy beetles, they seem to delay migration until the maximum amount of fat reserves for the winter has been accumulated. Because of the present practice of growing only spring oilseed crucifers in Finland, the beetles in the rape-growing areas appear to have difficulties in properly preparing for the winter: the time between adult emergence in August (determined by the phenology of the crop) and entering the winter diapause in September is very short for accumulating fat reserves (cf. Hokkanen 1993). In central Europe, for example, the new-generation beetles have about three months between emergence and winter diapause.

It can be concluded from this study that:

1) *Nosema meligethi* possibly occurs everywhere in Finland in the populations of *Meligethes aeneus*, but the level of infection is practically zero in most of the areas where oilseed crucifers are cultivated. The highest levels of infection and highest probability of occurrence are in the central and eastern parts of the country among *Meligethes* populations subsisting on cruciferous weeds.
2) Infection levels remain relatively low even in the areas of highest incidence of the disease, reaching about half that is considered ideal. Practical methods of augmentation of the disease would be critical for increasing the impact of this pathogen.
3) Levels of infection at a particular location steadily increase during the summer, approximately doubling by the autumn.
4) There appears to be at least some horizontal transmission of the disease among the adult beetles throughout the summer. It may be that this transmission is not so effective as it could be, due to the fact that the beetles feed on the highly nutritious pollen, which results in only a minute amount of excrement. Artificial augmentation of *Nosema* could be used to ensure more effective horizontal transmission.
5) Infection of M. *aeneus* larvae was not detected. The most attractive hypothesis to explain this is latent infection, but this has to be shown experimentally prior to speculation on its nature.
6) *Nosema* infection changes the behaviour of the host beetle at least in the autumn; the infected beetles migrate earlier to their winter quarters than do healthy beetles. This may be an attempt to slow down the infection process, which is rather rapid in active beetles.
7) Infected beetles are, in the autumn, on average 13% lighter in weight than healthy beetles, which may have a drastic effect on their overwintering survival.
8) *Nosema meligethi* clearly has the potential for becoming an important agent in the integrated control of *Meligethes aeneus*, provided that practical methods of artificial augmentation can be developed. Research on the rape agroecosystem and its management should be aimed toward finding ways to make the most of this potential, and to ensure the compatibility of agronomic practices with the characterictics of *N. meligethi*.

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