Susceptibility of the June chafer, *Amphimallon solstitiale* to entomopathogenic nematodes

Marek Tomalak
Department of Biological Pest Control and Quarantine
Institute of Plant Protection
Miczurina 20, 60-318 Poznan, Poland
M.Tomalak@ior.poznan.pl

Introduction

SCARAB BEETLES (Coleoptera: Scarabaeidae)

PESTS OF PERIODICALLY GROWING IMPORTANCE TO:

- ⇨ various agricultural crops (cereals, corn, sugar beet, potato, carrot, etc.)
- ⇨ lawns in parks, gardens and recreation areas
- ⇨ forest nurseries and plantations
- ⇨ young trees in newly forested areas

✶ In Poland

particularly important problem in soils of originally agricultural lands, recently taken over by forestry

Our study on biological control of scarab beetles in forest and recreation areas

Started 1994

- ⇨ 1996 - 1999 national grant with Dr. A. Bednarek
- ⇨ 1999-2004 included Ph.D. student - J. Kowalska

⇨ Major areas: lawns in recreation areas, forest nurseries and new plantations

<u>Main objectives:</u>

- ⇨ to evaluate biocontrol potential of entomopathogenic nematodes against scarab beetles
- ⇨ to identify key factors affecting the nematode infectivity to examined scarab species

Scarab beetles in Poland

Some 40 species present (*Scarabaeidae pleurosticti*)

MOST COMMON SPECIES:

- ⇨ *Melolontha melolontha / M. hippocastani*
- ⇨ *Amphimallon solstitiale*
- ⇨ *Phylloperta horticola*

- ⇨ *Serica brunnea, Anomala dubia, Anisoplia segetum ...*

PRECISE IDENTIFICATION NEEDED !!!

Scarab beetles
Most common species

A. *Melolontha melolontha / hippocastani*

B. *Amphimallon solstitiale*

C. *Phylloperta horticola*

Scarab beetles
Most common species

A. *Anomala dubia*
B. *Scerica brunnea*
C. *Anisoplia segetum*

Scarab beetles
Most common species

A. *Melolontha melolontha*
B. *Amphimallon solstitiale*
C. *Phylloperta horticola*

June chafer, *Amphimallon solstitiale*

MAIN CHARACTERISTICS:

- ⇨ In western regions of Poland more important than *Melolontha melolontha*
- ⇨ Occasionally, up to 100 grubs per m^2
- ⇨ Larvae (grubs) feed on plant roots, 5-15 cm below the ground level
- ⇨ 2-year generation time
- ⇨ Insects overwinter as larvae L2 and L3
- ⇨ Pupation in May
- ⇨ Adults fly in June/July
- ⇨ Extensive damage usually every 2nd year

June chafer, *Amphimallon solstitiale*

MAIN CHARACTERISTICS cd.:

⇨ Difficult pest to control with chemical insecticides

- Carbosulfan: *Marshal 250 EC; Marshal SusCon 10GR*
- Carbofuran: *Furadan 5GR*
- Diazinon: *Diazinon 10GR; Basudin 10GR; Basudin 25EC*
- Imidacloprid: *experimental trials*

⇨ Usual application rates:

- GR - 80 - 120 kg/ha
- EC - 0.04 - 0.10 % ⇨ 0.5 l / m row

⇨ Environmental safety / limitations

Entomopathogenic nematodes as biocontrol agents against scarabs

⇨ Extensive research on *Popillia japonica* and *Phylloperta horticola*

⇨ Encouraging data available on control of *P. japonica, Phyllophaga hirticula*, *Ph. horticola, Aphodius contaminatus* (reviewed by Klein, 1990; Forschler & Gardner, 1991; Smits, 1994; Selvan *et al*., 1994; Ehlers *et al*., 1998) ....

and *Amphimallon solstitiale* (Pettersson, 1997)

⇨ Less encouraging on *A. solstitiale* (Ehlers *et al.,* 1998) and *Melolontha melolontha* (Peters, 2000)

⇨ Discouraging data on control of *A. solstitiale* (*Smits, 1992; 1996*).

Major factors affecting EPN efficacy against *Amphimallon solstitiale*

- ⇨ Nematode species
- ⇨ Insect development and available penetration routes
- ⇨ Insect moulting
- ⇨ Seasonal activity
- ⇨ Insect wounding (accidental biting)
- ⇨ Active elimination of IJs

Materials and Methods

I. Laboratory experiments

Infectivity tests:

- ⇨ soil columns: ∅=7.5 cm; h=10 cm (4 - 1 - 5 cm)
- ⇨ soil: sandy loam (8% H_2O)
- ⇨ individual insects in separate soil columns – no wounding !!!
- ⇨ nematode dose: 100 IJ/cm^2 (i.e.4400 IJ/column)
- ⇨ temperature: 20±2°C
- ⇨ exposure time: 7, 14, or 21 days
- ⇨ 6 replicates per treatment, 10 columns per replicate

Materials and Methods

INSECTS: *Amphimallon solstitiale*

- ⇨ L1, L2, L3 collected in the field – reared at 18 –20°C
- ⇨ Pupae, young adults – obtained from larvae at 18 - 20°C
- ⇨ 2 major grub collection and field experiment sites:
  - Poznan - recreation area around Malta lake - mostly lawns
  - Skrzynka - abandoned agricultural fields adjacent to pine forest and new forest plantation (100 km east from Poznan)

Materials and Methods

NEMATODES: *Steinernema* (8 spp.), *Heterorhabditis* (4 spp.) reproduced *in vivo* in *Galleria mellonella* (all) larvae and *in vitro* on solid media (selected)

1. *S. affinae* - PL
2. *S. arenarium* – Ryazan (St. Petersburg), (05PL)

3-4. *S. carpocapsae* - All, HU (Rutgers/Antibes)

5-7. *S. feltiae* - ScP, SN, UK76 (Rutgers/St. Albans)

8. *S. glaseri* NC / NC-selected (Rutgers)
9. *S. intermedium* - D (Kiel)
10. *S. riobrave* (Antibes)
11. *S. sp. 1* – IT (*S. affinae*-type) (Bari)

12-13. *H. bacteriophora* - HP88, Brecen (Maynooth)

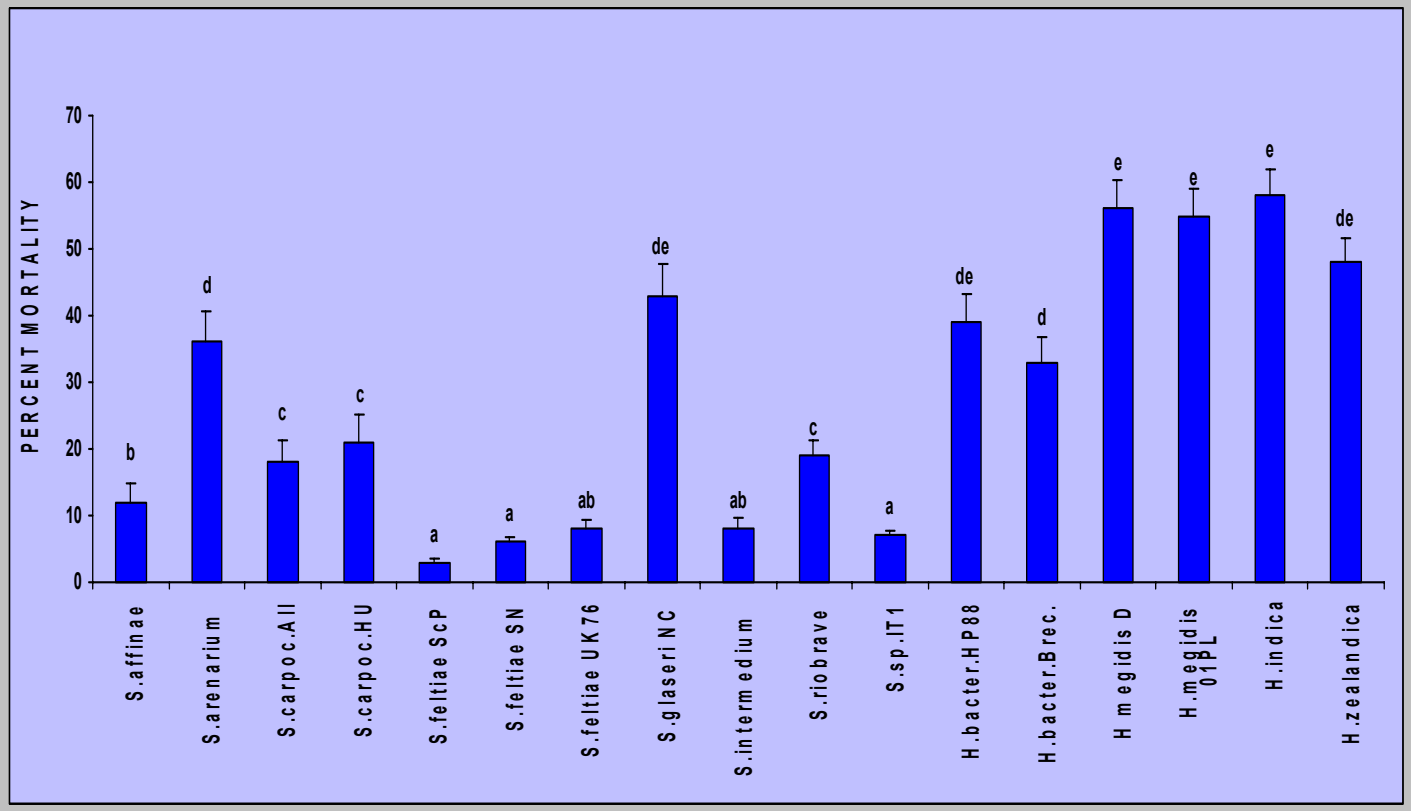
14. *H. indica* (Maynooth)

15-16. *H. megidis* - D (Kiel), 01PL (03PL)

17. *H. zealandica* (Maynooth)

Results

Nematode species



Mortality of *Amphimallon solstitiale* grubs (L3) caused by entomopathogenic nematodes - spring tests. (*Individual insect per soil column / 100 IJs per cm^2 / 21 day exposure*)

Materials and Methods

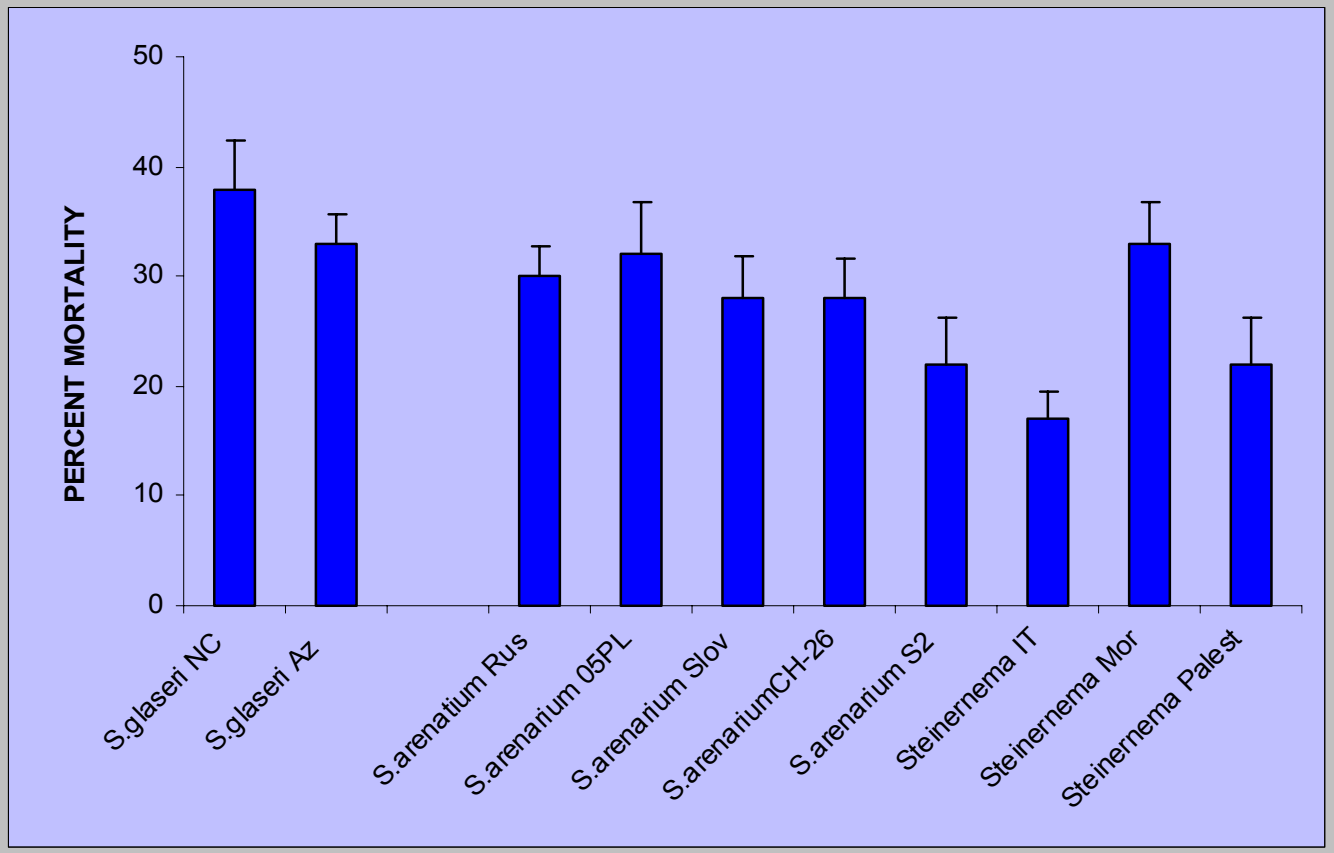
Theoretically, P R E A D A P T E D nematode species

Long IJ-type *Steinernema* (Deberecen group)

1. *S. glaseri* NC (Rutgers)
2. *S. glaseri* Azores
3. *S. arenarium* Ryazan (St. Petersburg),
4. *S. arenarium* 05PL
5. *S. arenarium* Slovak (Ceske Budejovice)
6. *S. arenarium* CH-26 (Vadensvil)
7. *S. arenarium* S2 (Barcelona)
8. *Steinernema sp.* Italy (Bari)
9. *Steinernema sp.* Morocco (Kiel)
10. *Steinernema sp.* Palestine (Kiel)

Results

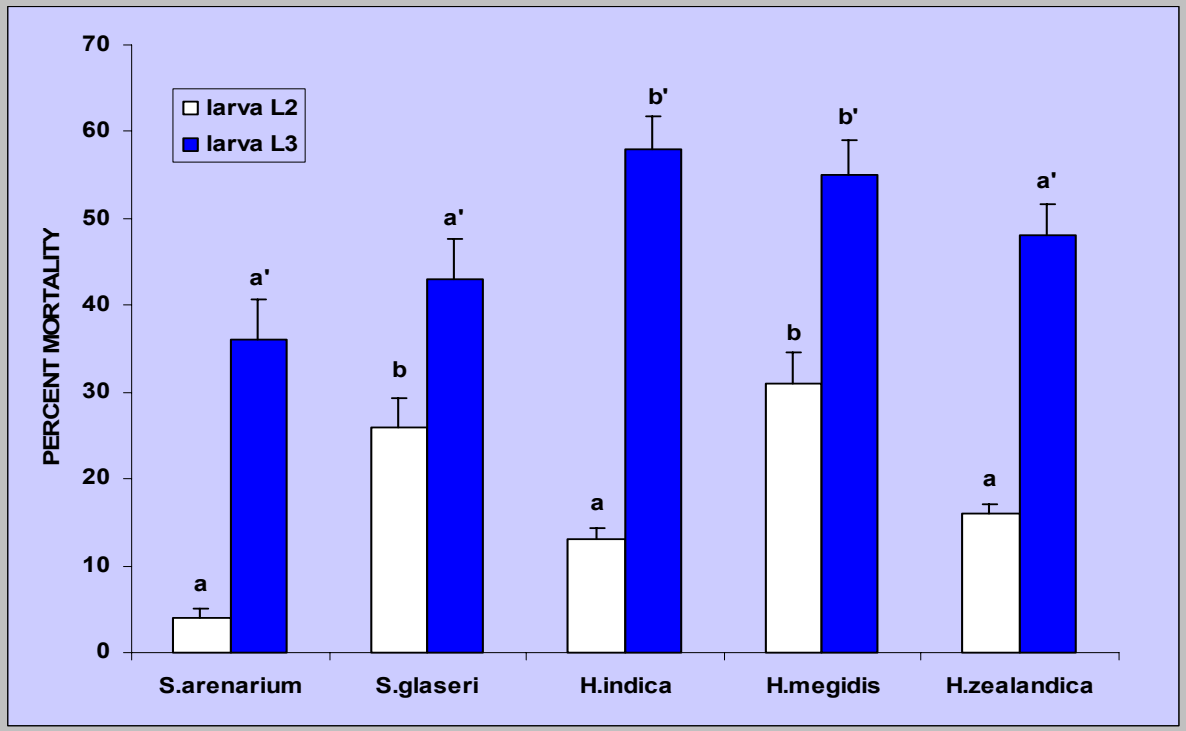
“Long IJ-type *Steinernema* - infectivity



Mortality of *Amphimallon solstitiale* grubs (L3) caused by „long IJ-type *Steinernema* - spring tests. (*Individual insect per soil column / 100 IJs per cm^2 / 21 day exposure*)

Results

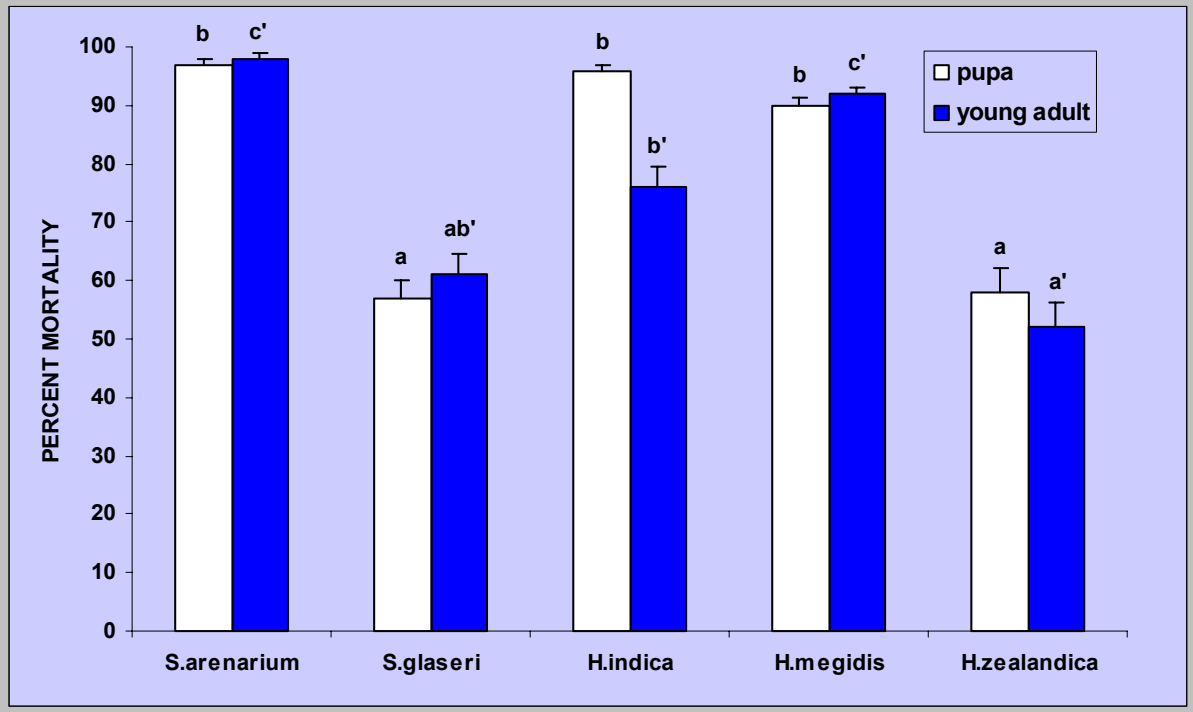
Insect development



Mortality of *Amphimallon solstitiale* grubs (L2 & L3) caused by entomopathogenic nematodes - spring tests. (*Individual insect per soil column / 100 IJs per cm^2 / 21 day exposure*)

Results

Insect development



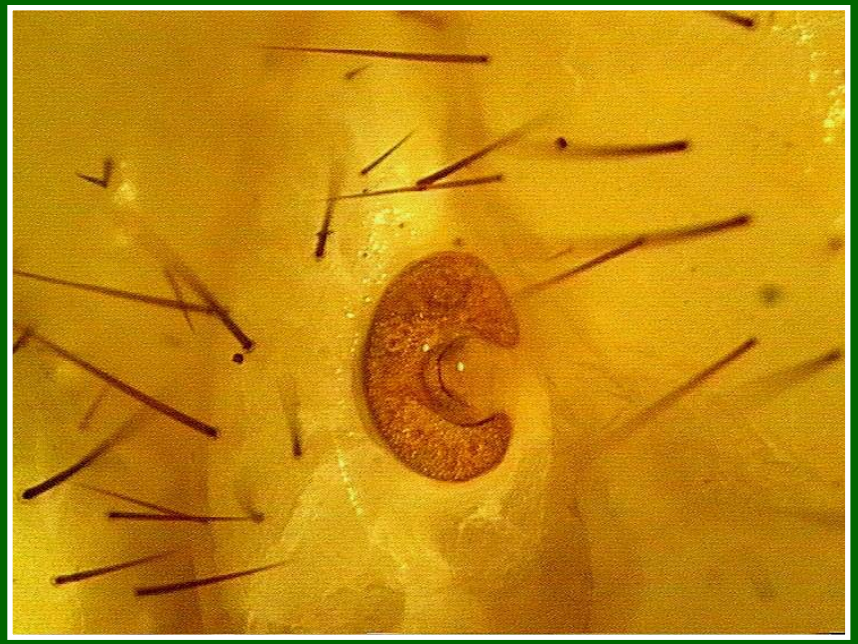
Mortality of *Amphimallon solstitiale* pupae and young adults caused by entomopathogenic nematodes - spring tests. (*Individual insect per soil column / 100 IJs per cm^2 / 7 or 12 day exposure for pupae and adults, respectively*)

Amphimallon solstitiale
Spiracles

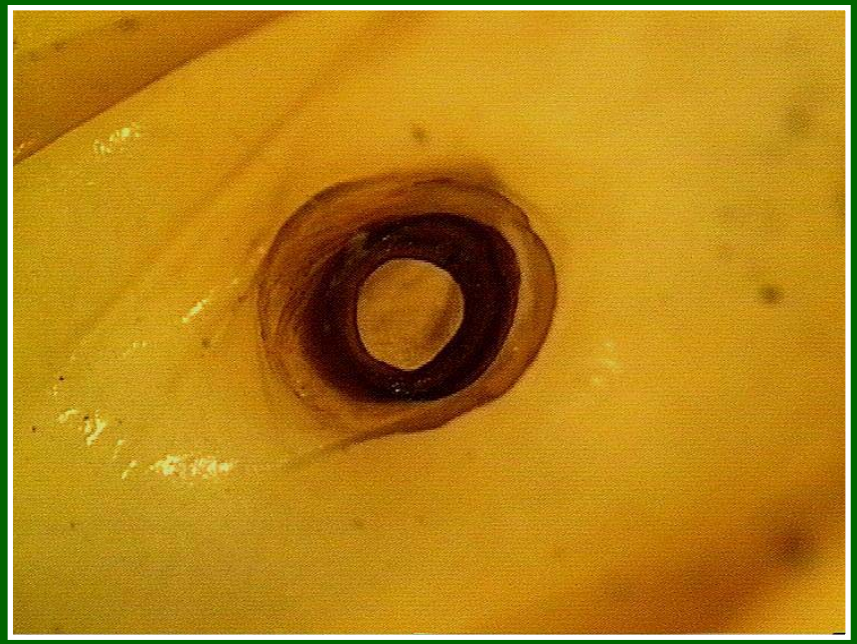
Larva L3

Pupa

Amphimallon solstitiale
Spiracles



Larva L3



Pupa

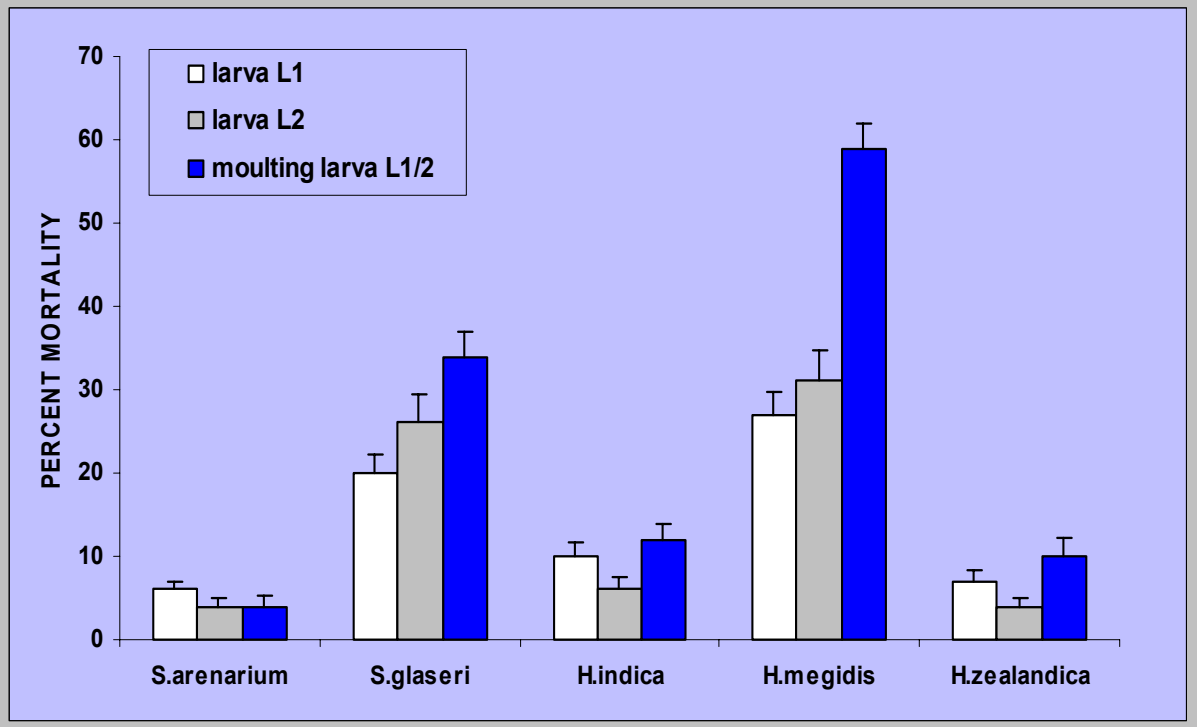
Pupa of *Amphimallon solstitiale*

Steinernema arenarium - penetration through spiracles

Pupa

Results

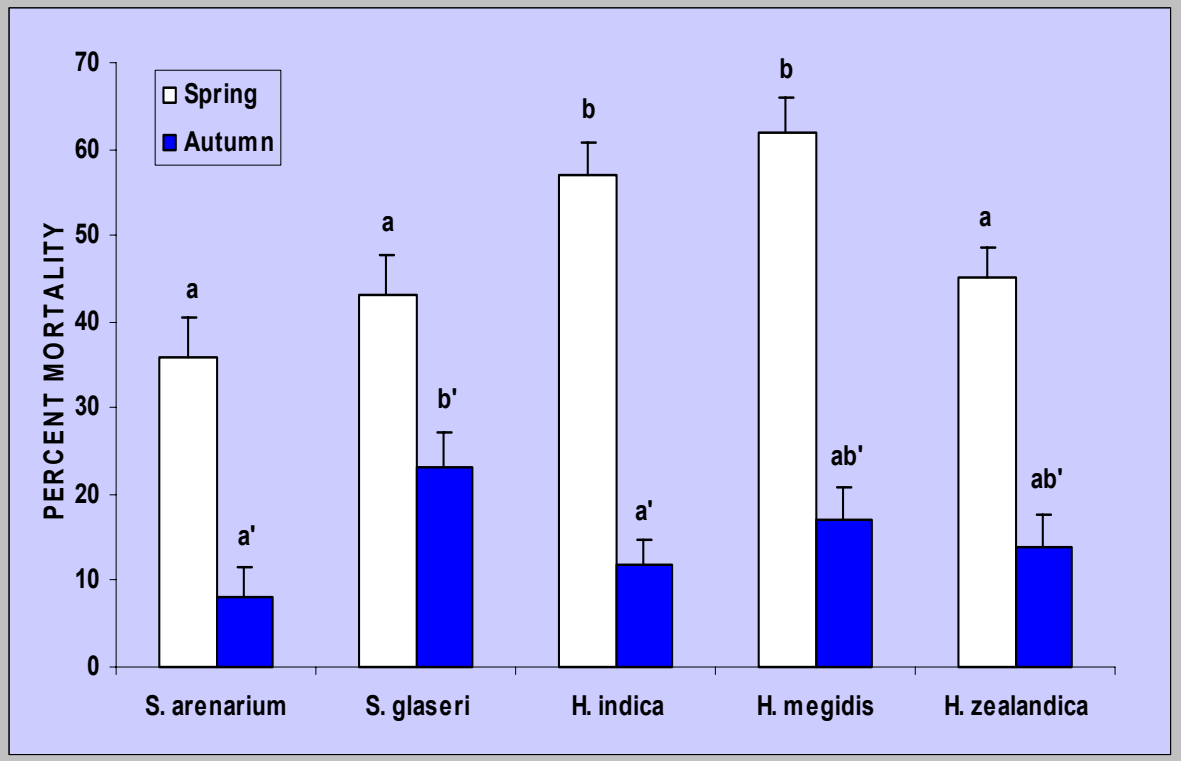
Insect moulting



Effect of L1/L2 larval moulting on susceptibility of *Amphimallon solstitiale* to entomopathogenic nematodes - summer tests. (*Individual insect per soil column / 100 IJs per cm^2 / 21 day exposure*)

Results

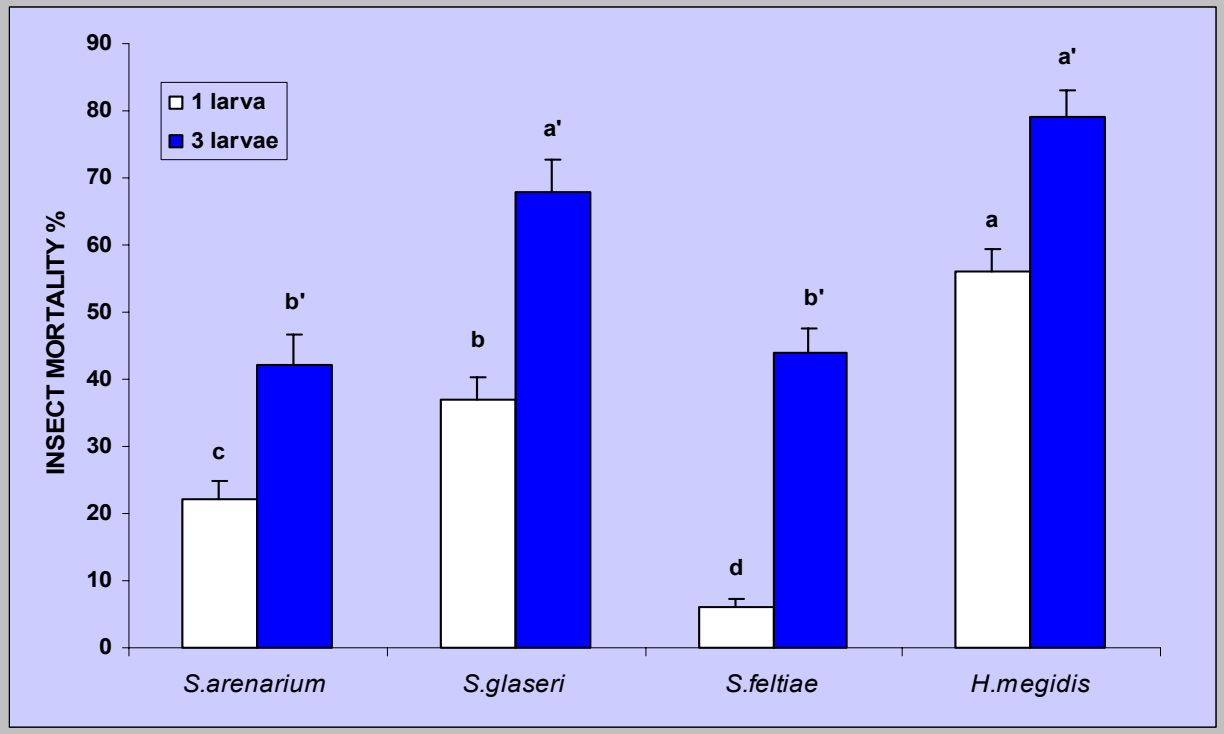
Seasonal activity



Mortality of non diapausing and diapausing *Amphimallon solstitiale* grubs (L3) caused by entomopathogenic nematodes. (*Individual insect per soil column / 100 IJs per cm^2 / 21 day exposure*)

Results

Insect wounding



Effect of accidental wounding on mortality of *Amphimallon solstitiale* grubs (L3) caused by entomopathogenic nematodes - spring tests. (Laboratory experiment: *1 or 3 insects per soil column / 100 IJs per cm^2 / 21 day exposure*)

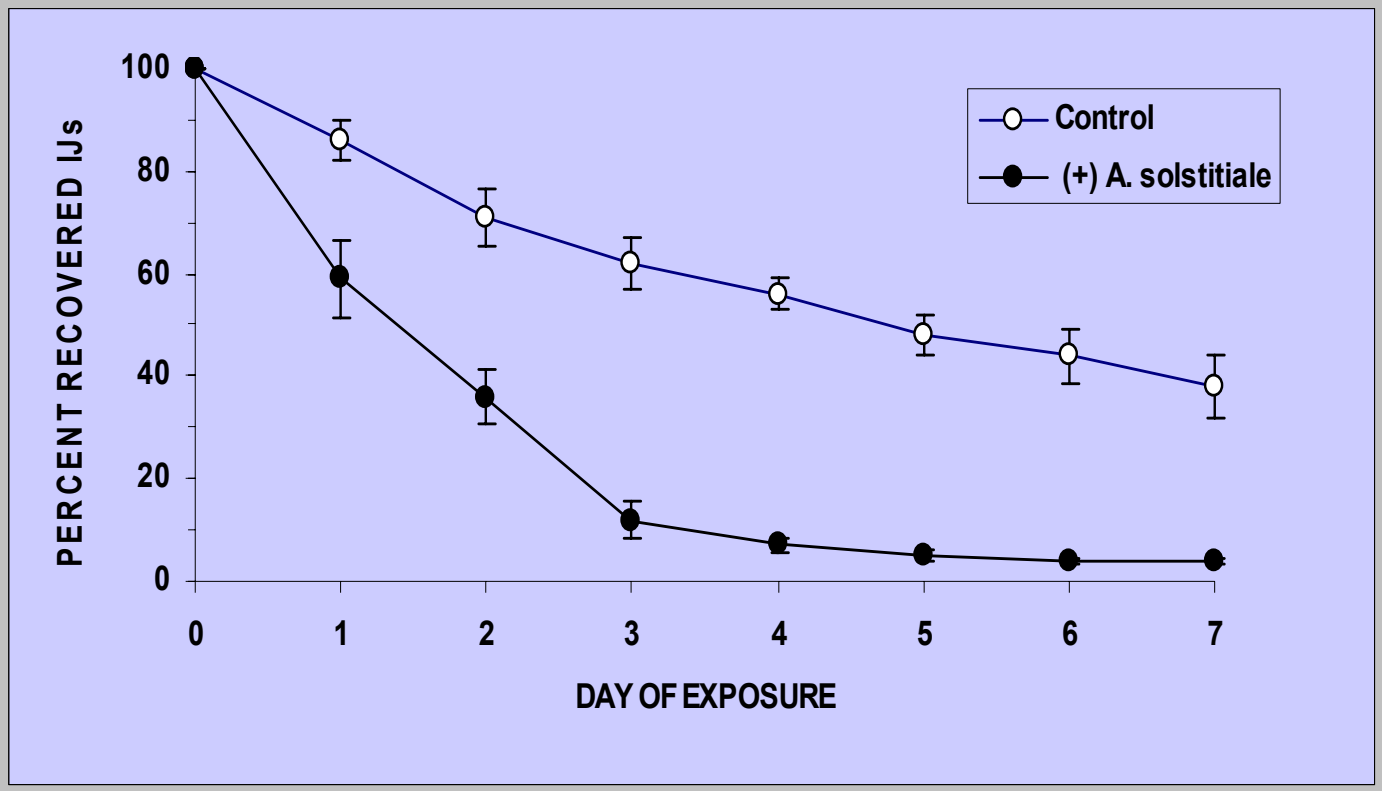
Materials and Methods

Active IJ elimination tests:

- ⇨ ∅ 5 cm Petri dishes + sand + carrot ring + 1 grub L3
- ⇨ exposure: 1,2,3,4,5,6 and 7 days
- ⇨ *S. glaseri* - 200 IJ/ dish
- ⇨ 10 replicates per treatment, 1 dish per replicate

Results

Active elimination of IJs



Effect of *Amphimallon solstitiale* grubs (F3) on survival of *Steinernema glaseri* in sand. (*sand: 200 IJ / ∅ 5cm Petri dish*)

Materials and Methods

II. Field experiment 2002

⇨ Location

- Poznan: recreation area around Malta lake (mostly lawns)

⇨ Experimental plots

- Treatments: randomly chosen 3 x 3 m plots 3 m apart
- Analysis: central 1 x 1 m square within each plot , dug to the depth of 30 cm

⇨ Nematodes

- *S. glaseri NC*
- *S. arenarium 05PL*
- *H. megidis 03PL*

⇨ Nematode dose: 0.5 or 1.0 x 10^6 IJ m^{-2}

⇨ Control: no nematodes

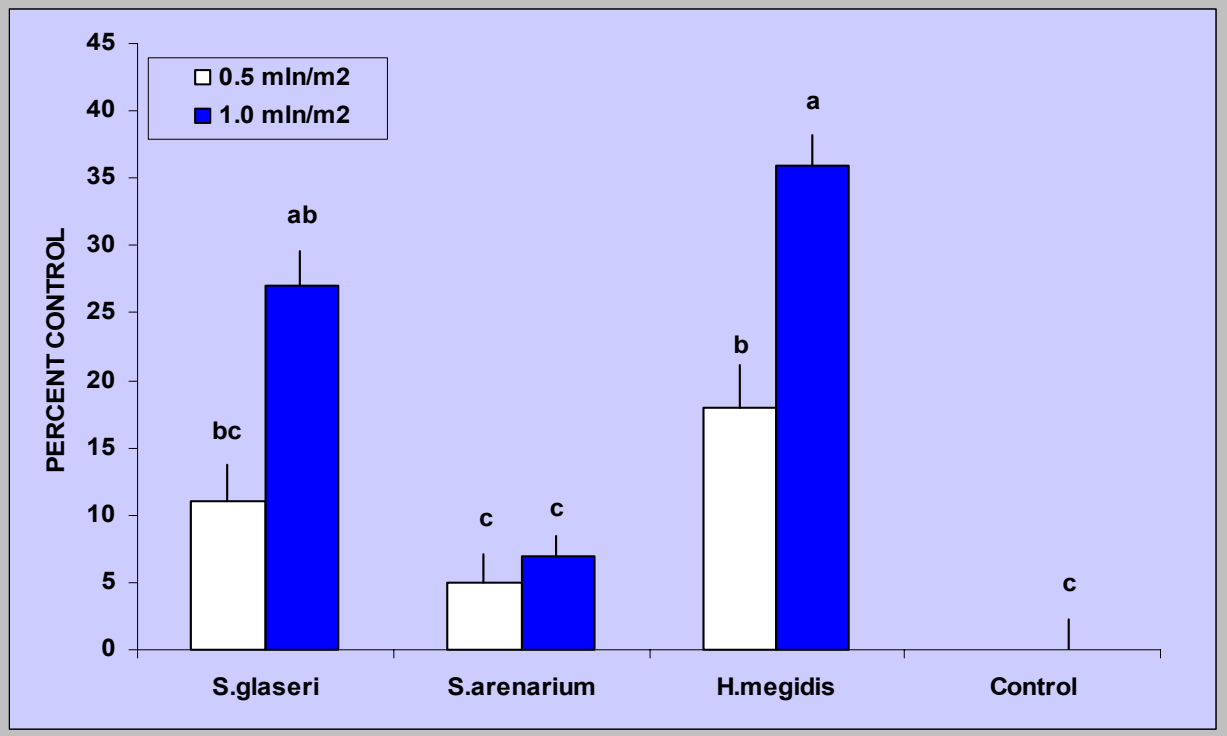
Materials and Methods

Field experiment cd:

- ⇨Application time: July 3, 2002
- ⇨ Examination time:
  - 0 and 60 - 63 days after treatment (grub and soil (nematode) sampling)
  - May of the following year - additional soil (nematode) sampling
- ⇨ 6 replicates per treatment

Results

Field experiment 2002 - nematode efficacy



Efficacy of *S. glaseri, S. arenarium* (05PL), and *H. megidis* (03PL) in control of *A. solstitiale* grubs (L3) in the soil. (Field experiment - Poznan - control: 18 grubs/m^2)

Results

Field experiment 2002/2003 - nematode recovery from the soil

TREATMENT **1 mln / m²**	**Before treatment**	**After 1 month**	**After 2 months**	**After 11 months**
S. glaseri	*S. feltiae 2/6*	*S. glaseri 3/6* *S. feltiae 2/6*	*S. glaseri 5/6* *S. feltiae 3/6*	*S. glaseri 2/6* *S. feltiae 2/6*
S. arenarium	*S. feltiae 3/6*	*S. arenarium 3/6* *S. feltiae 2/6*	*S. arenarium 2/6* *S. feltiae 3/6*	*S. arenarium 1/6* *S. feltiae 3/6*
H. megidis	*S. feltiae 4/6*	*H. megidis 6/6* *S. feltiae 4/6*	*H. megidis 6/6* *S. feltiae 2/6*	*H. megidis 5/6* *S. feltiae 4/6*
Control	*S. feltiae 3/6*	*S. feltiae 2/6*	*S. feltiae 3/6*	*S. feltiae 2/6*

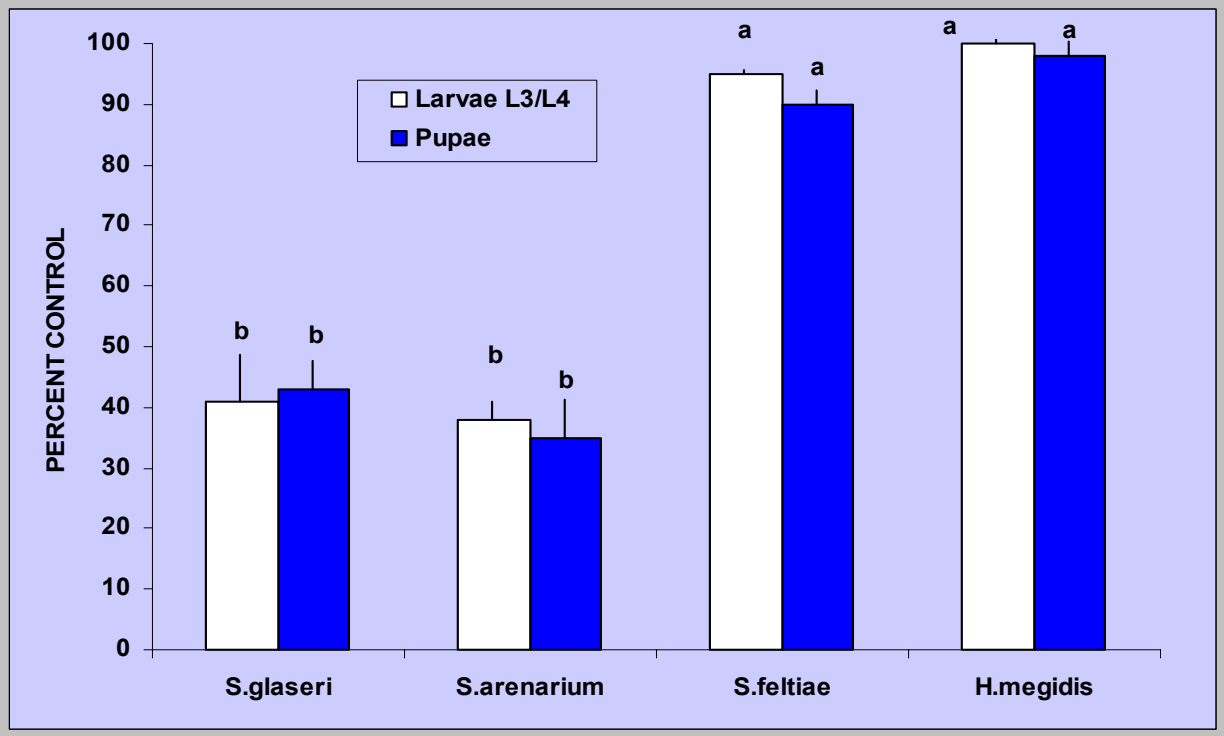
Alternative host

Phyllobius vespertinus

(Coleoptera: Curculionidae)

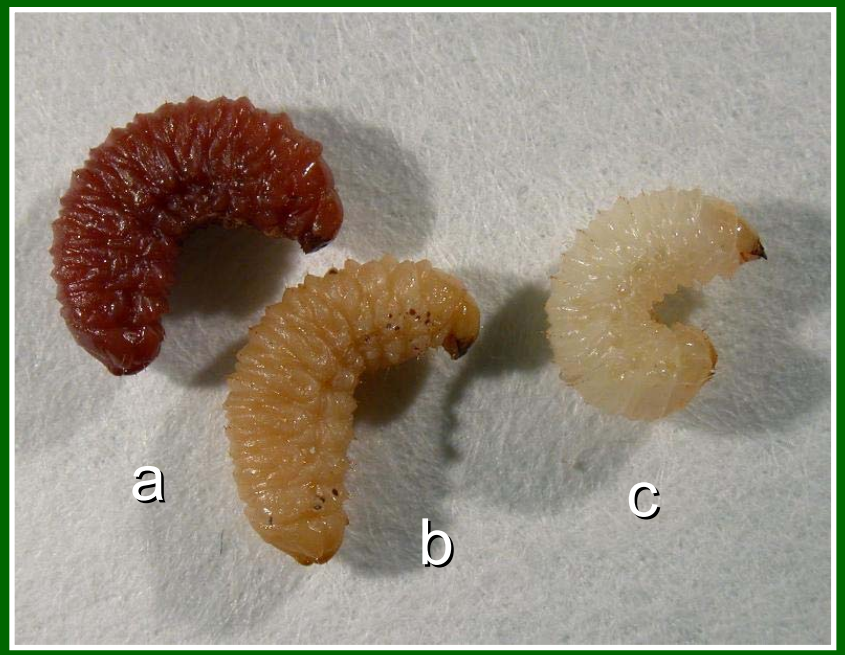
Results

Alternative host



Susceptibility of *Phyllobius vespertinus* to *S. glaseri, S. feltiae, S. arenarium,* and *H. megidis* (Laboratory experiment: *sand: 100 IJ / ∅5cm Petri dish - 10 insects / dish - 7 day exposure*)

Phyllobius vespertinus
(Coleoptera: Curculionidae)



Infected with *H. megidis* (a), *S. arenarium* (b), and uninfected (c)

Emerging nematodes

Nematode isolation from field sites heavily infested with *Amphimallon solstitiale*

Natural populations of EPNs:

⇨ Poznan: recreation area around Malta lake (mostly lawns)

1996 - 2004 *Steinernema feltiae* - always present at some locations (recycles in *Phyllobius vespertinus* and others)

⇨ Skrzynka: abandoned agricultural fields adjacent to pine forest and new forest plantation (100 km east from Poznan)

1997 - *Heterorhabditis megidis* (01PL) - 1 infested L3 grub out of some 4000 collected in October for winter storage - never re-isolated again from insects or soil samples - no apparent effect on the pest population

1998 - *Steinenrma arenarium* (05PL) - isolated from soil samples collected in April - occasionally re-isolated from soil samples in following years - no apparent effect on the pest population

Conclusions

⇨ *Amphimallon solstitiale* showed to be highly unpredictable host species for nematode infection.

⇨ The examined species and strains of entomopathogenic nematodes showed significant differences between their infectivity to *Amphimallon solstitiale*.

⇨ Detailed study on the most effective species (i.e. *H. megidis, H. indica, H. zealandica, S. glaseri* and *S. arenarium*) revealed that their infectivity was further affected by the insect

- development and available penetration routes;
- moulting,
- seasonal activity,
- accidental wounding,
- active elimination of IJs, and

Conclusions

- ⇨ Modification of spiracles clearly improved efficacy of nematode penetration into the hemocoel of pupae and adult *A. solstitiale*.
- ⇨ Direct penetration of IJs through the soft cuticle could explain the improved infectivity of *H. megidis* during the insect larval moulting.
- ⇨ Third instar grubs tested in the autumn or winter were much less susceptible to nematode infection than those tested in the spring.
- ⇨ The population of *S. glaseri* infective juveniles was rapidly reduced in the soil inhabited by *A. solstitiale* grubs.
- ⇨ The host – parasite relationships between *A. solstitiale* and entomopathogenic nematodes still remain only partially understood and require further investigations.